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Possible indices for the assessment of ecological stream quality based on macroinvertebrates in Euphrates tributaries (Turkey)

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ABSTRACT

The aim of this study was to support the development of ecological stream quality assessment tools in order to provide a method for sustainable water management in Turkey. Therefore, we present two new or adapted indices based on benthic invertebrates. To develop and adapt the indices, 17 streams were studied and separated into three quality classes, which were supported by four community indices (EPT [%], EPTCBO [%], number of Individuals, evenness), and 23 taxa were identified as indicators for these three quality classes. As a first biological index, we adapted the Hindu Kush-Himalaya biotic score (HKHbios) to the Euphrates catchment by establishing a new and ecoregion-specific score list (Euph-Scores) by scoring 93 taxa depending on their distribution between the quality classes. Based on these scores, several average score per taxon values (ASPT value) were calculated. All ASPT values of the Euph-Scores separated the quality classes significantly. After a comparison of the different ASPT values we recommend to use the weighted ASPT, because the weighting enabled a sharper differentiation between the quality classes and named it Euphrates Biotic Score (EUPHbios). As a second biological index, we propose the proportion of habitat specialists. To calculate this index, a habitat score was developed by analysing the habitat preferences of several benthic invertebrates. Habitat score values were assigned to the 20 most common taxa from the streams in the best quality class (natural streams). The proportion of habitat specialists, identified using the new habitat score, differed significantly between the three quality classes, with higher values in natural streams than in polluted streams. In the light of the results, the presented methods appear to be suitable for developing a multi-metric index for assessment programs for the mountainous regions of the Middle East.

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Introduction

Benthic invertebrates are the most commonly used biological indicators for assessing the ecological quality of running waters (Rosenberg and Resh 1993) and for estimating the intensity of anthropogenic impacts. European systems for assessing ecological quality based on benthic invertebrate composition are often multi-metric indices, where several different metrics are combined to indicate the ecological status class of a surface water (e.g. Böhmer et al. 2004; Hering et al. 2004a, 2004b). Although the general approach of bioindication with benthic invertebrates is the same, all national methods have been adapted to specific geographical regions and parametrised for specific aquatic fauna (e.g. Biological Monitoring Working Party-BMWP for the UK, Armitage et al. 1983; Belgian Biotic Index, De Pauw and Vanhooren 1983; PERLODES in Germany, Meier et al. 2004). In Turkey, a first assessment method using bioindication with benthic invertebrates, the Turkish-BMWP biotic index (TR-BMWP), was recently developed (Kazanci et al. 2016). However, due to limited data availability, it uses the family-level identification of benthic macroinvertebrate families for assessment and is based on the British-BMWP. Therefore, the degree of regional adaptation seems to be somewhat limited, because the original British scores were changed only slightly based on expert knowledge, again due to limited data availability. Consequently, further development of biotic indices for the assessment of ecological stream quality in Turkey is needed.

Currently, the national authorities in the Mediterranean part of Turkey use the 'Intercalibration Common Metrix (ICMi)' which includes, e.g. the ASPT (Average Score per Taxon, Armitage et al. 1983), the number of EPT and the total number of families and Shannon–Wiener Index (Bayrak Arslan 2015). Except for the ASPT, these assessment methods are relatively universal and easy to implement for Turkey. To calculate the ASPT, BMWP values that are not parameterised for Turkey are used. The BMWP and consequently ASPT were originally developed for Great Britain on the basis of the in-depth knowledge of experts on the environmental requirements of British taxa (Armitage et al. 1983). Later, BMWP was modified for several countries such as Canada (Barton and Metcalfe-Smith 1992), Spain (Zamora-Munoz and Alba-Tercedor 1996) or Poland (Czerniawska-Kusza 2005) and has repeatedly been used in Turkey (e.g. Kazanci et al. 1997, 2010a, 2010b, 2011, 2013; Duran et al. 2003; Kalyoncu and Zeybek 2011; Zeybek et al. 2014). However, a comparative study of the various BMWP values using the different national ASPT showed that the transfer of these country-specific indices to Turkey produces inaccurate results (Zeybek et al. 2014).

As a possible index in contrast to the BMWP, the Hindu Kush-Himalaya biotic score (HKHbios; Ofenböck et al. 2010) is the result of a clearly documented calculation method based on data from extensive benthic invertebrate sampling. Consequently, this biotic score can be adapted to different countries and catchment areas quite easily using the same calculation method with the specific data of a regional sampling campaign. Another big difference to the BMWP is that the HKHbios is not limited to family-level identification. All identified taxa – that is, family, genus or species level – can be used in the score list. By creating a specific score list for the region of interest, the HKHbios can easily be adapted and used worldwide by analysing the respective regional benthic community compositions. The first step in creating such a score list is the pre-classification of the studied streams into quality classes. A taxon-specific score is calculated based on the frequency of the respective taxon's occurrence in the different quality classes. In our view, these features make the HKHbios very well suited for the adaptation needed to start developing an assessment procedure for streams in different regions of the world, especially in countries where insufficient taxonomic work has been done so far.

Since multi-metric approaches require further metrics, the proportion of habitat specialists can also be used as a potential indicator for habitat loss. Habitat specialists are organisms that prefer or are even restricted to certain habitats and will therefore disappear with the destruction or degradation of these habitats (Futuyma and Moreno 1988; Devictor et al. 2010; Poisot et al. 2011; Kneitel 2018). Due to the high sensitivity of habitat specialists to habitat loss, such an index might specifically indicate hydromorphological degradation. However, to establish an index of habitat use, a better knowledge of the habitat preference characteristics of stream invertebrates is necessary. Until now, many faunistic studies have compiled detailed taxa lists and collected information about the distribution of species throughout Turkey (e.g. Kazanci 2001, Kazanci and Türkmen 2012; Darılmaz and Salur 2015; Salur et al. 2016). In addition, autecological information about several taxa has already been well documented (e.g. Graf et al. 2008, 2009; Buffagni et al. 2009; <https://www.freshwaterecology.info>). However, this information has mainly been collected on European water bodies. Especially on higher-order taxa (genera or families), the information might actually apply to other species than those that are common in Turkey. Due to the specific fauna of Eastern Turkey, it is necessary to gather additional autecological information and to integrate it into a habitat score in order to provide a solid database for a future multi-metric index for stream quality assessment in Turkey.

To contribute to the development of an ecological assessment procedure in Turkey, we aimed to develop a biotic score and a habitat score specifically adapted to the Euphrates catchment area. Therefore, we investigated the benthic invertebrate community composition of 17 streams with different intensities of anthropogenic pressure in their catchment areas in the upper regions of the Euphrates Basin in Eastern Turkey (Anatolia). Based on our data set, we determined the indicator taxa for different ecological quality classes by comparing the community structures of streams with different anthropogenic impact intensities. To verify the specified quality classes, abiotic factors and community indices were analysed. In the next step, we adapted the HKHbios (Ofenböck et al. 2010) to the upper Euphrates catchment area by creating a specific scoring list and comparing our own results with existing biotic indices. In addition, we determined the habitat use of macroinvertebrates in the six most natural streams in order to understand the importance of the different habitats and analysed the effect of stream degradation on the proportion of habitat specialists.

Methods

Study sites

The study was performed on 17 mountain streams (2nd to 3rd order) in the Upper Euphrates Basin near the cities of Erzincan, Erzurum and Tunceli in Eastern Anatolia (Turkey, Figure 1). Eastern Anatolia has a continental climate characterised by warm, dry summers and cold, snowy winters (Sensoy et al. 2008). All sampling sites were located between epirhithral or metarhithral zones of the streams at about 970–1940 m above sea level (Table 1). The size of the catchment area was calculated using the software ArcGIS 10.1 (ESRI). Fourteen of the streams drain directly into the Euphrates River; three streams drain into the Pülümür River, one of the main tributaries of the Euphrates River. Large proportions of the catchment areas are used for agriculture and pasture (e.g. 80% of the total area of the province Erzurum and 53% of Erzincan; Environmental Report of Province Erzincan 2016; Environmental Report of Province Erzurum 2016). The sampling sites represent different levels of habitat diversity and different levels of water pollution and structural degradation (Appendix 1).

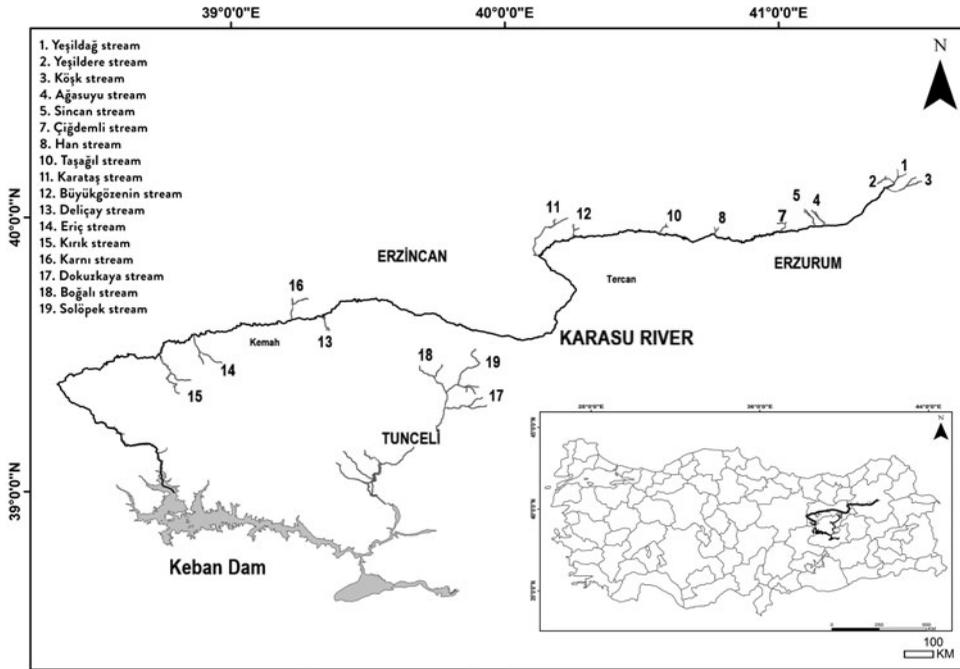


Figure 1. Location of stream sites.

Table 1. Stream characteristics of studied streams.

Stations	Altitude [m]	Catchment area [km ²]	Coordinates	Discharge Q [m ³ s ⁻¹]	Mean width [m]	Order
1	1898	78.2	40°08'12.52''	041°25'44.38''	0.42 ($n=9$)	4.83
2	1936	54	40°08'21.49''	031°24'24.99''	0.26 ($n=6$)	5.97
3	1896	74.6	40°05'45.62''	041°24'48.65''	0.37 ($n=7$)	3.33
4	1761	77.8	39°59'34.96''	041°08'55.69''	0.17 ($n=8$)	3.90
5	1767	77.2	39°59'31.64''	041°07'20.61''	0.06 ($n=6$)	2.95
7	1769	72.8	39°58'17.57''	041°01'22.27''	0.14 ($n=8$)	4.07
8	1699	114.5	39°56'53.17''	040°46'08.73''	0.26 ($n=5$)	5.17
10	1642	75.7	39°57'43.68''	040°34'40.43''	0.23 ($n=8$)	4.00
11	1596	71.7	39°56'12.91''	040°07'51.44''	–	3.17
12	1556	58.3	39°56'38.59''	040°15'03.27''	–	3.67
13	1122	128.8	39°38'07.61''	039°20'17.61''	0.11 ($n=9$)	8.85
14	1195	107.8	39°30'35.98''	038°53'13.75''	0.28 ($n=8$)	4.37
15	976	206.4	39°29'22.37''	038°44'36.53''	0.20 ($n=7$)	3.53
16	1271	76.4	39°40'23.95''	039°13'34.28''	0.19 ($n=8$)	4.00
17	1122	53.3	39°18'23.52''	039°46'59.07''	0.18 ($n=9$)	4.53
18	1350	112.5	39°24'34.15''	039°44'41.08''	0.64 ($n=9$)	9.83
19	1238	85.5	39°23'21.88''	039°49'45.32''	0.40 ($n=4$)	5.20

The measurement of discharge Q [m³ s⁻¹] was based on Carufel (1980). Mean width [m]: $n=3$.

Field sampling

We sampled all 17 streams at one site per stream in autumn (September 26th to October 5th 2013) and in spring (May 25th to May 31st 2014). At each site, the benthic community was sampled according to the modified AQEM protocol (Hering et al. 2004a). Within a 50 m reach of each stream, the relative proportions of substrates and organic

materials (% area coverage) were estimated, and 20 individual samples, each representing 5% of substrate coverage, were taken by kick sampling in front of a 25 × 25 cm dip net (1 mm meshes, 0.0625 m²) according to the habitat type distribution. Instead of pooling all 20 subsamples, as described in the AQEM protocol, only samples of the same substrate type were pooled to enable habitat-specific analyses of the invertebrate community. All subsamples were stored in 96% ethanol, which was replaced by 70% ethanol in the laboratory.

To characterise the stream sites, discharge (m³ s⁻¹) was determined by estimating the sectional stream area and current velocity using a velocity head rod (Carufel 1980). Samples for physical and chemical measures of the stream water were taken as three replicates from the middle of the stream during the sampling day. The environmental factors temperature [°C], O₂-content [mg·L⁻¹], O₂-saturation [%], pH and conductivity [μS/cm] were measured (2013: Professional Plus Multiparameter Probe (YSI, Ohio/USA), 2014: Oxi 330, (WTW GmbH, Weilheim/Germany) and WalkLAB TI 9000 (Trans Instruments Pte Ltd, Petro Centre/Singapore)). To analyse the NO₂-N, NO₃-N, NH₄-N and PO₄ concentrations, on both sampling occasions, water samples were taken from the middle of the stream and filtered (cellulose nitrate filter, 0.45 μm, Sartius Stedim Biotech GmbH, Göttingen/Germany) using a vacuum hand pump (Thermo scientific Nalgene, Waltham/USA). Samples were thereafter stored at 4 °C during the sampling day and at -20 °C until further processing.

Laboratory analyses

The NO₂-N, NO₃-N, NH₄-N and PO₄ concentrations in the water samples taken in September 2013 were analysed in the laboratory of Hacettepe University (Ankara/Turkey) with an ion chromatography system (DIONEX LC25 and ICS-1000, Thermo Fisher Scientific Inc. Sunnyvale/USA) using standard methods (Clesceri et al. 1989). Water samples from May 2014 were analysed using continuous flow analyses (CFA) in a laboratory at the University of Koblenz-Landau (Koblenz/Germany) with an AA3 HR Autoanalyzer (Seal Analytical, Norderstedt/Germany). All benthic macroinvertebrates were identified to the lowest feasible taxonomic level and counted using a stereo microscope (TSO Thalheim, Pulsnitz/Germany).

Data analysis

The flow velocity V (ms⁻¹) was calculated with the formula $= \sqrt{2 \cdot g \cdot h}$, where 'g' is gravity and 'h' is the velocity head. Based on the cross-sectional areas (A in m²) and the stream velocities of the individual sections (0.5 or 1 m wide), we calculated the corresponding discharges using the formula: $Q = A \cdot V$. The total discharge was calculated from the sum of the individual sections.

To analyse the taxonomic data, taxa with <10 individuals per sample and taxa occurring in only one season were combined with taxa of the same genus or family that occurred in other samples, resulting in more solid information for higher taxonomic units (family or genus). To differentiate the benthic communities of the streams into different quality classes, the similarity of benthic community composition was analysed by employing a cluster analysis based on Bray-Curtis similarities (%) after fourth-root transformation of the abundance data using the Software Primer (version 6). Samples with a minimum similarity of 35% were grouped into the same quality class. Next, based on the level of anthropogenic stressors (Appendix 1), the quality classes were defined. The streams with few or no negative impacts were defined as natural streams (Quality class I);

the streams with negative impacts were defined as slightly polluted streams (Quality class II) or moderately polluted streams (Quality class III). To identify indicative taxa for the three quality classes, we used a method by Dufrene and Legendre (1997) practically applied in the function ‘indval’ (R package labdsv: Roberts 2015; R Development Core Team 2017) for both seasons separately. All taxa that were characterised as indicator taxa in a quality class by our analysis with an indicator value >0.5 were presented.

Nutrient concentrations [mg L^{-1}] of nitrate-nitrogen ($\text{NO}_3\text{-N}$), ammonium-nitrogen ($\text{NH}_4\text{-N}$) and total phosphate (PO_4) were classified into quality classes using LAWA threshold values (Environmental Federal Office of Germany 2019). The total number of taxa, total number of individuals, Shannon Index and evenness were calculated with the software Past 3.21 (2018). The EPT [%] was calculated as the ratio of individuals belonging to the insect orders Ephemeroptera, Plecoptera and Trichoptera to total benthic abundance. EPTCBO [%] including Coleoptera, Bivalvia and Odonata in addition to EPT were calculated accordingly. To determine the differences between the community indices of the three quality classes, the indices were compared using a one-way ANOVA. The values were square-root transformed. If normality could not be reached, a Kruskal–Wallis ANOVA was performed on ranks (Sigma Plot 12.5).

As one metric for the assessment procedure, we adapted the biotic score for the Euphrates tributaries (EUPHbios) based on the calculation method of the Hindu Kush-Himalaya biotic score (HKHbios; Ofenböck et al. 2010). Firstly, the so-called Euphrates biotic scoring list was created. To this end, all taxa that did not occur in at least three streams were excluded, reducing the taxa list for this analysis from 134 to 93 taxa. The taxa on the list were identified to species, genus and family level, except Nematoda, which were not identified. An additional list was compiled by reducing the resolution to family level (57 families and one phylum Nematoda) in order to compare the results of this study to other existing biotic scores based on family level. To distinguish between the two groups, ASPT for genus/species was named ASPT, and ASPT for families was named ASPT_{FAM} (families shortened to ‘FAM’).

For each taxon the ‘guide score’ was calculated according to Sharma and Moog (1996), which was adapted by Ofenböck et al. (2010) to create a five-class system. However, due to the lack of IV and V quality classes among the studied streams, the calculation was shortened to three quality classes in this study:

$$\text{Guidescore} = S_{\text{I}} / S_{\text{tot}} * 10 + S_{\text{II}} / S_{\text{tot}} * 7.5 + S_{\text{III}} / S_{\text{tot}} * 5.5$$

S_{I} , S_{II} and S_{III} are the number of streams in which the taxon was found in each quality group. S_{tot} is the number of streams in which the taxon occurred in total. Because the obtained guide scores differed from the HKHbios, they are called ‘Euph-Scores’ in the following text. The ASPT values for the Euphrates are based on this list, including the weighted ASPT value, which represents the ‘Euphrates Biotic Score (EUPHbios)’ proposed here. Using these adapted scores, the variation of ASPT values – such as the family-based value (ASPT_{FAM}), the weighted value ($\text{ASPT}_{\text{W}} = \text{EUPHbios}$) and the value-based weighted-abundance class (ASPT_{WA}) – were calculated (see Ofenböck et al. 2010, for details).

To increase the difference between the quality classes and, in turn, allow a clearer assessment, the ASPT values were weighted by assigning higher weights to clear representatives of Qc I and Qc III. The weighting factor of 5 was assigned to all taxa with a Euph-Score of 10 or 5.50 because these taxa showed a very high level of occurrence in Qc I or Qc III. All taxa with a score between 5.51–6.99 and 8.50–9.99 were weighted with 3 because these taxa were mainly found in neighbouring quality classes.

Weighting was not possible for the ASPT_{FAM} due to the fact that there were always several genera with different scores in any one family. For weighting based on abundance,

abundance classes were assigned (Class 1: 1–10; Class 2: 11–100, Class 3: 101–1000; Class 4: 1001–10,000; Class 5: >10,000, see Ofenböck et al. 2010) and the class number was used as the factor. The Euph-Scores of six higher-order taxa were extremely different from the guide scores from the HKHbios (Diptera; Chironomidae, Dolichopodidae, Muscoidae, Oligochaeta, Psychodidae and Nematoda). For these values, the HKHbios guide score was 1 or 2, whereas the value of the Euph-Scores varied between 6 and 10. The ASPT und ASPT_{FAM} were additionally calculated without these six extremes. The EUPHbios and ASPT_{WA} were only calculated with the complete list. In addition, other ASPT values were calculated from the HKH scores (ASPT_{HKH}), Turkish BMWP scores (ASPT_{TR}) and the original BMWP scores (ASPT_{OR}). All ASPT values were compared using a two-way ANOVA with the factors ‘quality class’ and ‘index’. If normality and/or the equality of variance condition were/was not met, the data were log (10) transformed.

In order to quantify the use of different habitats by common taxa, we used data from natural streams and included only taxa that were present in at least three streams with a minimum abundance of 10 individuals m⁻² per stream in each sampling season. To calculate the mean habitat-specific abundance of a given species for a specific habitat type, the abundance of each taxon (ind m⁻²) was calculated for each stream and each habitat type by taking into account the number of samples specifically in this habitat type. In addition, the total abundance of all taxa was calculated (sum of all abundances for each stream, Table 2, Step A). Next, the relative abundance of each taxon for each habitat was calculated (percentage of total abundance for the stream, Table 2, Step B) and averaged over the sampled streams.

To describe habitat use, we assigned a habitat score to different classes of relative abundances, whereby relative abundances of 10% corresponded to a score of 1 and the total habitat scores over all habitats added up to 10. However, due to rounding, sometimes only a total score of 9 was reached. For instance, one taxon was distributed as follows: 12%, 14% and 74%. In this case, scores of 1, 1 and 7 were assigned, adding up to a total score of 9. If the abundance differed clearly between the habitats, as in this example, the habitat with the highest abundance was assigned a higher score value (example: 74% = 8).

Table 2. Calculation method of the relative abundances.

	Number of individuals	Number of samplings in a habitat 1	Number of individuals	Number of samplings in a habitat 2	Number of individuals	Number of samplings in a habitat 3
Stream 1	20	5	1	5	0	5
Stream 2	50	10	2	5	1	5
Stream 3	10	5	3	10	1	10
Step A						Sum Σ
Stream 1	20/(5 × 0.0625) = 64		1/(5 × 0.0625) = 3.2		0/(5 × 0.0625) = 0	67.2
Stream 2	50/(10 × 0.0625) = 80		2/(5 × 0.0625) = 6.4		1/(5 × 0.0625) = 3.20	89.6
Stream 3	10/(5 × 0.0625) = 32		3/(10 × 0.0625) = 4.8		1/(10 × 0.0625) = 1.60	38.4
Step B						
Stream 1	(64 × 100)/67.2 = 95.24		(3.2 × 100)/67.2 = 4.76		(0 × 100)/67.2 = 0	100
Stream 2	(80 × 100)/89.6 = 89.29		(6.4 × 100)/89.6 = 7.14		(3.2 × 100)/89.6 = 3.57	100
Stream 3	(32 × 100)/38.4 = 83.33		(4.8 × 100)/38.4 = 12.50		(1.6 × 100)/38.4 = 4.17	100
Sum Σ	267.86		24.40		7.74	300
Mean Σ	(267.86 × 100)/300 = 89.29		(24.40 × 100)/300 = 8.13		(7.74 × 100)/300 = 2.58	
Habitat score	9		1		+	

As an additional metric for the assessment procedure, the proportions of specialists and generalists in each stream were calculated, and compared between sampling campaigns and quality classes using a one-way ANOVA. Generalist and specialist taxa were separated based on the habitat scores. Taxa with a score ≥ 4 in any one habitat were considered as specialists. When the scores were always ≤ 4 in all habitat types, the taxa were assigned to the group of habitat generalists. The only exception was *Hydraena* spp., which had a score of 6 when summing roots and xylal (Appendix 7). Because these habitat types were very similar, this taxon was also considered to be a habitat specialist. The relative abundances of all habitat specialists and generalists, respectively, were added for each stream and sampling occasion. To perform the statistical tests and construct plots, the software Sigma Plot 12.5 (Systat Software GmbH, Erkrath/Germany) was used.

Results

Ecological quality classes

The cluster analysis resulted in three groups of stream communities (Figure 2) which were assigned to the quality classes Qc I (natural streams), Qc II (slightly polluted streams) and Qc III (moderately polluted streams) based on additional information related to anthropogenic pressure on the studied streams (Appendix 1). Streams no. 12, 14, 15, 17, 18 and 19 were assigned to Qc I, streams no. 5, 7, 8, 10 and 11 to Qc II and streams no. 1, 2 and 3 to Qc III. Streams no. 4, 13 and 16 showed no consistent results; they were classified in different groups for each season or even represented an own cluster in the case of stream no. 4. Consequently, these communities were excluded from further analyses.

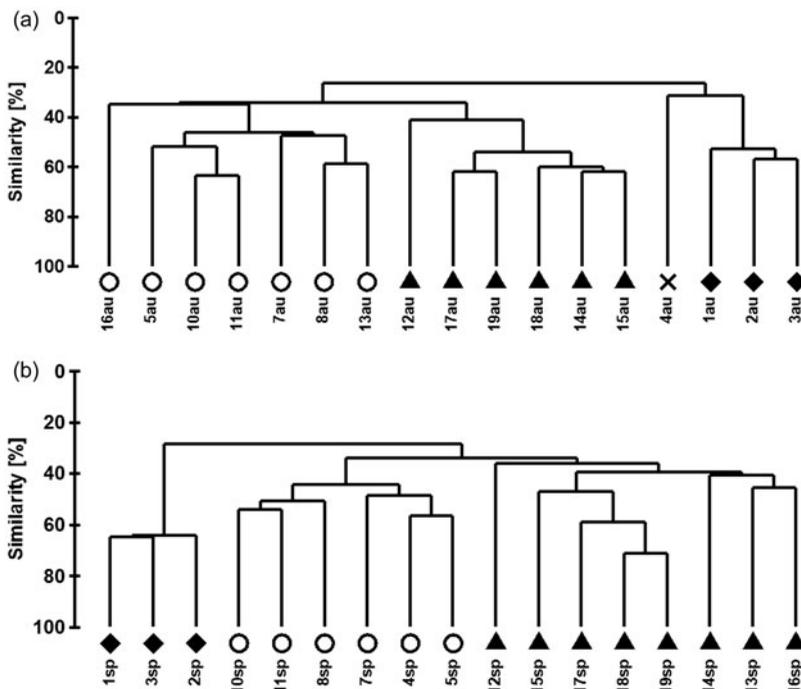


Figure 2. Cluster analyses of benthic community of all the sites from both seasons based on Bray-Curtis similarity: autumn (au) 2013 and spring (sp) 2014. Quality class I = \blacktriangle , Quality class II = \circ , Quality class III = \blacklozenge , X = own group because of 35% similarity to the other three groups.

Independent of their quality class assignment, the streams were characterised by high oxygen concentrations and alkaline pH values (Appendix 2). The temperatures differed greatly and ranged between 5.9 and 18.6 °C in autumn 2013 and between 9.1 and 20.4 °C in spring 2014.

Most of the nutrient concentrations of the studied streams match their classification according to the LAWA chemical quality classes (Environmental Federal Office of Germany 2019). However, the nitrite levels of several streams of Qc II and III were rather high (autumn: Qc III stream no. 1, Qc II stream no. 7, 8 and 10; spring: Qc II streams no. 7 and 13). The nitrate levels of some streams classified in Qc II were higher than in streams of the other quality classes (autumn: stream no. 7; spring: streams no. 4 and 5). The ammonium concentration of most streams was very high in autumn (up to max. 2.32 mg·L⁻¹). The phosphate concentrations were below the detection limit of the analysis (<0.01 mg·L⁻¹ in autumn and <0.003 mg·L⁻¹ in spring) in both seasons, except in Qc III (spring: streams no. 1, 2, 3).

Twenty-three potential indicator taxa were assigned to different quality classes. These taxa clearly occurred predominantly in one class, as shown by the indicator values (R function 'indval', Appendix 3). In four of the six calculated fauna-based community indices, the three quality classes differed significantly (Appendices 4 and 5). EPT (total abundance of Ephemeroptera, Plecoptera and Trichoptera) and EPTCBO (EPT and Coleoptera, Bivalvia and Odonata) were highest in Qc I and differed significantly from Qc II. The highest evenness score was recorded in Qc I and differed significantly from that of Qc III. The number of individuals was highest in Qc III and decreased in the direction of Qc I (Kruskal-Wallis one-way analysis, $H = 16.73$, $p < 0.001$; Dunn's method, Qc I ($n = 12$) × Qc III ($n = 6$), $Q = 3.83$, $p < 0.05$, Dunn's method, Qc I ($n = 12$) × Qc II ($n = 10$), $Q = 2.82$, $p < 0.05$). The quality classes did not differ regarding the number of taxa and Shannon diversity.

Euphrates biotic score

The Euph-Scores of 93 taxa, their respective weights (Table 3) and abundance classes were used to calculate several different versions of ASPT values. However, as assumed, the EUPHbios, based on the Euph-Scores (Table 3), showed the sharpest separation among the investigated indices, indicated by higher differences between the means of the quality classes than other scores (Figure 3). A comparison of the EUPHbios indices to the other ASPT values of the Euph-Scores showed differences between the quality classes in the selected indices (ANOVA, quality × index, $p < 0.001$, Appendix 6). Weighting of the Euph-Scores regarding indication strength of the taxa resulted in a sharper separation of the quality classes, because the values of Qc I were higher, and those of Qc III were lower (EUPHbios, Figure 3 and Appendix 6). On the other hand, using abundance weighting (ASPT_{WA}) did not improve the separation, because the ASPT_{WA} values did not differ significantly from those of the EUPHbios (Figure 3 and Appendix 6). Similarly, the ASPT values without extreme taxa did not differ from the ASPT with extreme taxa (ASPT, Figure 3 and Appendix 6).

In contrast to the EUPHbios, for three out of four ASPT values of other scores (ASPT_{HKH}, ASPT_{TR}, and ASPT_{OR}), the class separation between Qc II and Qc III was not significant (ASPT_{HKH}: Holm Sidak post-hoc test, Qc II ($n = 10$) vs. Qc III ($n = 6$), $p = 0.29$; ASPT_{OR}: Holm Sidak post-hoc test, Qc II ($n = 10$) vs. Qc III ($n = 6$), $p = 0.68$). ASPT_{TR} did not differ between the quality classes (ANOVA, $H = 5.182$, $p = 0.07$, $n = 12/10/6$; Qc I/II/III). Overall, most ASPT values of other scores were significantly lower than

Table 3. Euph-Score list of 93 taxa. Taxa written in bold were considered to be 'extreme' based on the calculations of the ASPT-EUPHbios.

Order	Family	Taxon	Score	Weight
Acari	HYDRACHNIDIAE	Gen. spp.	8	1
Amphipoda	GAMMARIDAE	Gen. spp.	8	
Amphipoda	Gammaridae	<i>Gammarus</i> spp.	8	1
Bivalvia	SPHAERIIDAE	Gen. spp.	7	
Bivalvia	Sphaeriidae	<i>Pisidium</i> spp.	7	3
Coleoptera	DYTISCIDAE	Gen. spp.	8	
Coleoptera	Dytiscidae	<i>Platambus lunulatus</i>	9	3
Coleoptera	Dytiscidae	<i>Platambus</i> sp.	7	3
Coleoptera	Dytiscidae	<i>Nebrioporus stearinus</i>	9	3
Coleoptera	ELMIDAE	Gen. spp.	9	3
Coleoptera	Elmidae	<i>Esolus</i> sp.	8	1
Coleoptera	Elmidae	<i>Grouvellinus caucasicus</i>	10	5
Coleoptera	Elmidae	<i>Limnius</i> sp.	9	3
Coleoptera	Elmidae	<i>Normandia nitens</i>	10	5
Coleoptera	Elmidae	<i>Riolus</i> sp.	9	3
Coleoptera	GYRINIDAE	Gen. sp.	8	
Coleoptera	Gyrinidae	<i>Gyrinus</i> sp.	8	1
Coleoptera	HELODIDAE	Gen. sp.	7	1
Coleoptera	HYDRAENIDAE	Gen. spp.	8	
Coleoptera	Hydraenidae	<i>Hydraena</i> spp.	9	3
Coleoptera	Hydraenidae	<i>Limnebius</i> spp.	7	3
Coleoptera	Hydraenidae	<i>Ochthebius</i> spp.	9	3
Coleoptera	HYDROPHILIDAE	Gen. spp.	8	
Coleoptera	Hydrophilidae	<i>Helophorus</i> spp.	8	1
Coleoptera	Hydrophilidae	Hydrophilidae	7	1
Coleoptera	Hydrophilidae	<i>Laccobius</i> sp.	8	1
Diptera	ATHERICIDAE	Gen. sp.	8	
Diptera	Athericidae	<i>Atherix</i> sp.	8	1
Diptera	BLEPHARICERIDAE	Gen. sp.	7	1
Diptera	CERATOPOGONIDAE	Gen. sp.	7	1
Diptera	CHIRONOMIDAE	Gen. spp.	8	1
Diptera	DIXIDAE	Gen. sp.	9	3
Diptera	DOLICHOPODIDAE	Gen. sp.	8	1
Diptera	EMPIDIDAE	Gen. sp.	8	1
Diptera	LIMONIIDAE	Gen. sp.	9	3
Diptera	MUSCOIDAE	Gen. sp.	10	5
Diptera	PSYCHODOIDAE	Gen. sp.	8	1
Diptera	RHAGIONIDAE	Gen. sp.	6	3
Diptera	SIMULIIDAE	Gen. spp.	8	
Diptera	Simuliidae	<i>Prosimilium</i> sp.	8	1
Diptera	Simuliidae	<i>Simulium</i> spp.	8	1
Diptera	STRATIOMYOIDAE	Gen. sp.	10	
Diptera	Stratiomyoidae	<i>Stratiomys</i> sp.	10	3
Diptera	TABANIDAE	Gen. sp.	8	1
Diptera	TIPULIDAE	Gen. spp.	8	1
Diptera	Tipulidae	<i>Hexatoma</i> sp.	7	1
Ephemeroptera	BAETIDAE	Gen. spp.	8	
Ephemeroptera	Baetidae	<i>Baetis lutheri</i> -group	8	1
Ephemeroptera	Baetidae	<i>Baetis rhodani</i>	8	1
Ephemeroptera	Baetidae	<i>Baetis</i> spp.	8	1
Ephemeroptera	CAENIDAE	Gen. spp.	8	
Ephemeroptera	Caenidae	<i>Caenis macrura</i>	8	1
Ephemeroptera	Caenidae	<i>Caenis</i> sp.	8	1
Ephemeroptera	EPHEMERELLIDAE	Gen. spp.	8	
Ephemeroptera	Ephemerellidae	<i>Ephemerella</i> sp.	8	1
Ephemeroptera	Ephemerellidae	<i>Serratella ignita</i>	6	3
Ephemeroptera	EPHEMERIDAE	Gen. sp.	8	
Ephemeroptera	Ephemeridae	<i>Ephemera</i> spp.	10	5
Ephemeroptera	HEPTAGENIIDAE	Gen. spp.	8	
Ephemeroptera	Heptageniidae	<i>Ecdyonurus dispari</i>	6	5
Ephemeroptera	Heptageniidae	<i>Ecdyonurus macani</i>	8	1

(continued)

Table 3. Continued.

Order	Family	Taxon	Score	Weight
Ephemeroptera	Heptageniidae	<i>Ecdyonurus</i> sp.	8	1
Ephemeroptera	Heptageniidae	<i>Ecdyonurus starmachi</i>	8	1
Ephemeroptera	Heptageniidae	<i>Electrogena</i> sp.	7	1
Ephemeroptera	Heptageniidae	<i>Epeorus caucasicus</i>	9	3
Ephemeroptera	Heptageniidae	<i>Epeorus zaitzevi</i>	8	1
Ephemeroptera	Heptageniidae	<i>Heptagenia</i> sp.	7	1
Ephemeroptera	Heptageniidae	<i>Rhithrogena puytoraci</i>	8	1
Ephemeroptera	Heptageniidae	<i>Rhithrogena</i> sp.	8	1
Ephemeroptera	SIPHONURIDAE	Gen. sp.	6	5
Gastropoda	HYDROBIIDAE	Gen. sp.	6	3
Gastropoda	LYMNAEIDAE	Gen. sp.	6	
Gastropoda	Lymnaeidae	<i>Radix</i> sp.	6	3
Gastropoda	PLANORBIDAE	Gen. spp.	6	
Gastropoda	Planorbidae	<i>Ancylus fluvialitis</i>	6	5
Gastropoda	Planorbidae	<i>Gyraulus</i> sp.	7	1
Gastropoda	TATEIDAE	Gen. sp.	6	
Gastropoda	Tateidae	<i>Potamopyrgus</i> sp.	6	3
Heteroptera/Hemiptera	NEPIDAE	Gen. sp.	6	
Heteroptera/Hemiptera	Nepidae	<i>Nepa</i> sp.	6	3
Hirudinea	ERPOBDELLIDAE	Gen. sp.	7	
Hirudinea	Erpobdellidae	<i>Erpobdella</i> sp.	7	3
Hirudinea	GLOSSIPHONIIDAE	Gen. sp.	7	
Hirudinea	Glossiphoniidae	<i>Batracobdella</i> sp.	7	3
Nematoda	NEMATODA	Gen. spp.	8	1
Odonata	AESHNIDAE	Gen. spp.	9	
Odonata	Aeshnidae	<i>Caliaeschna microstigma</i>	9	3
Odonata	CALOPTERYGIDAE	Gen. sp.	8	
Odonata	Calopterygidae	<i>Calopteryx splendens</i>	8	1
Odonata	GOMPHIDAE	Gen. spp.	8	
Odonata	Gomphidae	<i>Onychogomphus</i> spp.	8	1
Odonata	Gomphidae	<i>Ophiogomphus</i> sp.	8	1
Oligochaeta	OLIGOCHAETA	Gen. sp.	6	
Oligochaeta	Oligochaeta	<i>Eiseniella</i> sp.	6	3
Plathelminthes	TURBELLARIA	Gen. spp.	7	1
Plecoptera	CHLOROPERLIDAE	Gen. spp.	7	
Plecoptera	Chloroperlidae	<i>Chloroperla</i> sp.	8	1
Plecoptera	Chloroperlidae	<i>Siphonoperla</i> sp.	6	3
Plecoptera	LEUCTRIDAE	Gen. sp.	8	
Plecoptera	Leuctridae	<i>Leuctra</i> sp.	8	1
Plecoptera	NEMOURIDAE	Gen. spp.	9	
Plecoptera	Nemouridae	<i>Amphinemura</i> sp.	9	3
Plecoptera	Nemouridae	<i>Protonemura</i> sp.	9	3
Plecoptera	PERLIDAE	Gen. sp.	8	
Plecoptera	Perlidae	<i>Perla</i> sp.	8	1
Plecoptera	PERLODIDAE	Gen. spp.	9	
Plecoptera	Perlodidae	<i>Isoperla</i> sp.	9	3
Plecoptera	Perlodidae	<i>Perlodes</i> sp.	9	3
Trichoptera	BRACHYCENTRIDAE	Gen. sp.	10	
Trichoptera	Brachycentridae	<i>Micrasema</i> sp.	10	5
Trichoptera	GLOSSOSOMATIDAE	Gen. sp.	8	
Trichoptera	Glossosomatidae	<i>Glossosoma</i> sp.	8	1
Trichoptera	HYDROPSYCHIDAE	Gen. spp.	9	
Trichoptera	Hydropsychidae	<i>Hydropsyche instabilis</i> -gr.	9	3
Trichoptera	Hydropsychidae	<i>Hydropsyche</i> spp.	9	3
Trichoptera	HYDROPTILIDAE	Gen. sp.	8	1
Trichoptera	LEPIDOSTOMATIDAE	Gen. sp.	7	3
Trichoptera	LEPTOCERIDAE	Gen. spp.	10	
Trichoptera	Leptoceridae	<i>Adicella</i> sp.	10	5
Trichoptera	Leptoceridae	<i>Ceraclea</i> sp.	10	3
Trichoptera	LIMNEPHILIDAE	Gen. sp.	8	1
Trichoptera	PSYCHOMYIIDAE	Gen. sp.	7	
Trichoptera	Psychomyiidae	<i>Psychomyia</i> sp.	7	3

(continued)

Table 3. Continued.

Order	Family	Taxon	Score	Weight
Trichoptera	RHYACOPHILIDAE	Gen. sp.	9	
Trichoptera	Rhyacophilidae	<i>Rhyacophila</i> sp.	9	3
Trichoptera	SERICOSTOMATIDAE	Gen. spp.	9	
Trichoptera	Sericostomatidae	<i>Schizopelex</i> sp.	10	5
Trichoptera	Sericostomatidae	<i>Sericostoma</i> sp.	8	1

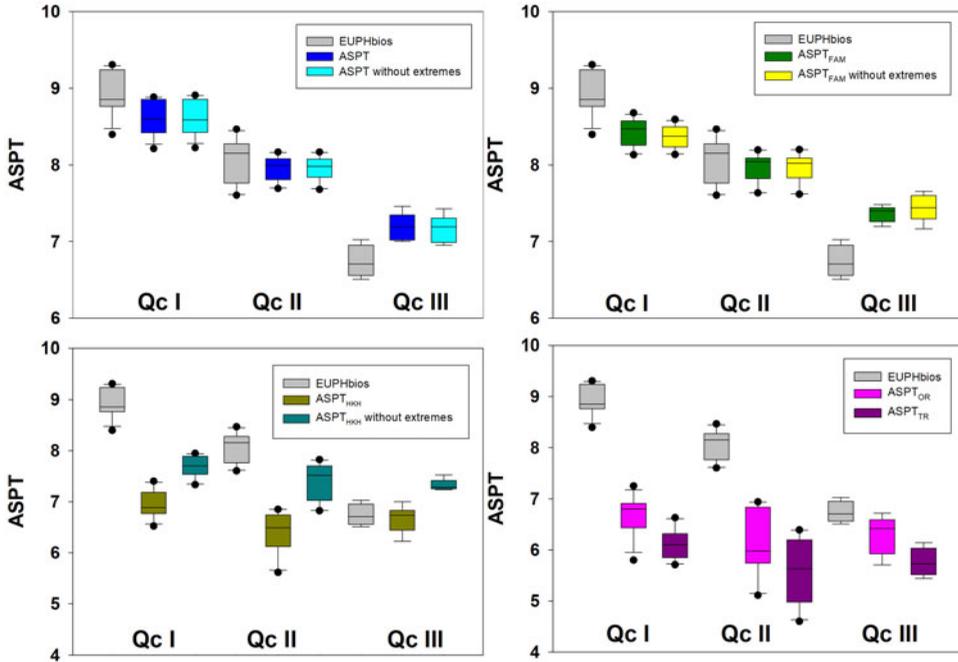


Figure 3. Box-Whisker plots (median, quartiles, 5th and 95th percentiles, outliers) of the ASPT values in the three different quality classes (I, II, III). ASPT = Average Score per Taxon, EUPHbios = weighted ASPT ($ASPT_w$), ASPT without extremes = ASPT values without extreme taxa, $ASPT_{FAM}$ = ASPT values of family level without extreme taxa, $ASPT_{FAM}$ without extremes = ASPT values of the HKHbios without extreme taxa, $ASPT_{HKH}$ = ASPT values of the Hindu Kush-Himalaya biotic index (HKHbios), $ASPT_{HKH}$ without extremes = ASPT values of the HKHbios without extreme taxa, $ASPT_{OR}$ = ASPT values of the original biological monitoring working party (BMWP) and $ASPT_{TR}$ = ASPT values of the Turkish BMWP.

those of the EUPHbios (Figure 3). For these reasons, they did not seem to be suitable for assessment in this study.

Habitat specialisation as a biotic index

We were able to describe the habitat use of 20 taxa sampled in the streams of Qc I (Appendix 7). Among the investigated habitats, lithal habitats were mostly preferred by the analysed taxa. Despite the low presence of xylal and root habitats compared to other habitat types in the studied streams, at least two taxa (*Hydraena* spp.: Coleoptera, *Stratiomys* sp.: Diptera) preferred clearly these habitats with scores ≥ 6 for xylal and roots together (Appendix 7). The habitats Akal, CPOM, Psammal, Macrophytes and FPOM can be considered to be of minor importance for these stream communities. Although they were sampled with the same relative effort, only few taxa seemed to prefer these habitat types specifically or even use them at a moderate level. (Appendix 7).

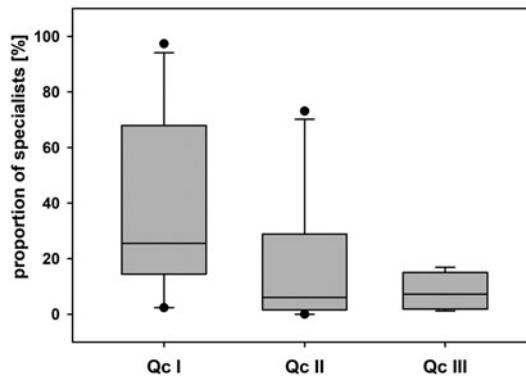


Figure 4. Box-Whisker plots (median, quartiles, 5th and 95th percentiles, outliers) of the proportion of specialists in the different streams (Quality classes: Qc I; $n = 12$, Qc II; $n = 10$, Qc III; $n = 6$) in the three different quality classes.

To analyse the potential effect of habitat degradation on benthic community composition, we compared the proportion of habitat specialists in the different quality classes. Based on the habitat score (score ≥ 4 in one of the habitats, Appendix 7), the following taxa were considered to be specialists: *Epeorus* sp., *Epeorus caucasicus*, *Epeorus zaitzevi*, *Ephemera* sp., *Perla* sp., *Hydraena* spp., Limoniidae and *Stratiomys* sp. The remaining twelve taxa, *Beatis* spp., *Rhithrogena* sp., *Leuctra* sp., *Protonemura* sp., *Elmis* sp., *Hydropsyche instabilis*-gr., *Hydropsyche* spp., *Rhyacophila* sp., *Atherix ibis*, Chironomidae, *Psychoda* sp. and *Simulium* spp., were considered to be generalists because they did not show a clear preference for one of the habitats (score ≤ 4 , Appendix 7). The proportion of specialists differed significantly between the three quality classes (ANOVA, $F = 3.69$, $p = 0.039$, Figure 4). The habitat specialists showed a tendency towards higher abundances in natural streams than in slightly or moderately polluted streams (ANOVA, $p = 0.087$, $n = 12/10$; Qc I/II and $p = 0.072$, $n = 12/6$; Qc I/III). In Qc II and Qc III, the proportions of specialists were similar (ANOVA, $p > 0.05$, $n = 10/6$; Qc II/III, Figure 4).

Discussion

The aim of this work was to support the development of methods for the assessment of ecological stream quality in Turkey and to illustrate the good adaptability of the HKHbios, which might be useful for the development of regionalised multi-metric indices. We were able to show that the EUPHbios and the proportion of habitat specialists are promising indices and recommend their use as part of a multi-metric index in regions where assessment approaches using benthic invertebrates are being developed. The calculation method of both indexes is universal and can be used easily by other scientists. This study is the first adaptation of the HKHbios in the Middle East and clearly confirms the general applicability and adaptability of this biotic score in different ecoregions of the world.

There are two advantages of the EUPHbios compared to the BMWP/ASPT indices. Firstly, the taxa list is specifically for the ecoregion. Secondly, the level of identification can vary from phylum to species level, extending the list compared to the BMWP score list. Thus, more precise results can be obtained. The newly adapted EUPHbios proved to be a suitable biotic score for the Euphrates region and is easily adaptable to different ecoregions as described by Ofenböck et al. (2010). In the regions of Nepal and Central Himalaya, the HKHbios was successfully applied shortly after its development (e.g. Shah

and Shah 2012; Sharma et al. 2015), and it has already been adapted to Ethiopia (ETHbios, Aschalew and Moog 2015). In addition, the ASPT is basically a mean of taxa scores, which can be weighted by the abundance or the indication value of the single taxa. We suggest weighting values by the indication value, because this increased the Qc I scores and decreased the Qc III scores significantly, thereby increasing differentiation between the quality classes.

Although the currently used indicator in Turkey (TR-BMWP) is also calibrated for Turkey, this calibration is based on expert knowledge. In addition, the indicator includes only the family level and is therefore possibly less sensitive. In fact, the ASPT_{TR} values resulting from the TR-BMWP are lower than the original ASPT values (ASPT_{OR} without any calibration for Turkey) and, more importantly, do not differentiate clearly between the quality classes of our study. The fact that both the original and the adapted BMWP yield significantly lower values than the EUPHbios might be due to the lack of Qc IV and V in this study. Therefore, more heavily impacted sites will have to be included before using the EUPHbios for stream quality assessment. Some taxa, especially those introduced as 'extremes' in the methods, need probably to be assigned much lower scores than the scores reported here. Therefore, we recommend continuing the process of adapting the EUPHbios. We expect that after nationwide and ecoregion-specific samplings and assessments, a more realistic EUPHbios or even a national biotic score (TRbios) can be developed.

The second potential indicator, the proportion of habitat specialists, appears to be suitable for assessing the ecological stream quality of the stream types analysed here, because it reacted clearly to degradation or pollution. In general, the presence of specific benthic macroinvertebrates strongly depends on habitat characteristics and spatial and temporal variability (e.g. Southwood 1977, 1988; Townsend 1989; Townsend and Hildrew 1994). A high percentage of xylal (defined as tree trunks, branches, roots) is one of the habitat indicators for the very good hydromorphological status of German streams (Feld 2004). We assume that the xylal and living roots in the streams of the Euphrates Basin might be important habitats that influences the benthic community, because they were used most intensely among the organic habitats in our study. However, due to the sparsely wooded riverbanks, their spatial proportion was often low (median between 5 and 10%). Indeed, there is already a remarkable amount of knowledge regarding the habitat preferences of benthic invertebrates (www.freshwaterecology.info). However, it does not include data on habitat preferences in Eastern Turkey, and especially data on the preferences of higher-order taxa are usually ecoregion specific.

The biotic indices of this work, based on data from samples taken two times a year, represent the difference between the quality classes more clearly than the recorded environmental conditions. Above all, a higher percentage of sensitive EPT/EPTCBO taxa in Qc I appears to be a useful indicator in our study; the proportion of these taxa is considered to be an indicator of reference streams in the literature (e.g. Moog et al. 2004; Meier et al. 2006). The number of individuals was highest in the moderately polluted streams, whose largest proportion consisted more of less sensitive taxa. Most of the identified indicator taxa were found in Qc III, because tolerant species usually occur in high densities (e.g. Pearson and Rosenberg 1978; Rygg 1985). Consequently, a drawback of our analysis of indicator values is that taxa such as *Epallage fatime* or *Epeorus znojkoii*, which occurred in very small abundances and only in Qc I, were not identified as indicator taxa although they might possibly have a high indicator value due to their especially high environmental requirements. Therefore, although the data basis was too small to draw further conclusions concerning the indicator value of rare taxa, these taxa should be regarded as potential indicator taxa and their distribution should be studied further.

In conclusion, we suggest that this pilot project might be used as blue print for similar studies in other catchment areas of Turkey. The methods, including the explained calculation methods seem useful for assessing the ecological stream/river quality and can be applied in each ecoregion. For the Euphrates region, by solidifying and enlarging the data base, more indicator taxa and habitat specialists can be defined, improving the quality of the suggested indices further.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Appendix 1: Anthropogenic stressors of studied streams.

Anthropogenic stressors							
Stream	Agriculture	Allotment	Banks fixed/ Riverbed straightened	Extraction (chrome and stone)	Livestock farming	Waste water	Irrigation
1	XXX				XXX		
2	XX				XX	XX	XXX
3	XXX				XXX	XXX	
4	XXX		XXX		XXX	XXX	XXX
5	XXX		XXX		XXX	XXX	XXX
7	XXX		XXX		XXX	XXX	
8	XX			XX	XX	XX	
10	XX			XX	XX	XX	
11	X				X	X	
12				XX	X		
13		X				X	
14		X					
15							
16		X			X	X	
17							
18							
19							

X = impact, XX = slight impact, XXX = moderately strong impact.

Appendix 2: Physico-chemical conditions and nutrient concentrations [$\text{mg}\cdot\text{L}^{-1}$] of the studied streams on the sampling days in both seasons (autumn 2013 and spring 2014).

Sampling-day	Time	Streams	Temperature [°C]	O ₂ -	O ₂ -	pH	Conductivity [$\mu\text{S}/\text{cm}$]	NO ₂ -N	NO ₃ -N	NH ₄ -N	PO ₄
				content [$\text{mg}\cdot\text{L}^{-1}$]	saturation [%]						
01.10.2013	10:35 AM	1	11.0	11.77	107	8.83	210	0.13	0.81	0.33	<0.01
01.10.2013	12:32 PM	2	12.9	9.49	90	8.61	46	0.02	0.65	0.01	<0.01
01.10.2013	4:06 PM	3	15.1	8.99	90	8.47	130	<0.01	0.74	0.14	<0.01
02.10.2013	10:50 AM	4	12.1	14.18	132	8.84	246	0.06	1.81	<0.01	<0.01
02.10.2013	13:02 PM	5	13.8	11.48	111	8.67	310	0.09	0.48	0.40	<0.01
02.10.2013	02:24 AM	7	13.2	9.96	95	8.74	283	0.25	4.37	0.46	<0.01
03.10.2013	10:32 AM	8	13.4	9.41	90	8.69	442	0.11	1.52	2.32	<0.01
03.10.2013	2:24 PM	10	16.1	9.65	98	8.70	719	0.17	0.2	1.08	<0.01
05.10.2013	10:28 AM	11	5.9	12.66	101	9.07	310	0.09	0.39	0.25	<0.01
05.10.2013	1:50 PM	12	12.2	10.40	95	9.04	362	0.07	1.35	0.41	<0.01
29.09.2013	11:57 AM	13	16.2	9.39	96	8.39	969	<0.01	0.48	0.29	<0.01
28.09.2013	4:31 PM	14	13.6	10.10	98	9.10	503	<0.01	2.56	0.48	<0.01
28.09.2013	11:45 AM	15	12.1	10.52	98	8.78	400	<0.01	1.38	0.22	<0.01
29.09.2013	9:36 AM	16	12.9	9.57	91	8.57	501	<0.01	<0.01	0.30	<0.01
27.09.2013	11:03 AM	17	11.1	10.27	93	8.39	419	<0.01	0.32	0.33	<0.01
27.09.2013	4:25 PM	18	13.0	9.40	90	8.57	700	<0.01	0.35	0.63	<0.01
26.09.2013	1:48 PM	19	18.6	8.28	89	8.48	1044	<0.01	0.48	0.78	<0.01
28.05.2014	9:00 AM	1	13.4	9.02	112	7.68		0.073	0.413	0.176	0.040
28.05.2014	11:00 AM	2	11.5	10.22	115	7.45		0.062	0.901	0.003	0.023
28.05.2014	3:00 PM	3	12.9	8.04	94	6.9		0.067	0.609	0.014	0.180
30.05.2014	10:50 AM	4	15.2	10.5	128	7.89		0.099	4.360	0.372	<0.003
29.05.2014	9:38 AM	5	14.9	8.15	96	8.2		0.066	3.155	0.010	<0.003
29.05.2014	12:00 PM	7	20.4	8.25	115	8.07		0.124	1.082	0.005	<0.003
29.05.2014	2:00 PM	8	20.3	10.5	145	7.95		0.068	0.608	0.022	<0.003
30.05.2014	2:24 PM	10	20.3	8	107	7.93		0.033	0.033	0.000	<0.003
27.05.2014	12:40 PM	11	12.6	10.6	129	8.06		0.021	0.592	0.007	<0.003
27.05.2014	4:00 PM	12	12.3	11.2	128	8.28		0.016	1.266	0.024	<0.003
27.05.2014	9:00 AM	13	9.1	11.4	114	8		0.321	0.565	0.026	<0.003
26.05.2014	2:30 PM	14	13.6	8.33	90	8.36		0.076	0.86	0.028	<0.003
26.05.2014	11:00 AM	15	12.1	8.08	84	8.30		0.012	0.43	0.010	<0.003
27.05.2014	7:50 AM	16	11.4	10.7	118	8.1		0.010	0.375	0.016	<0.003
31.05.2014	3:30 PM	17	18.1	12.70	157	8.15		0.02	0.29	0.019	<0.003
31.05.2014	10:00 AM	18	11.4	11.20	122	8.22		0	1.01	0.006	<0.003
31.05.2014	12:40 PM	19	19.8	11.80	146	8.01		0.018	0.47	0.019	<0.003

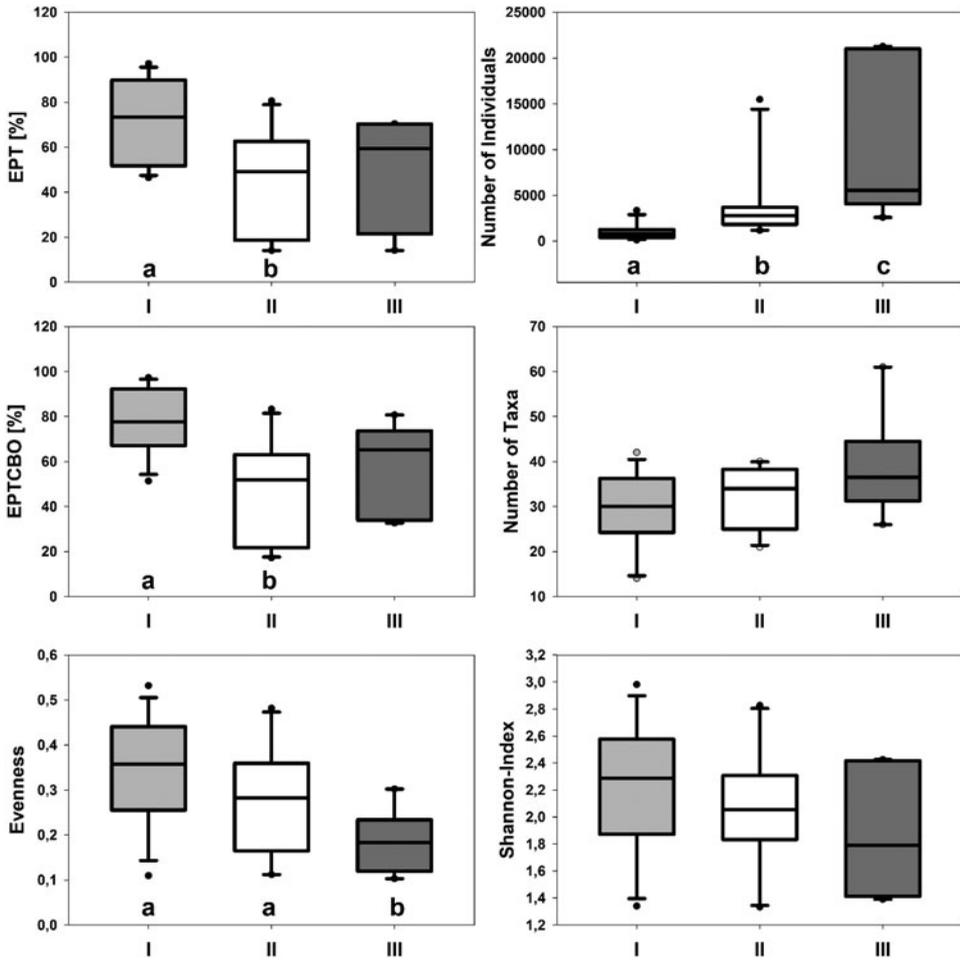
Nutrients [$\text{mg}\cdot\text{L}^{-1}$]	Quality classes of LAWA						
	I	I-II	II	II-III	III	III-IV	V
Total-P	0.05	0.075	0.1	0.2	0.4	0.8	0.8
NH ₄ -N	0.04	0.075	0.1	0.2	0.4	0.8	0.8
NO ₃ -N	1	1.5	2.5	5	10	20	20

Appendix 3: All taxa that were significantly defined as indicator taxa for a specific quality class (Qc) resulting from the function “indval” (indicator values and *P* values given).

Species	Autumn				Spring			
	Qc	Indicator value	<i>P</i> value	Freq	Qc	Indicator value	<i>P</i> value	Freq
<i>Leuctra</i> sp.	1	0.642	0.032	5	3	0.985	0.001	5
<i>Perlodes</i> sp.	1	0.638	0.026	1				
<i>Ancyclus fluviatilis</i>	3	1.0	0.004	10	3	1.0	0.001	1
<i>Baetis rhodani</i>	3	0.800	0.012	10				
<i>Baetis</i> spp.	3	0.736	0.047	5	3	0.904	0.010	2
<i>Batracobdella</i> sp.	3	0.862	0.007	1				
<i>Ecdyonurus dispari</i>	3	1.0	0.002	1	3	1.0	0.002	3
<i>Ecdyonurus starmachi</i>	3	0.667	0.029	3				
<i>Eiseniella</i> sp.	3	1.000	0.003	2				
<i>Epeorus zaitzevi</i>	3	0.772	0.007	2				
<i>Limnebius</i> spp.	3	0.973	0.002	5				
<i>Platambus</i> sp.	3	0.997	0.003	3				
<i>Potamopyrgus</i> sp.	3	1.0	0.002	6	3	0.867	0.006	4
<i>Protonemura</i> sp.					1	0.975	0.001	2
<i>Atherix</i> sp.					1	0.669	0.033	9
<i>Hydropsyche instabilis</i>-gr.					2	0.748	0.025	7
<i>Baetis lutheri</i> -gr.					2	0.729	0.046	4
<i>Nepa</i> sp.					3	1.0	0.003	1
<i>Serratella ignita</i>					3	1.0	0.002	16
<i>Radix</i> sp.					3	0.993	0.004	3
<i>Electrogena</i> sp.					3	0.873	0.004	1
<i>Limnebius</i> spp.					3	0.790	0.006	2
<i>Coelostoma orbiculare</i>					3	0.667	0.018	1
<i>Psychomyia</i> sp.					3	0.667	0.019	4

Written in bold, taxon represent a clear quality class. Freq is the number of times the species was present among the samples (not abundance).

Appendix 4: Proportion of community indices in the three different quality classes (I, II, III) visualized by Box-Whisker plots (median, quartiles, 5th and 95th percentiles, outliers). EPTCBO = Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata. “a”, “b” and “c” showed significant differences between the plots.



Appendix 5: Comparison of the biological indices in three quality classes (Qc).

Community indices	Test	Source of variation	DF	MS	F	P	Method	Comparison	Diff of means	t	P	P < 0.050
EPT	One-way ANOVA	Between groups	2	12.564	4.862	0.016	Holm-Sidak method	Qc I × Qc II	2.060	2.992	0.018	Yes
		Residual	25	2.584				Qc I × Qc III	1.586	1.973	0.116	No
EPTCBO	One-way ANOVA	Between groups	2	13.343	7.385	0.003	Holm-Sidak method	Qc III × Qc II	0.474	0.571	0.573	No
		Residual	25	1.807				Qc I × Qc II	2.199	3.82	0.002	Yes
Evenness	One-way ANOVA	Between groups	2	0.0495	4.334	0.024	Holm-Sidak method	Qc I × Qc III	1.260	1.875	0.14	No
		Residual	25	0.0114				Qc III × Qc II	0.939	1.352	0.188	No
Number of taxa	One-way ANOVA	Between groups	2	1.304	2.163	0.136		Qc I × Qc III	0.156	2.917	0.022	Yes
		Residual	25	0.603				Qc II × Qc III	0.0869	1.575	0.239	No
Shannon-diversity	One-way ANOVA	Between groups	2	0.0276	1.003	0.381		Qc I × Qc II	0.0689	1.506	0.145	No
		Residual	25	0.0275								

EPTCBO = Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata.

Appendix 6: Comparison of the ASPT values via two-way ANOVA.

Comparable indexes	Source of Variation	DF	SS	MS	F	P
EUPHbios * ASPT	Quality	2	26.37	13.19	241.90	<0.001
	Index	1	0.00	0.00	0.01	0.92
	Quality × Index	2	1.21	0.61	11.13	<0.001
	Residual	50	2.73	0.05		
EUPHbios * ASPT without extremes	Quality	2	26.85	13.43	245.07	<0.001
	Index	1	0.00	0.00	0.00	0.99
	Quality × Index	2	1.11	0.56	10.13	<0.001
	Residual	50	2.74	0.05		
EUPHbios * ASPT _{WA}	Quality	2	38.42	19.21	294.68	<0.001
	Index	1	0.02	0.02	0.31	0.58
	Quality × Index	2	0.00	0.00	0.01	0.99
	Residual	50	3.26	0.07		
EUPHbios * ASPT _{FAM}	Quality	2	21.30	10.65	206.97	<0.001
	Index	1	0.00	0.00	0.00	0.99
	Quality × Index	2	2.59	1.29	25.14	<0.001
	Residual	50	2.57	0.05		
EUPHbios * ASPT _{FAM} without extremes	Quality	2	19.55	9.78	190.13	<0.001
	Index	1	0.00	0.00	0.00	0.979
	Quality × Index	2	3.24	1.62	31.53	<0.001
	Residual	50	2.57	0.05		
EUPHbios * HKHbios	Quality	2	13.26	6.63	75.66	<0.001
	Index	1	19.92	19.92	227.37	<0.001
	Quality × Index	2	7.63	3.81	43.54	<0.001
	Residual	50	4.38	0.09		
EUPHbios * HKHbios without extremes	Quality	2	13.29	6.64	94.47	<0.001
	Index	1	2.61	2.61	37.10	<0.001
	Quality × Index	2	6.77	3.38	48.12	<0.001
	Residual	50	3.52	0.07		
EUPHbios * ASPT _{TR}	Quality	2	0.05	0.02	35.29	<0.001
	Index	1	0.22	0.22	324.044	<0.001
	Quality × Index	2	0.02	0.01	15.43	<0.001
	Residual	50	0.03	0.00		
EUPHbios * ASPT _{OR}	Quality	2	14.40	7.20	46.66	<0.001
	Index	1	30.30	30.30	196.34	<0.001
	Quality × Index	2	6.73	3.36	21.80	<0.001
	Residual	50	7.72	0.15		

Abbreviations: GS = genus/species, FAM = family, W = weighted, WA = weighted and abundance-classed, TR = Turkey, OR = original. Bold values indicate significant values ($P < 0.05$).



Appendix 7: Habitat use by macroinvertebrate taxa in the studied streams in the Euphrates River Basin based on the percentage of abundances in a specific habitat.

Taxa	n	Megalithal	Macrolithal	Mesolithal	Microlithal	Alkal	Psammal	Algae	Macrophytes	Roots	Xylal	CPOM	FPOM
Specialists													
<i>Epeorus</i> sp.	3	2	2	6	+								
<i>Epeorus caucasicus</i>	4	3	5	1	1								
<i>Epeorus zaitzevi</i>	4	4	2	1	3		+	+		+			
<i>Ephemerella</i> sp.	3	+	4	+	+		+	1		5			
<i>Perla</i> sp.	4	4	1	2	2			1			+		
<i>Hydraena</i> spp.	5	2		1				1	+	3	3		+
Limoniidae	3	1	+	+	1	5	1	1	+	0	0	+	1
<i>Stratiomys</i> sp.	4	+	+	+	+	+	+			4	5	1	+
Generalists													
<i>Beatis</i> spp.	6	1	1	1	2	+	+	1		3	1	+	+
<i>Rhithrogena</i> sp.	3	3	2	+	3	2							
<i>Leuctra</i> sp.	6	+	1	1	2	+	0	1	+	3	2	+	+
<i>Protonemura</i> sp.	8	1	2	1	+			2	+	3	1		+
<i>Elmis</i> sp.	4	1	3	1	2	+	+	1	1	+	1		+
<i>Hydropsyche instabilis</i> -gr.	11	1	3	1	2		+	1	+	2	+	+	+
<i>Hydropsyche</i> spp.	7	2	2	2	1	+	1	+	+	1	1	+	+
<i>Rhyacophila</i> sp.	6	1	2	2	1		1	+	+	+	3		+
<i>Atherix ibis</i>	5	1	1	1	1	+		+		3	2	1	+
Chironomidae	7	1	1	1	1	+	+	1	+	2	1	1	1
<i>Psychoda</i> sp.	4	1	1	+	1	1		2	1	2	1		+
<i>Simulium</i> spp.	6	2	1	1	1		+	1	1	1	2		+

Habitat use was scored within a range of 1 to 10, increasing with an increase of use (all habitats summed up to 10), " " = no presence in the habitat, " + " = odd presence in the habitat (< 5%). n represents the number of samplings in both seasons together (autumn 2013 and spring 2014). Taxa were included when they were present at a minimum of three samplings.