The effects of road crossings on stream macro-invertebrate diversity

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Abstract
Although it is well known that the increasing size of the human population has a negative effect on freshwater biodiversity, the subject of whether or how the intersection of roads and streams (hereafter road crossings) influence the diversity of stream macro-invertebrates is under-researched. To fill this gap in our knowledge, we collected stream macro-invertebrates from road crossings (bridges and culverts) and compared their diversity with upstream and downstream sections. We found that road crossings had negative effects on the richness and abundance of native macro-invertebrates, as well as on the number of protected taxa. Our results showed also that alien individuals were more abundant at road crossings. These findings support the assumption that road crossings contribute to the spread of alien species. The assessment of environmental variables indicated that road crossings caused habitat modifications, and based on these it can be assumed that habitat modifications and associated phenomena (e.g. pollutants and storm events) were the major drivers of the observed patterns in biodiversity. Our results fill a knowledge gap and contribute to the deeper understanding of the effect of road crossings on freshwater biodiversity.

Keywords Diversity decline · Freshwater biodiversity · Human impact · Macro-invertebrates · Road crossings

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Introduction

One of the greatest challenges facing society today is the need to halt biodiversity decline, while promoting social and economic benefits. The increasing size of the human population is related to pollution, habitat degradation, species invasions, change in land cover as well as the increasing number of different human infrastructures. Unfortunately, these human-induced phenomena are frequently associated with a decline in biodiversity (Vörösmarty et al. 2010; Gál et al. 2019). Linear infrastructure such as roads, highways, railway lines, canals and pipelines are among the most widespread manifestations of human activity (Forman and Alexander 1998). Despite the fact that roads can promote diversified societal and economic benefits, they can also generate high environmental costs (Laurance et al. 2009). This problem could not be more timely, because by 2050 it is estimated that there will be a 60% increase in the total length of the road and rail network worldwide over that in 2010 (Dulac 2013).

Roads and associated crossings of roads and streams (bridges and culverts) can modify and degrade the natural flow and biodiversity of streams (Forman and Alexander 1998; Wemple et al. 2018). Roads can increase the extent of impermeable surfaces which reduces the volume of water infiltrating into the soil during rainfall events. Therefore, roads contribute to large volumes of surface run-off in urban areas (Walsh et al. 2012). Road-crossings are often associated with stormwater-drainage systems which rapidly deliver stormwater into streams, resulting in flashy hydrology. Moreover, unpaved roads in forests and rural areas can also generate overland flow during storm events (Ziegler et al. 2000; MacDonald et al. 2001). The intersection of roads and streams (hereafter road crossings) facilitate the introduction of sediment into streams due to erosive disturbances and mass wasting from adjacent hillslopes (Croke et al. 2005; Brown et al. 2014). Therefore, road crossings can contribute massively to sedimentation even during low magnitude rainfall events (Ziegler et al. 2001). The removal of macrophytes and riparian vegetation is also common practice in urban areas (e.g. nearby bridges and culverts), and results in an increased amount of sediment run-off. Besides fine sediment, road run-off may include various other pollutants like heavy metals, deicing salt, nutrients and pesticide depending on the surrounding land use (Boxall and Maltby 1997; Hopkins et al. 2013; Sebastiao et al. 2017; Wang et al. 2017).

Culverts and bridges may cause modifications in channel morphology due to altered hydraulics, and consequently change habitat quality. They can increase or decrease flow velocity, turbulence, the deposition of sediment downstream from the road crossings (aggradation), and degradation of the riverbed and channel enlargement (Douglas 1985; Roy and Sahu 2018). The substrate composition of the riverbed at road crossings might be structurally different from the natural upstream and downstream river sections. The riverbed at road crossings is frequently constructed of concrete and riprap, and this artificial surface is covered mostly by deposited silt and sand. All of these factors may reduce habitat heterogeneity at road crossings (Wellman et al. 2000; Bouska et al. 2010). Finally, road crossings can be potential barriers to the movement of fish and other aquatic organisms (Maitland et al. 2016).

Macro-invertebrates (i.e. invertebrate animals > 0.25 mm in length, Rosenberg & Resh, 1993) play an important role in freshwater ecosystems by feeding on various food sources (e.g. algae, coarse detritus or fine particulate organic matter, Cummins, 1974), by ecosystem engineering (Mermillod-Blondin 2011), as well as by providing food for higher trophic levels (Nery and Schmera 2016). Due to their taxon-specific sensitivity, macro-invertebrates are the most widely used organisms in freshwater biomonitoring of human
impact (Bonada et al. 2006). Although macro-invertebrates show sensitivity to different human-induced factors such as habitat modification (Wirth et al. 2010) or species invasions (Schmidlin et al. 2012), only sporadic and indirect information is available on how road-crossing structures influence community diversity (see Peterson 2010 and Cocchiglia et al. 2012). A possible explanation might be that the phenomenon was examined mostly on fish rather than macro-invertebrate communities.

Despite this knowledge gap, we have several predictions on how road crossings affect the diversity of stream macro-invertebrates. We already know that the type and abundance of freshwater invertebrates are controlled by the particle size of the stream-bottom substrate. Cobble and pebble, for instance, support a greater abundance and diversity of macro-invertebrates than sand or silt (Cummins and Lauff 1969; Erman and Erman 1984). Regarding run-off, Smith and Kaster (1983) found that pollutant-sensitive taxa are half as abundant at sites with an intermediate amount of highway run-off than at control sites. Moreover, sedimentation influences food resources such as periphyton quality and quantity for algivorous macro-invertebrates (Yamada and Nakamura 2002; Suren and McMurtrie 2005). The filter feeding apparatus of collectors can also become clogged by fine sediment, which reduces feeding efficiency (Vuori and Joensuu 1996). The consequence is that the community composition of macro-invertebrates will be altered towards burrowers and detritus feeders (Larsen et al. 2011). Macro-invertebrates may drift passively if they are dislodged due to anthropogenic disturbances. However, drift can also be active as a behavioral response to some stimulus (Hershey et al. 2010). There are studies suggesting that a slow-flow velocity can induce active or direct drift (Pearson and Franklin 1968; Minshall and Winger 1968; Waters 1972). This result is obviously due to the fact that there is less oxygen in the water at a decreased flow velocity, thereby individuals actively dislodged from the substrate seek better flow conditions and more oxygenated water. Changes in sedimentation and flow regimes can support drifting behavior of macro-invertebrate populations (Imbert and Perry 2000; Shaw and Richardson 2001; Gibbins et al. 2010), which can also influence community structure.

From the perspective of water quality degradation related to road run-off, most studies have investigated the impacts of heavy metals and deicing salt. Road maintenance is frequently accompanied by deicing salts which can significantly increase ion concentrations. Deicing salts such as sodium chloride (NaCl) and magnesium chloride (MgCl₂) are toxic to many macro-invertebrate taxa, especially Ephemeroptera and Plecoptera (Kotalik et al. 2017). Heavy metals and other chemicals may accumulate in stream sediments leading to a loss of macro-invertebrate diversity (Maltby et al. 1995; Boxall and Maltby 1997; Beasley and Kneale 2002). The majority of aquatic insects disperse along stream corridors and thus the connectivity of the stream channel and the riparian zone at the stream bank are essential for colonization processes (Petersen et al. 2004). However, road crossings may also act as physical and optical barriers for adult winged aquatic insects. Road crossings may physically block insect pathways for upstream flight, and thus reduce the density of larvae at upstream sections (Blakely et al. 2006). Road crossings may also change polarization patterns at the water surface, thereby affecting flight paths of aquatic insects (Kriska et al. 1998, 2007), and potentially forcing female insects to lay eggs downstream from bridges (Málnás et al. 2011) Bridges can also become ecological traps for nocturnal insects due to light sources (e.g., street-lamps). These unpolarized light sources may attract flying nocturnal insects (Kriska et al. 2007). Unpolarized light from the street-lamp may also become horizontally polarized through reflection from smooth, dark surfaces like asphalt, mimicking the appearance of a natural water body. This may result in massive numbers of insects laying eggs on the asphalt.
road of the bridge instead of in the river (Szaz et al. 2015). Based on these observations we hypothesized that the abiotic habitat at road crossings is different from the unaltered upstream and downstream sections, and that road crossings have a negative effect on the diversity of freshwater macro-invertebrates.

In the present study, we examined the effects of road crossings on the diversity of stream macro-invertebrates. In particular, we asked (1) whether the abiotic habitat at road crossings is different from the unaltered upstream and downstream sections, (2) whether the road crossings decrease the diversity of native macro-invertebrates, (3) whether the road crossings attract more alien taxa than the unaltered stream sections and (4) whether the road crossings alter the community composition of macro-invertebrates.

**Methods**

**Study sites**

Hungary (area: 93,030 km²) is a country in Central Europe. Approximately 68% of the area is situated below an altitude of 200 m a.s.l. (lowland). All running waters belong to the river Danube system. We selected 9 study sites (Suppl. Table 1) based on the following criteria: (1) sites located in lowland areas, (2) sites where the stream width is less than 10 m, and (3) sites situated outside urban areas. Within each site (e.g. Suppl. Fig. 1), we defined a road-crossing section located directly below a bridge, where the length of the sampling reach corresponded to the bridge width (hereafter road crossing section), and two 50-m long sections, one upstream (hereafter upstream section) and one downstream (hereafter downstream section). Upstream and downstream sections were separated by 100 m from the road crossing sections.

**Assessment of environmental variables**

The assessment of environmental variables was performed in each stream section in October (2016), April (2017) and July (2017). Water chemistry parameters e.g. temperature, pH, conductivity (μS/cm corrected to 25 °C) and salinity (ppt-parts per thousand) were measured with a Hanna Combo pH/EC/TDS/Temp tester (HI 98129 model). Stream sections were also characterized by nine visually estimated environmental variables considering water depth, current velocity and substrate composition (Suppl. Table 2).

**Sampling and identification of macro-invertebrates**

A kick and sweep sampling technique was used to collect macro-invertebrates using a hand net (500 μm mesh). At each section (upstream, road crossings, downstream) and sampling date (October 2016, April 2017 and July 2017), we took 3 replicate three-minute samples covering most microhabitats present in the section. Samples were kept separately and preserved in 70% ethanol, returned to the laboratory on the same day and identified to the lowest possible taxonomic level (usually species) according to the relevant references (Soós 1957, 1963; Richnovszky and Pintér 1979; Savage 1989; Csabai 2000; Csabai et al.
We followed the taxonomical nomenclature of the Fauna Europaea Web Service (de Jong et al. 2014). Taxa were classified as non-native (alien) or native based on DAISIE (DAISIE 2008) and other sources (grey literature). Protected species are listed in the corresponding 100/2012. (IX.28.) decree of the Ministry of Rural Development.

Data analysis

Constrained Analysis of Principal Coordinates (CAP, Anderson and Willis 2003) with Euclidean Distance (Podani 2000) was used to test the separation of stream sections (upstream, road crossing and downstream) using standardized abiotic variables (all values were standardized between 0 and 1). We ran an ANOVA-like permutation to test for the significance of the separation of stream sections in multivariate space. In this test, the number of permutations is controlled by the targeted “critical” P value (alpha) and accepted Type II or rejection error (beta). If the results of permutations differed from the targeted alpha at the risk level given by beta, the permutations were terminated (Oksanen et al. 2010). Our ANOVA-like permutation tests were terminated at 999 permutations.

Principal Component Analysis (PCA; Podani 2000) was used to reveal which environmental variables separate upstream, road crossings and downstream sections.

We used linear models to examine whether the numbers of native taxa (log-transformed) and individuals (log-transformed) were influenced by the stream section (i.e. upstream, road crossing and downstream), study sites and seasons. We selected the best-fit models using an information theoretic approach based on the Akaike Information Criterion corrected for the number of cases and parameters estimated (AICc), and Akaike weights (Garamszegi and Mundry 2014). Delta AICc indicates the difference in the fit between a particular model considered and that of the best fit model. Models with delta AICc < 10 are presented in the Results section. AIC weight was calculated among all possible pairs. If the ANOVA table of the best fit model revealed significant differences, then a Tukey test (Zar 1999) was used as multiple comparison method. The individual-based rarefaction, originally suggested by Sanders (1968) and corrected by Hurlbert (1971), was used to compare taxa richness independently from the number of individuals collected. For rarefaction analyses, site, abundance data for site, seasonal and replicate samples were pooled. Generalized linear models (GLMs) with Poisson distributions were used to test whether stream section (i.e. upstream, road crossings and downstream), study sites and seasons influence the numbers of protected taxa, protected individuals, alien taxa and alien individuals. The application of a Poisson distribution was necessary because of many zeros in the response variables. As in linear models, the best-fit models were selected using an information theoretic approach based on the Akaike Information Criterion corrected for the number of cases and parameters estimated (AICc) and Akaike weights.

Constrained Analysis of Principal Coordinates (CAP, Anderson and Willis 2003) with Bray–Curtis distance (Podani 2000) was used to test the separation of the macro-invertebrate community in different stream sections (upstream, road crossing and downstream). We ran an ANOVA-like permutation to test for the significance of the separation of stream sections in multivariate space. Statistical analyses were performed in R (R Core Team 2016) using the car (Fox and Weisberg 2011), multcomp (Hothorn et al. 2008), MuMln (Barton 2016) and vegan (Oksanen et al. 2016) packages.
Results

Environmental variables

CAP showed overall environmental differences between upstream, road crossing and downstream sections (ANOVA-like permutation: F = 12.860, P = 0.001). The ordination plot showed that road crossing sections were clearly separated (positive side of CAP1 axis) from upstream and downstream sections (both at the negative side of CAP1 axis, Suppl. Fig. 2). PCA confirmed this separation and showed that road crossings are characterized by a high proportion of concrete and riprap habitat with high current velocity, while upstream and downstream sections are in general deeper, covered mostly by pebble, and characterized by terrestrial and aquatic plants (Fig. 1).

Taxa richness and abundance of native macro-invertebrates

Altogether 157 taxa were found among the 32,507 identified individuals (Suppl. Table 3). We identified 7 protected (Argyroneta aquatica, Dolomedes fimbriatus, Aeshna isocela, Orthetrum brunneum, Calopteryx virgo, Borysthenia naticina, Anisus (Disculifer) vorticus) and 4 non-native taxa (Dikerogammarus bispinosus, Echinogammarus ischnus, Limnomysis benedeni and Potamopyrgus antipodarum). Six protected species were present exclusively in upstream and downstream sections while a single protected species was present only at one road crossing section. The most abundant taxa were Simuliidae sp. (6346 individuals), Asellus aquaticus (3811 individuals), Baetis rhodani (3717 individuals) and Chironomidae sp. (3262 individuals).

The best fit model (with the lowest AICc) revealed that the native taxa richness of macro-invertebrates was influenced by the stream section, site and season (Table 1). Alternative
statistical models are not plausible (Table 1). The ANOVA table of the best fit model identified the significant effect of stream section, site and season (Table 2). The Tukey test showed that upstream sections had the highest number of native taxa followed by downstream and road crossing sections (Fig. 2). Regarding the number of native individuals, the best fit model revealed the effect of stream section and site (Table 1). Alternative statistical models explaining the number of native individuals are not plausible (Table 1). The ANOVA table of the best fit model identified the significant effect of stream section and site (Table 3).

**Table 1** Best fit linear models explaining the effects of stream section, site and season on the numbers of native taxa and individuals of macro-invertebrates

| Response variable                      | Predictors                          | df  | AICc    | Delta AICc | Weight |
|---------------------------------------|-------------------------------------|-----|---------|------------|--------|
| Number of native taxa                 | Stream section + site + season       | 14  | 421.1   | 0.00       | 0.986  |
|                                       | Stream section + site                | 12  | 429.5   | 8.49       | 0.014  |
| Number of native individuals          | Stream section + site                | 12  | 645.1   | 0.00       | 0.806  |
|                                       | Stream section + site + season       | 14  | 647.9   | 2.87       | 0.192  |

Only models with delta AICc < 10 are displayed

**Table 2** Summary output of ANOVA table explaining the effect of stream section, site and season on the taxonomic richness of native macro-invertebrates

| Predictor       | Df  | Sum Sq | Mean Sq | F value | P      |
|-----------------|-----|--------|---------|---------|--------|
| Stream section  | 2   | 14.510 | 7.255   | 23.445  | < 0.001|
| Site            | 8   | 26.523 | 3.315   | 10.713  | < 0.001|
| Season          | 2   | 3.904  | 1.952   | 6.308   | 0.002  |
| Residuals       | 230 | 71.173 | 0.309   |         |        |

**Fig. 2** Effect of stream section (upstream [white], road crossing [dark grey] and downstream [light grey]) on the number of native macro-invertebrate taxa. Different letters indicate differences by Tukey test. Bars indicate mean values, whiskers standard errors

**Table 3** Summary output of ANOVA table explaining the effect of stream section and site and on the number of native macro-invertebrate individuals

| Predictor       | Df  | Sum Sq | Mean Sq | F value | P      |
|-----------------|-----|--------|---------|---------|--------|
| Stream section  | 2   | 12.66  | 6.333   | 8.062   | < 0.001|
| Site            | 8   | 78.279 | 9.785   | 12.456  | < 0.001|
| Residuals       | 232 | 182.251| 0.786   |         |        |
while the Tukey test showed that upstream sections harbor a larger abundance of native individuals than road crossing and downstream sections (Fig. 3). Rarefied taxa richness revealed that upstream sections had the largest taxa richness independent of the number of individuals collected, followed by downstream and road crossing sections (Fig. 4).

**Taxa richness and abundance of protected and alien taxa**

The best fit GLMs revealed that the number of protected taxa was influenced mostly by the stream section and site, and that alternative statistical models are not plausible (Suppl. Tables 4 and 5). A Tukey test showed that road crossings had a negative effect on the number of protected taxa (Fig. 5). The most plausible statistical model showed that the number of protected individuals was influenced by site and season (Suppl. Tables 4 and 6).
Regarding the number of alien taxa, we found several plausible statistical models (Suppl. Table 4). Most of the models suggested the importance of site (4 models) while two models showed the importance of stream section and season (Suppl. Table 4). Our information theoretic approach revealed only a single statistical model to explain the number of alien individuals (Suppl. Table 4). This statistical model indicates the significant effects of stream section, site and season (Suppl. Table 7). A Tukey test showed that road crossings had a positive effect on the number of alien individuals (Fig. 6).
Community composition

Constrained analysis of principal coordinates showed that the different stream sections differed in the composition of macro-invertebrate communities (ANOVA-like permutations: $F_{2,240} = 2.827, P = 0.001$). The ordination plot showed that the community composition of road crossings (black) differed from the community composition observed in upstream (white) and downstream (gray) sections (Fig. 7).

Discussion

There is a substantial lack of studies that have examined the effects of road crossings on the diversity of stream macro-invertebrates. To fill this gap, we collected macro-invertebrates from road crossings, as well as from upstream and downstream sections and compared their diversity. We found that road crossings have negative effects on the richness and abundance of native macro-invertebrates, as well as on the taxonomic richness of macro-invertebrates with conservation importance (protected taxa). Our results also showed that alien individuals were more abundant in road crossings than in upstream and downstream sections. These findings suggest that road crossings negatively influence the integrity of stream macro-invertebrate communities.

Habitat alteration is probably the greatest anthropogenic threat to freshwater ecosystems (Ogren and Huckins 2015). Our results are in line with previous studies stating that road crossings cause changes in stream channel geomorphology, and thus modify the natural stream bed habitat (Bouska et al. 2010; Roy and Sahu 2018). The most obvious explanation for the difference among habitats at road crossings and the unaltered downstream and
upstream sections is the use of concrete stream bed, as well as riprap with gabion (woven wire mesh unit filled with stones) at road crossings. These artificial structures reduce habitat heterogeneity and present conditions that are notably different from those present in natural stream ecosystems.

Our results suggest that road crossings have negative effects on native macro-invertebrate diversity. The native taxon richness and abundance clearly decreased under the road crossing sections compared to the upstream sections, where the diversity was the greatest. The low diversity was associated with environmental variables reflecting poor habitat quality and low habitat heterogeneity. A possible explanation is that modified habitats, such as road crossings, may have a reduced available surface area compared to natural and more complex habitats. Obviously, the reduced available surface area decreases the diversity of stream macro-invertebrates (Brauns et al. 2007). Moreover, the complex and stable habitats can serve as a refuge during storm events. This is particularly important considering the fact that urban areas and roads can create large volumes of surface run-off from the impermeable surfaces, resulting in flashy floods (Wemple et al. 2018). Moreover, the road surface run-off may contain different chemicals (Davis et al. 2001) which can also negatively impact diversity (Beasley and Kneale 2002; Robson et al. 2006). The differences between the diversity in upstream and downstream sections might be explained by the phenomenon that the run-off at road crossings may have had impacts not just directly at the road crossings but also at downstream sections (Fig. 2). Finally, the local hydrological and geomorphological conditions can also be important for the accumulation and dispersion of road-sourced contamination in sediment. Consequently, downstream sections might be exposed to contamination which can lead to many adverse effects on aquatic ecosystems (Sebastiao et al. 2017).

The most abundant macro-invertebrate taxa in our study are relatively tolerant of human disturbances. However, we collected protected taxa almost exclusively from upstream and downstream sections, and found also that road-crossing structures have a negative effect on the number of the protected macro-invertebrate taxa (Fig. 5). This result is not surprising because protected taxa are sensitive to anthropogenic stress related to habitat modification (Ilmonen et al. 2012). We collected only a single protected Mollusca species, *Borysthenia naticina* at one road crossing section (we also found this species at the upstream and downstream sections). A possible explanation for this observation is that riprap with gabion may offer adequate habitat for this species. For instance, the riprap allowed some submerged macrophytes to grow beneath the road crossing and the bottom was covered with sand and a thin detritus layer. Although one of the main conclusions of this study is that road crossings had a negative effect on protected taxa richness, evidence from previous studies suggests that human-impacted waterbodies can also maintain several macro-invertebrate taxa with conservation importance (Vermonden et al. 2009; Schmera and Baur 2011; Hill et al. 2016), and thus the appearance of a protected species at road crossing section is not surprising.

In contrast, alien species frequently occupy these modified and disturbed stream habitats. Riverbed modification can provide uncolonized habitats that may allow newly-arriving alien macro-invertebrates to establish (Wirth et al. 2010). Low habitat heterogeneity (Kestrup and Ricciardi 2009), fluctuating resources (Davis et al. 2000) and declines in water quality may present conditions that are more adequate for invaders than native community members (Karatayev et al. 2009). Many studies have found that alien species can use these fragments as stepping stones for dispersal. Consequently, these spatially separated but altered habitats present corridors in the landscape and may assist in the spread of alien species (Parenedes and Jones 2000; Rahel 2002; Havel
et al. 2005). We found that alien taxa are present in the studied streams and that road crossings had a positive impact on the abundance of alien specimens. The observed significant effect of site can be explained by the patchy distribution of alien individuals among the studied sites, while that of season by the natural flow regime. At low water levels, tributaries can filter population dispersal and population persistence (Wilson and McTammany 2014; Tonkin et al. 2018; Milner et al. 2019). High water levels, in contrast, can promote the dispersal of species in such a way that it abates the filtering effects by decreasing the differences between the tributaries and mainstreams (Czeglédi et al. 2016). In agreement with this assumption, most of the alien species that we found originated from the River Danube (Bódis et al. 2012; Borza 2014; Borza et al. 2017).

Our study provided clear evidence that the composition of macro-invertebrates in road crossings differ from upstream and downstream sections. We assume that compositional differences of macro-invertebrates reflect differences in environmental conditions between stream sections. The Hydropsychidae family, for instance, take advantage of stable water flow and use nets to capture suspended organic matter as food (Hershey et al. 2010), while modified flow may break apart their filter feeding-nets (Boon 1993). The abundance of Ephemeroptera is determined also by habitat requirements, thus the growth of algae and moss increase the abundance of some species (Baetis), while others that need clean rock surfaces and use suckers or friction pads for attachment (Heptageniidae) are excluded from these habitats (Ward 1976; Ellis and Jones 2013). Plecoptera are highly sensitive to human disturbances in such a way as their abundance increases with distance from human developments (Ward and Stanford 1995; Milner et al. 2019). Chironomidae and Oligochaeta are frequently more common near human development, where the rate of habitat modification is the largest. Finally, some snails (Basommatophora) are able to resist the altering environmental factors and show great abundance in regulated streams (Jones 2012). In sum, we found that road crossings harbor a distinct and poorer community than natural stream sections. Generally, it can also be considered that the most abundant macroinvertebrate taxa (Simuliidae sp., Asellus aquaticus, Chironomidae sp.) were not sensitive to the human disturbances; in addition, the most abundant Ephemeroptera taxa (Baetis rhodani) is also a relatively tolerant species.

There is a common view among freshwater ecologists that minor alterations have negligible effects on aquatic ecosystems (Jennings et al. 1999; McGoff et al. 2013). Even though road crossings seem to alter habitats and natural stream ecosystems only moderately, these changes together have a significant impact on freshwater biodiversity. This insightful study investigated the effects of road crossings on macro-invertebrate diversity. We found that road crossings caused habitat alterations and contributed to a decrease in the numbers of native and protected macro-invertebrate species. Our results suggest also that road crossings might contribute to the spread of alien taxa. In conclusion, our study provides evidence of the negative impacts of road crossings on stream macro-invertebrate diversity.

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