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Reassessing the indicator value of the EPT group in karst rivers under hydromorphological pressure

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ABSTRACT

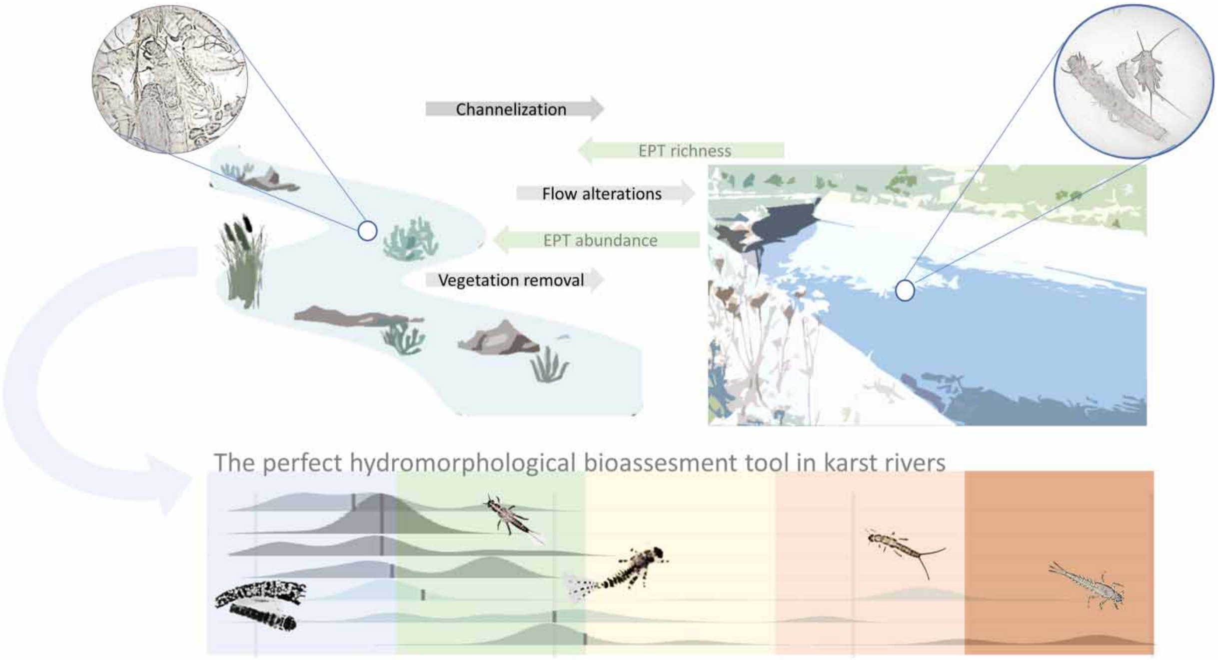
Hydromorphological degradation is one of the most common stressors to freshwater ecosystems nowadays. Rivers lose riparian vegetation, habitat heterogeneity, natural flow velocity, etc., due to hydromorphological alterations. We analyzed macroinvertebrate communities in a wide range of hydromorphological conditions – from near natural sites to significantly altered water bodies, focusing on Ephemeroptera, Plecoptera, and Trichoptera (EPT). Considering that the EPT group is a quite sensitive and generally stenovalent group, we wanted to examine which of the hydromorphological pressures affects them the most. We also wanted to identify indicator taxa for different levels of degradation: minor, moderate, and severe. We collected samples from 84 karst rivers sites in Croatia. We found 52 taxa of EPT (Ephemeroptera – 21, Plecoptera – 11, Trichoptera – 20). Changes in river morphology proved to be the most important stressor affecting the distribution of the EPT group. Hydrological regulation did not show significance toward the EPT community, possibly due to the karst nature of the rivers studied. The most sensitive EPT taxa were those with the greatest preference for macrophytes and lithal habitats. More tolerant EPT taxa were those with a wide range of habitat preferences and/or taxa that feed on particulate organic matter.

Key words: caddisflies, hydromorphological degradation, mayflies, microhabitat, morphology, stoneflies

HIGHLIGHTS

- Our research underscores the pivotal role of morphological changes in rivers as the primary stressor impacting EPT communities.
- We identify indicator taxa for different levels of hydromorphological degradation.
- Focusing on the understudied karst rivers, our research provides unique insights into the complex and heterogeneous habitats of these ecosystems.
- Reliable metrics for assessing river health.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Anthropogenically unaffected river ecosystems are rare in Europe, apart from highly protected areas such as national parks, which are most likely also under the influence of climate change (Dorić *et al.* 2023; Pozojević *et al.* 2023). It is also highly unlikely that there is a single anthropological pressure burdening the functioning of aquatic communities in rivers. Therefore, the majority of *in situ* freshwater ecological research are multistressor impact related (Birk *et al.* 2020; Vos *et al.* 2023). Hydromorphological degradation is considered one of the main drivers of community change in aquatic ecosystems and is by definition, a multistressor as it combines several intertwined environmental features: hydrology, morphology and longitudinal connectivity, which consequently influence other environmental factors as well. Hydromorphologically degraded rivers contribute significantly to the overall global river network. As such, they have an official designation – heavily modified water bodies (HMWBs). HMWBs are defined as freshwater habitats, whose natural hydrology or morphology is altered to maintain a major human purpose and function (WFD CIS 2003).

Disturbance of natural hydrological regime reduces the heterogeneity of freshwater habitats, it can affect the connectivity between surface and groundwater and the lateral connectivity of freshwater habitats (Pavlek *et al.* 2023). Removal of vegetation and woody debris in the riparian zone reduces the habitat heterogeneity essential for emerging insects and reduces the stability of riverbanks (Palt *et al.* 2023). In freshwater environments, a positive correlation between biodiversity and habitat heterogeneity and complexity is generally recognized, and it has already been shown that morphological man-made alterations generally reduce this heterogeneity (Friberg *et al.* 2009; Garcia *et al.* 2012). Flow regulation alters the river ecosystem in two ways (Blinn *et al.* 1995): (1) an increase in laminar flow (i.e., current velocity) can lead to the accidental drift of macroinvertebrates and food sources (Miliša *et al.* 2006); (2) on the other hand, a decrease in flow velocity can lead to sediment deposition (both organic and inorganic) in certain parts of the river, but also to oxygen reduction, which negatively affects the river fauna (Englund & Malmqvist 1996; Kennen *et al.* 2014).

From the ecosystem services point of view, habitat alterations can be considered one of the biggest problems related to freshwater habitats, as they interrupt the process of providing drinking water, energy, nautical, and tourism activities in altered hydrosystems (Russi *et al.* 2013). To achieve sustainability, bioassessment of freshwater ecosystems is an indispensable tool (Dolédec & Statzner 2010), and it is generally carried out by analyzing communities of several groups of organisms. However, it has been shown that macroinvertebrates are one of the best known and most reliable assessment groups (Bonada *et al.* 2006). Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa are generally stenovalent taxa that usually cannot tolerate nutrient and organic matter enrichment and are sensitive to changes in hydrological and morphological riverine features and loss of microhabitat heterogeneity (Ab Hamid & Rawi 2017). Among macroinvertebrates, EPT taxa are particularly good and widespread bioindicators of environmental conditions in lotic habitats (Rosenberg & Resh 1993). EPT is an abbreviation for the three insect orders mentioned above, which are usually considered and expressed together as EPT metrics because of their similarities in condition and habitat preferences. Therefore, many EPT representatives are among the first to disappear from artificially modified habitats that have been altered to serve human interests more efficiently (Ghani *et al.* 2016). Overall, sensitive, stenovalent taxa such as the EPT are not expected to be abundant and diverse at HMWBs (Vilenica *et al.* 2022). HMWBs are primarily affected by channelization (as a morphological stressor) and hydropeaking (significant discharge fluctuations causing hydrological stress). Stream channelization results in a loss of the natural mosaic of lotic and lentic habitats for EPT taxa; flow regulation and hydropeaking can have dramatic negative effects on passive drifting during upwelling and cause acute egg-mortality particularly in EPT taxa lay their eggs near the shoreline and partly emerged rocks, while removal of vegetation results in a direct loss of shelter and food for most of these sensitive taxa (Ekka *et al.* 2020; Salmaso *et al.* 2021).

Karst is the result of complex hydrological and geological processes in which water-soluble rock forms and reshapes over a long period of time. Because of this karst rivers provide heterogenous habitats, due to the different forms and shapes of karst. Therefore, these rivers are inhabited by numerous and diverse animal species (Ivković & Plant 2015; Ridl *et al.* 2018).

We hypothesized that hydromorphological (HYMO) degradation has a significant influence on the composition and abundance of EPT groups in karst rivers. Our first objective was to determine the main features of hydromorphological degradation that contribute to the variability of EPT groups. Our second objective was to determine how highly sensitive to less sensitive EPT taxa respond to a gradient of HYMO characteristics assessed as significant in the previous step. Finally, our third objective was to determine taxa that are indicators of (a) little or no, (b) moderate, and (c) severe degradation, with potential implications for monitoring of karst rivers.

2. MATERIALS AND METHODS

2.1. Study area and sampling

Benthic macroinvertebrates were sampled at 84 sampling sites in the Dinaric Western Balkans Ecoregion in Croatia (Figure 1 – list of sites in Supplementary material, Table S1). According to the Köppen–Geiger climate classification, the study area is characterized by Mediterranean climate (Beck *et al.* 2018), defined by Csa type. More specifically, the average temperature of the warmest month is above 22 °C and above −3 °C in the coldest month, while the precipitation of the driest month is less than 30 mm, i.e., less than one-third of the wettest month (Zaninović *et al.* 2008). In 2018, the average monthly air temperature in the study area ranged from 13 to 15 °C, while the total annual precipitation ranged from 900 to 1,300 mm (DHMZ 2019). The geology consists mainly of karst bedrock (Illies 1978; Šegota & Filipčić 2003).

To eliminate as much as possible the influence of other stressors present in the karst rivers studied, sites with little or no other anthropogenic pressures were selected on a gradient of hydromorphological degradation. This was done following the criteria set by Feio (2011) in establishing reference sites for rivers in this specific region, with no restrictions on hydromorphological scores.

Samples were collected in spring 2018 following the 'multi-habitat sampling' approach: at each site, 20 subsamples were collected from microhabitats covering at least 10% of the area, in proportion to their coverage at each sampling site (AQEM Consortium 2002). Kick-sampling was conducted using a hand net (500 µm mesh). Each subsample covered an area of 0.0625 m^2 of the river bottom. Samples were immediately fixed in 96% ethanol.

In the laboratory, subsampling was done to reduce the effort of sorting and identification. Benthic organisms from one-sixth of the whole field sample were identified and counted. At least one subsample was sorted until the minimum targeted number of 700 individuals was reached. If it contained less individuals, further subsamples were analyzed until the total abundance count reached 700 or more individuals. If needed, the whole sample was analyzed. The rest of the sample (if remained) was also inspected for rare macroinvertebrates, i.e., which were not part of the analyzed subsample(s), with the use of a binocular stereomicroscope (Olympus SZX9 and SZX10). All invertebrate groups were identified to species and genus level where possible, with the exception of some Oligochaeta, Diptera, and Hydrahnidia individuals. For all early instars and/or damaged specimens (including EPT), identification usually remained at the family level – as is sometimes the case with regular bioassessments due to different instars of invertebrates and sometimes even specimen damaging in the sampling process. We used

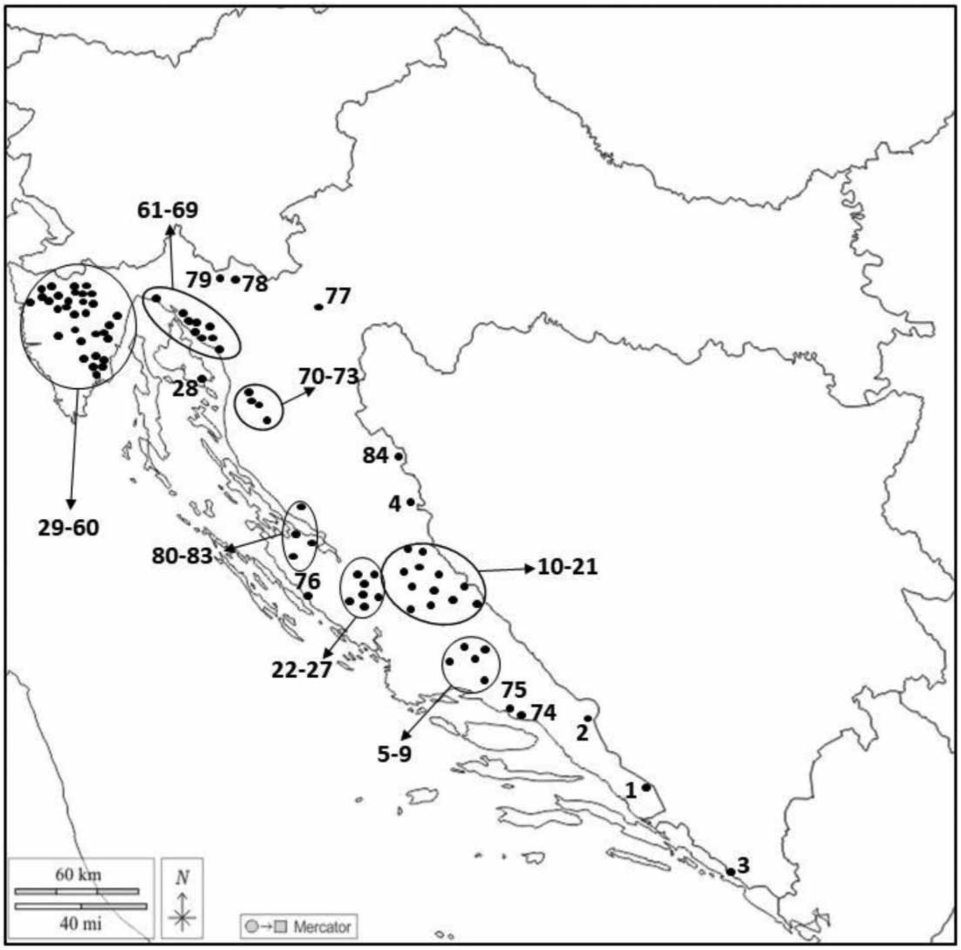


Figure 1 | Study area with 84 sampling sites on karst rivers in the Dinaric Western Balkans Ecoregion in Croatia. Due to the large number of sites investigated, individual numbering was impractical in this visualization. Therefore, the sites were grouped to roughly represent the position of a site, while the exact coordinates of the sites by site number can be found in Supplementary material, Table S1.

Campaioli *et al.* (1994) for all EPT groups, Müller-Liebenau (1969), Malzacher (1984) and Bauernfeind & Humpesch (2001) for identification of Ephemeroptera; Nilsson (1997) and Zwick (2004) for Plecoptera and other non-EPT taxa; Waringer & Graf (2011) for Trichoptera.

2.2. Hydromorphological evaluation

Hydromorphological alterations were assessed using the European Standard EN 156843:2010 ‘Water quality – Guidance standard on determining the degree of modification of river hydromorphology’ (DIN 2010). This assessment includes a total of 16 hydromorphological parameters; two hydrological parameters (Impact of artificial in-channel structures within the reach and Effect of catchment-wide modifications to natural flow); 12 morphological parameters (Planform, Channel section, Extent of artificial material, ‘Natural’ substrate mix or character altered, Bank structure and modifications, Aquatic vegetation management, Extent of woody debris, Erosion/deposition character, Vegetation type/structure on banks and adjacent land, Land use and associated features, degree of lateral connectivity of river and floodplain, and degree of lateral movement of river channel). Remaining two parameters are related to longitudinal connectivity and flow stability. The parameters listed represent quantitative or qualitative variables. Therefore, quantitative variables were expressed by five possible scores: 1 (near natural), 2 (slightly modified), 3 (moderately modified), 4 (extensively modified), and 5 (extremely modified). Qualitative variables have three possible scores: 1 (near natural), 3 (moderate alteration), and 5 (severe alteration) (Zaharia *et al.* 2018; Pavlek *et al.* 2023).

2.3. Data analysis

All hydromorphological parameters evaluated were used in an 'interactive forward analysis' based on redundancy analysis (RDA) followed by a Monte Carlo permutation test. The abundance of all EPT taxa was used in this analysis, which was performed using the CANOCO package version 5.0 (Ter Braak & Šmilauer 2012). This analysis was conducted to identify the main features of hydromorphological degradation that contribute to the variability of EPT groups. After determining the key hydromophological features, a simple polynomial regression was performed using these significant features as explanatory variables and the number of EPT taxa (S) and EPT group abundance (N) as response variables using Statistica 14.0 (TIBCO Software Inc. 2020). Polynomial regression was used to determine the 'flow' of the relationship between hydromorphological features and EPT taxa richness and abundance.

To evaluate how sensitive and tolerant EPT taxa respond to a gradient of significant HYMO features, two analyses were conducted: (1) the TITAN or Threshold Indicator Taxa Analysis (TITAN2 package; Baker *et al.* 2015). TITAN combines indicator species analysis and change-point analysis (Qian *et al.* 2003) to identify taxa whose occurrence increases or decreases along an environmental gradient. TITAN analyses were conducted using 500 bootstraps on taxa abundance matrices. Only taxa occurring in more than three samples were included in this analysis according to Qian *et al.* (2003). (2) The multi-level pattern analysis (R package *indicspecies*, De Cáceres *et al.* 2010.) was also chosen to include taxa with fewer occurrences on a gradient of HYMO features, and also because it is not as affected by the abundance of dominant species. Indicator taxa were tested at sites within three groups of morphology scores (little or no disturbance – 31 sites, scores: 1.00–2.25; moderate disturbance – 28 sites, scores 2.25–3.75; and severe disturbance – 25 sites, scores: 3.75–5.00). R software 2.2.4 (R Core Team 2022) was used to perform the above analyses.

3. RESULTS

A total of 52 EPT taxa was recorded at 84 sampling sites in karst rivers in Croatia: 21 taxa of Ephemeroptera, 11 taxa of Plecoptera, and 20 taxa of Trichoptera (Supplementary material, Table S3). Hydrology scores ranged from 1 to 5 for most features, with the worst overall HYMO score for a given site being 4.66 (Site 66, Figure 2(a)) and the best being 1 (Site 4, Figure 2(b)). Supplementary material (Table S2) shows all HYMO scores.

In the 'interactive forward RDA analysis', 12 HYMO parameters explained 20.6% of the total variation in EPT assemblages (Table 1). The only statistically significant parameter that influenced the whole EPT assemblage formation was the morphological score.

The morphological score, as the only significant HYMO feature, was tested as an explanatory variable with the number of EPT taxa (S) and the abundance of the EPT group (N) as response variables in a multiple polynomial regression (Figure 3). Both regressions were statistically significant and resembled a declining liner regression (Figure 3).

The morphological score was also tested as an environmental gradient in the TITAN analysis. The TITAN analysis revealed only decreasing taxa (z–) and no increasing taxa (z+) with increasing morphological degradation in the sampled rivers (Figure 4). In addition, TITAN identified seven taxa as significant indicators of changes in river morphology (Figure 5). The values of all other EPT taxa from this analysis are listed in Supplementary material, Table S3.

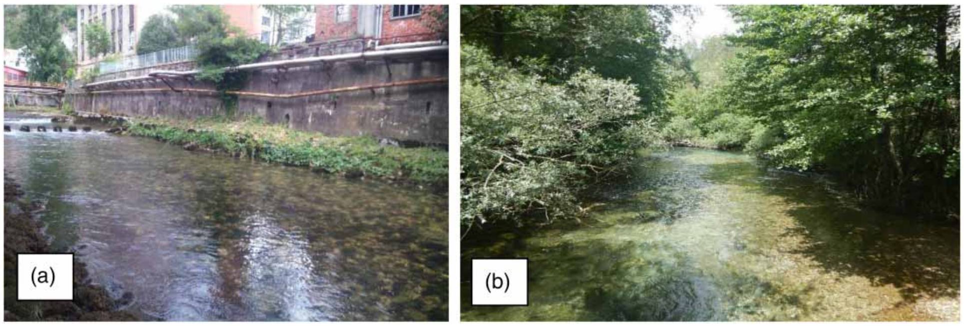


Figure 2 | Sites having (a) the worst total HYMO score at the Rječina stream (site 66) and (b) the best total HYMO score at the Krka spring (site 4).

Table 1 | Values of individual HYMO features in the 'interactive forward analysis' based on the RDA method that explain more than 20% of the total variation in EPT assemblages

Parameter	Variation explained %	Contribution in analysis %	Pseudo-*F*	*p*
3. Morphology	2.9	14.2	1.7	0.002
3.2.3. Bank structure and modifications	2.1	10.2	1.2	0.068
3.1.1. Planform	1.9	9.1	1.1	0.234
1.1. Impacts of artificial in-channel structures within the reach	1.7	8.2	1.0	0.498
3.1.2. Channel section	1.6	8.0	1.0	0.536
3.2.2. 'Natural' substrate mix or character altered	1.6	7.9	1.0	0.592
Total HYMO score	1.6	7.8	1.0	0.562
2.1. Longitudinal continuity	1.7	8.5	1.0	0.424
3.2.1. Extent of artificial material	1.4	6.7	0.8	0.868
1. Hydrology	1.5	7.0	0.9	0.790
1.2. Effects of catchment-wide modifications to natural flow	1.3	6.3	0.8	0.836
3.3.4. Vegetation type/structure on banks and adjacent land	1.2	6.0	0.7	0.950

Only parameters explaining more than 1% of the total variation are listed.

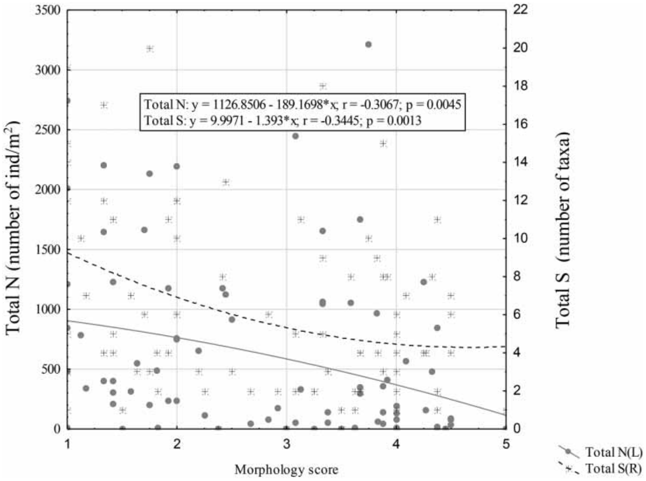


Figure 3 | Multiple polynomial regressions of morphology scores (*x*-axis) of karst rivers in Croatia against total abundance (left *y*-axis, Total *N*) and total taxa richness (right *y*-axis, Total *S*) of EPT taxa.

The results of the multi-level pattern analysis yielded six indicator species for sites with little or no morphological disturbance and two indicator species for sites with severe morphological disturbance (Table 2). No indicator species were detected for sites with moderate morphological disturbance while one indicator species was common for both groups with low and no disturbance to moderate morphological disturbance.

4. DISCUSSION

Our results revealed the overall morphological score parameter as the most significant driver of EPT variability among the assessed hydromorphological features of the studied karst rivers. We acknowledge that it is possible that other HYMO scores were not as responsive to EPT assemblages as expected due to of coarser gradients that comprised integer numbering. However, this was not the case for hydrology and the final HYMO score, which showed finer gradients, but were also not found to be significant drivers of EPT variability. In the studied region, i.e., the Dinaric Western Balkans, rivers are often characterized by natural flow intermittence that is not of anthropogenic origin, but rather occurs due to the hydrological

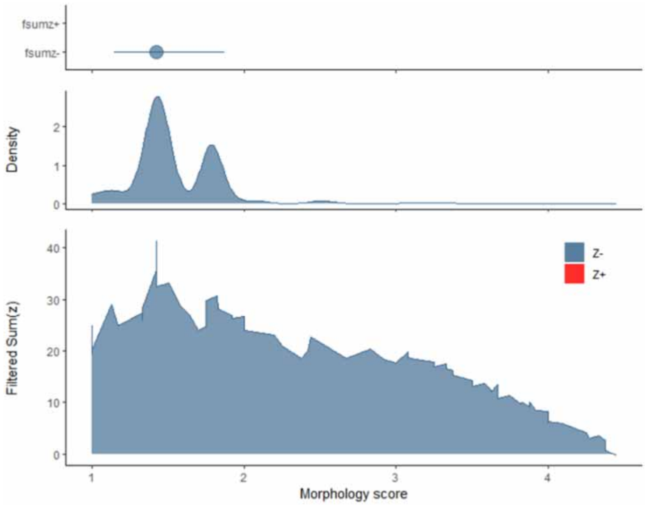


Figure 4 | EPT assemblage level change identified with TITAN, showing the magnitude of change in taxa that decrease along the gradient (z–) and those that increase along the gradient (z + , no taxa identified as such in this analysis). The peaks of the values indicate points along the environmental gradient that cause large changes in the EPT assemblage.

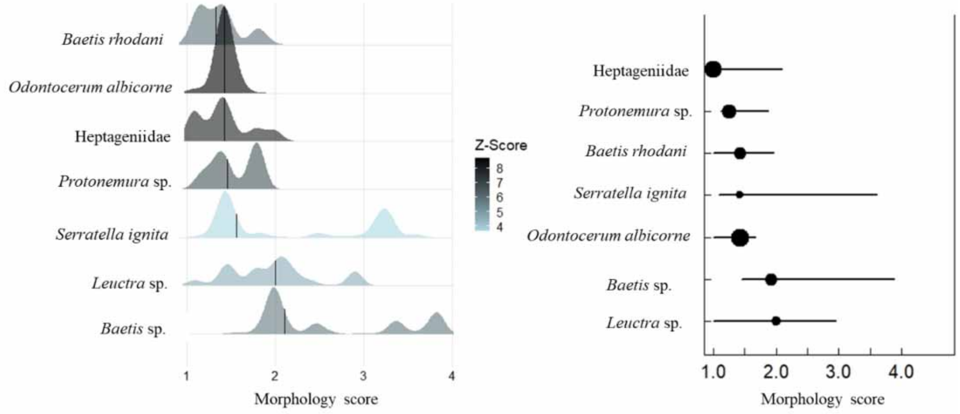


Figure 5 | Significant responses of specific EPT taxa to morphological degradation in karst rivers calculated using TITAN. Circles in the right graph indicate mean change-points in occurrence of each taxon (larger circles indicate more significant changes), while horizontal lines delineate 5th and 95th quantiles calculated based on 500 bootstraps. For all taxa occurrence decreased with increasing morphological disturbance (z– or ‘sensitive’ taxa).

traits of the karst bedrocks (Bonacci 2015). With this in mind, it is possible that most of the EPT taxa present in karst rivers of this region are naturally adapted to changes in hydrological regime (Suren & Jowett 2006), as recently documented for several Ephemeroptera species (Vilenica *et al.* 2021).

In determining the relationship between the morphological score and the number of EPT taxa and their abundance, we used multiple polynomial regression to avoid possible changes in community structure that would eventually lead to a ‘recovery’ in both abundance and taxa richness. However, this was not the case as the regressions were straightforward declining linear regressions showing that greater deterioration in morphology would be expected to result in a loss of EPT taxa richness and abundance, with no apparent offsetting or compositional changes within the community (Buffagni *et al.* 2004; Sabater *et al.* 2018). This presumption derived from the regression model was confirmed to some extent by the TITAN analysis and even by the multi-level pattern analysis (with two exceptions). The TITAN analysis showed that among the EPT taxa tested with increasing morphological degradation, only decreasing, i.e., sensitive taxa are present.

Several EPT taxa showed clear intolerance to increasing morphological degradation: *Baetis* sp., *Baetis rhodani*, Heptageniidae, *Serratella ignita*, *Leuctra* sp., *Protonemura* sp., and *Odontocerum albicorne*. Although morphological degradation

Table 2 | Results of the Multi-level pattern analysis – indicator taxa for three groups of morphological disturbance: little or no disturbance, scores: 1.00–2.25; moderate disturbance, scores 2.25–3.75; and severe disturbance, scores: 3.75–5.00

	Group 1 – Little or no disturbance	
	Stat	***p*-value**
Leuctra sp.	0.473	0.004**
Heptageniidae	0.469	0.01**
Protonemura sp.	0.44	0.016*
Odontocerum albicorne	0.397	0.013*
Dinocras sp.	0.359	0.042*
Oxyethira sp.	0.359	0.034*
	Group 2 – Moderate disturbance	
No taxa		
	Group 3 – Severe disturbance	
	stat	*p*-value
Habrophlebia fusca	0.416	0.013*
Nemoura sp.	0.346	0.031*
	Group $1+2$	
	Stat	*p*-value
Baetis sp.	0.776	0.006**

Multi-level pattern analysis, $\alpha = 0.05$*; 0.01**.

includes multiple parameters (see 'Materials and methods'), homogenization of the riverbed substrate is one of the most common alterations, since it provides more efficient manipulation of water flow (van Denderen *et al.* 2022). Therefore, the variety of rocky microhabitats such as micro-, meso-, and macrolithal are replaced by other, more homogeneous, flatter substrates. Such anthropogenic activities could explain the intolerance of *Leuctra* sp., as this genus has a strong preference not only for habitat diversity, but also for micro- and macrolithal specifically (Graf *et al.* 2009). Furthermore, a considerable proportion of species of the Heptageniidae family also preferably inhabit lithal substrates (Leitner & Lorenz 2020). Similar traits are found within the genus *Protonemura*, which prefers habitats with diverse rocky substrates and macrophytes – a significant shelter, oviposition and nutrient enrichment. Macrophyte removal and management is an ongoing and widespread stressor since macrophytes notably overlap with multiple water management purposes (Thomaz *et al.* 1999). *B. rhodani* is a rather tolerant and widespread species in lotic habitats (Ab Hamid & Rawi 2017), but it has the highest demands for microhabitats with macrophytes and lithal (Schmedtje & Colling 1996). Similarly, *S. ignita* occurs on a variety of substrates, with macrophytes being the most preferred (Schmedtje & Colling 1996). *B. rhodani* and *S. ignita* are both reported as rheophilic – if morphological degradation leads to reduced water flow, both species could suffer from such changes (i.e., decrease in abundance) (Vilenica *et al.* 2018). *O. albicorne* is the most complex species in this context, inhabiting a wide range of substrate types equally, from psammal, akal to microlithal, macrolithal and woody debris (Graf *et al.* 2008). Aside from the aforementioned lithal habitat homogenization in water management, woody debris disturbs regulated river flows and also presents a 'threat' to water management (Shields & Smith 1992). Consequently, woody debris is removed from regulated streams, regardless of its role in riverbank stabilization, food chain and freshwater ecosystem energy flow (Benke *et al.* 1984). Keeping that in mind, *O. albicorne* has a wide range of preferred habitats, but more than half of them conflict with anthropogenic river management objectives and are therefore being modified or even removed.

In the multi-level pattern analysis, seven taxa were associated with little or no morphological degradation: *Baetis* sp., Heptageniidae, *Dinocras* sp., *Leuctra* sp., *Protonemura* sp., *O. albicorne*, and *Oxyethira* sp. Most, i.e., five of these taxa are explained in the previous paragraph due to their sensitivity to morphological degradation. This analysis further confirmed their sensitivity through their relationship to little or no morphological degradation. The remaining taxa from this analysis that have not been previously explained are *Dinocras* sp. and *Oxyethira* sp. The *Dinocras* genus includes predator species that also prefer rocky habitats with macrophytes or moss (Graf *et al.* 2002). Thus, this taxon not only avoids anthropogenically altered habitat types, but also feeds on Baetidae (Figueroa *et al.* 2015), a family that has shown undeniably negative trends with increasing morphological alteration and degradation. Finally, *Oxyethira* sp. individuals require macrophytes in their habitats, because their larvae feed on green algae that cover macrophytes. Furthermore, the larvae of these taxa attach themselves to the leaves or stems of macrophytes before pupation (Ito & Kawamula 1984). Since the removal of aquatic vegetation is very common in all river morphology interventions (Thomaz *et al.* 1999), it can be assumed that *Oxyethira* sp. is sensitive to the morphological degradation of the river ecosystem.

On the other hand, the two species found in the habitat group associated with heavy disturbance are *Habrophlebia fusca* and *Nemoura* sp. *H. fusca* shows little preference toward any particular habitat distribution longitudinally (Graf *et al.* 2021) and may inhabit a variety of microhabitats ranging from those with pelal, psammal, lithal, macrophytes to those with particulate organic matter. This species preferably occurs in lotic habitats, although it was occasionally recorded also from standing waterbodies. As a predominant gatherer (Buffagni *et al.* 2009) is not connected to a narrow range of substrates (Wright *et al.* 1998). All of these characteristics make *H. fusca* an extremely adaptable species, even occurring in highly morphologically disturbed sites in this study.

The occurrence of *Nemoura* sp. in habitat group 3 (or morphologically highly disturbed) was very unexpected, as it is usually characterized as a very sensitive taxon (Harper 1973). It is difficult to find an explanation for this because it is a genus with a large number of ecologically diverse species, some of which colonize a wide range of habitats, while others are tied to macrophytes and lithal (Leitner & Lorenz 2020). However, almost all species are shredders that feed on leaf litter, possibly indicating that alterations are tolerable to some degree for this taxa as long as food sources are abundant (Graf *et al.* 2009).

5. CONCLUSION

This study presents an adjusted approach to biomonitoring by emphasizing the precision of morphology oriented assessments in karst rivers. The refined tool enables a more sophisticated understanding of the effects of hydromorphological alterations on EPT taxa in karts rivers and a more precise assessment of the relationship between pressure and response in biomonitoring. The EPT taxa most sensitive to morphology impairment are those with the greatest preference for microhabitats with macrophytes and lithal, which are generally the first to be affected by anthropogenic interventions to natural river morphology. Hydrology did not show significant effects on EPT community variability, as a possible result of acquired adaptations to dynamic hydrology in karst rivers. This study provides a novel perspective to biomonitoring practice and emphasizes the importance of morphology for comprehensive and accurate assessments of karst freshwater ecosystems.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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