ECOLOGICAL ASSESSMENT APPROACHES BASED ON BENTHIC INVERTEBRATES IN EUPHRATES TRIBUTARIES IN TURKEY

by

Zuhal Gültekin
from Tunceli, Turkey

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Doctor of Natural Sciences
Fachbereich 3: Mathematik/Naturwissenschaften
Universität Koblenz-Landau

Reviewer:
Dr. habil. Carola Winkelmann
Apl. Prof. Dr. Thomas Wagner

Examiner:
Dr. habil. Carola Winkelmann
Apl. Prof. Dr. Thomas Wagner
Prof. Dr. Wolfgang Imhof

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1 Preliminary note

In the following text, the first-person singular is used. However, it should be noted that each chapter of this thesis involved the work of more than one person. The names of the co-authors of the respective articles or manuscripts are given at the beginning of each chapter. In accordance with the applicable doctoral regulations, the author contributions are set out in a separate document.

An important note in order to understand the stream numbers: The streams defined as no. 1, 2, 3, 4 and 5 in the first study were renamed no. 14, 15, 17, 18 and 19 in the second study. In the second study, the streams no. 1, 2 and 3 are different streams to in the first study. Please pay attention to this note while reading the doctoral thesis.

2 Summary

Sustainable water management requires methods for assessing ecological stream quality. Many years of limnological research are needed to provide a basis for developing such methods. However, research of this kind is still lacking in Turkey. Therefore, the aim of this doctoral thesis was to provide basic research in the field of aquatic ecology and to present methods for the assessment of ecological stream quality based on benthic invertebrates. For this purpose, I selected 17 tributaries of the Euphrates with a similar typology/water order and varying levels of pollution or not affected by pollution at all. The characterisation of the natural mountain streams was the first important step in the analysis of ecological quality. Based on community indices, I found that the five selected streams had a very good ecological status. I also compared the different biological indications, collected on two occasions – once in spring (May) and once in autumn (September) – to determine the optimal sampling time. The macroinvertebrate composition differed considerably between the two seasons, with the number of taxa and Shannon index being significantly higher in autumn than in spring. In the final step, I examined the basal resources of the macroinvertebrates in the reference streams with an isotope analysis. I found that FPOM and biofilm were the most relevant basal resources of benthic invertebrates. Subsequently, based on the similarity of their community structures, I divided the 17 streams into three quality classes, supported by four community indices (EPT [%], EPTCBO [%], number of individuals, evenness). In this process, 23 taxa were identified as indicators for the three quality classes. In the next step, I presented two new or adapted indices for the assessment of quality class. Firstly, I adapted the Hindu Kush-Himalaya biotic index to the catchment area of the Euphrates and created a new, ecoregion-specific score list (Euph-Scores) for 93 taxa. The weighted ASPT values, which were renamed the Euphrates Biotic Score (EUPHbios) in this study, showed sharper differentiations of quality classes compared to the other considered ASPT values. Thus, this modified index has proved to be very effective and easy to implement in practical applications. As a second biological index, I suggested the proportion of habitat specialists. To calculate this index, the habitat preferences of the 20 most common benthic invertebrates were identified using the new habitat score. The proportion of habitat specialists differed significantly among the three quality classes with higher values in natural streams than in polluted streams. The methods and results presented in this doctoral thesis can be used in a multi-metric index for a Turkish assessment programme.

3 Zusammenfassung

Nachhaltiges Gewässermanagement erfordert Methoden zur Bewertung der ökologischen Gewässerqualität. Die Basis dafür zu entwickeln setzt langjährige limnologische Forschung voraus, die jedoch in der Türkei bisher nicht ausreichend vorhanden ist. Daher war es das Ziel dieser Doktorarbeit, Grundlagenforschung im Bereich der Gewässerökologie durchzuführen und Methoden zur Bewertung der ökologischen Gewässerqualität in der Türkei anhand der Untersuchung von benthischen Invertebraten bereitzustellen. Hierfür habe ich 17 Nebenflüsse des Euphrat mit ähnlicher Typologie/Gewässerordnung ausgewählt, die unterschiedlichen anthropogenen Belastungen bis gar keiner Beeinträchtigung ausgesetzt waren. Die Charakterisierung der natürlichen Bergbäche war der erste wichtige Schritt zur Analyse der ökologischen Qualität. Anhand von Gemeinschaftsindizes konnte ich feststellen, dass die hierfür ausgewählten fünf Bäche einen sehr guten ökologischen Zustand aufwiesen. Des Weiteren verglich ich die verschiedenen biologischen Indizes zwischen Frühling (Mai) und Herbst (September), um den optimalen Zeitpunkt der Beprobung festzustellen. Dabei zeigten sich erhebliche Unterschiede in der Makroinvertebratenzusammensetzung zwischen den beiden Jahreszeiten: Die Anzahl der Taxa und der Shannon-Index waren im Herbst deutlich höher als im Frühjahr. Anschließend untersuchte ich bei den Referenzbächen die Nahrungsressourcen des Makrozoobenthos mittels einer Isotopen-Analyse. Als wichtigste Basalressourcen für die benthischen Wirbellosen stellte ich FPOM und Biofilm fest. Infolgedessen unterteilte ich die 17 Bäche anhand der Ähnlichkeit ihrer Gemeinschaftsstruktur in drei Qualitätsklassen, die von vier Gemeinschaftsindizes (EPT [%], EPTCBO [%], Anzahl der Individuen, Evenness) unterstützt worden sind. Hierbei wurden 23 Taxa als Indikatoren für die drei Qualitätsklassen identifiziert. Im nächsten Schritt habe ich zwei Möglichkeiten für die Bewertung der Qualitätsklassen entwickelt bzw. angepasst. Als erstes adaptierte ich den biotischen Index Hindu Kush-Himalaya an das Einzugsgebiet des Euphrat, in dem ich eine neue und ökoregionspezifische Score Liste (Euph-Scores) für 93 Taxa erstellt habe. Die gewichteten ASPT-Werte, die in der vorliegenden Arbeit in Euphrat Biotischer Score (EUPHbios) umbenannt worden sind, zeigten im Vergleich zu den anderen ASPT-Werten schärfere Differenzierungen der Qualitätsklassen. Somit erwies sich dieser modifizierte Index in der praktischen Anwendung als sehr aussagekräftig und gut umsetzbar. Als zweiten biologischen Index habe ich den Anteil der Habitat-Spezialisten vorgeschlagen. Um diesen Index zu berechnen, wurden Habitatpräferenzen der 20 häufigsten Makroinvertebraten anhand des neuen Habitat-Scores identifiziert. Der Anteil der Habitat-Spezialisten unterschied sich deutlich zwischen den drei Qualitätsklassen, mit höheren Werten in natürlichen Bächen als in belasteten. Die in dieser Doktorarbeit vorgestellten Methoden und Ergebnisse können in einem multimetrischen Index für ein türkisches Bewertungsprogramm für Fließgewässer verwendet werden.

4 General Introduction

The transboundary Euphrates and Tigris catchment area covers six countries (Iraq, Turkey, Iran, Syria, Saudi Arabia and Jordan (Lehner et al., 2008, Food and agriculture organization of the United Nations, 2009, Fig. 1). It is the largest river catchment area of Turkey, and one third of the annual flow in Turkey originates from the Euphrates-Tigris catchment area (National strategy for the management of catchment areas of Turkey, 2014). The Euphrates has a length of 2700 km, consists of the tributaries Murat and Karasu and flows together with the Tigris into the Persian Gulf at Schatt al-Arab (Food and agriculture organization of the United Nations, 2009).

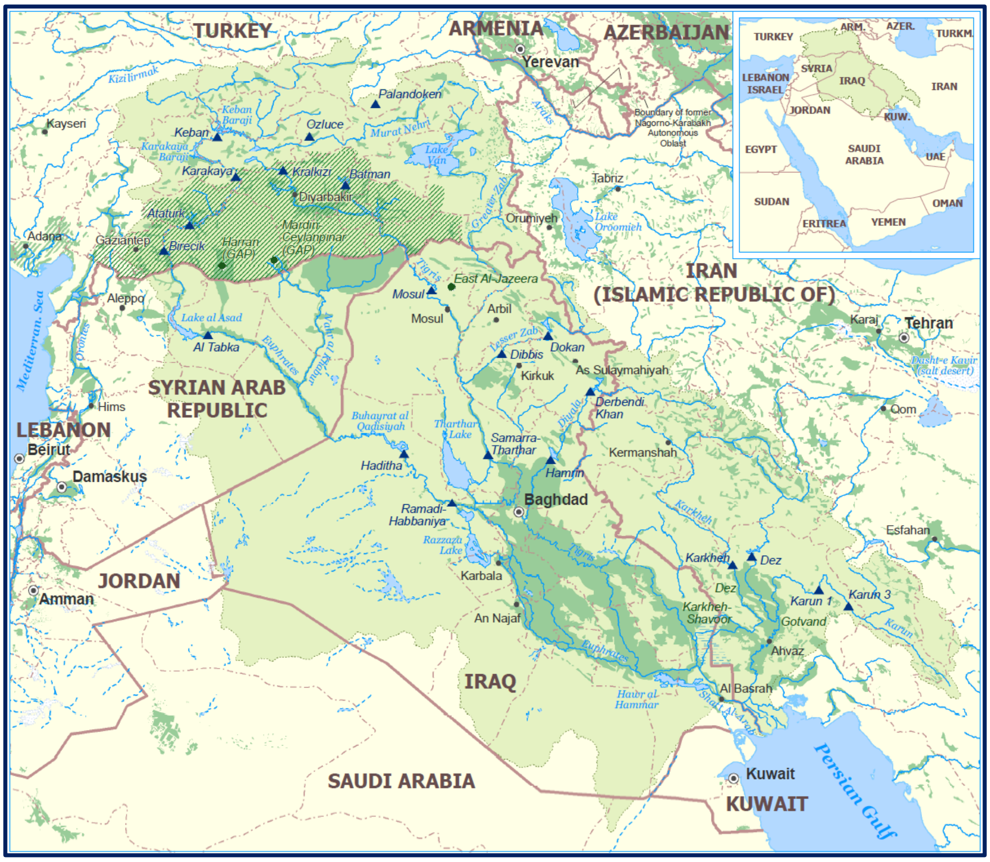

Fig. 1: Euphrates and Tigris catchment area. Source: FAO – AQUASTAT (2009).

There is a scarcity of freshwater in the Middle East which can lead to water conflicts between the riparian countries (e.g. Kliot, 1994; Wolf, 1998; Amery and Wolf, 2000; Freemann, 2001, Wolf and Newton, 2007; Voss et al., 2013). Due to its warm climate, the lower catchment area of the Euphrates and Tigris is more affected by water scarcity than the upper basin (Ohora et al., 2011). Turkey controls the water quantity with a high number of reservoirs and hydrological hydroelectric power plants and thereby holds the role of the decision maker regarding the water supply. Cooperation between Turkey and Iraq, which is the downstream neighbour of Turkey, is currently under negotiation, with Turkey in a more powerful position than Iraq because of its geographical location and experience in water management (Hürriyet Daily news, 2019). In fact, international cooperation in the catchment area of the Euphrates and Tigris would contribute to sustainable and peaceful water management in the Middle East. Monitoring of the surface waters is the one of the indispensable steps in this process in order to supply sufficient clean water to all riparian countries. It is also in the interest of the European Union (EU) that Turkey is moving in this direction.

The protection of Turkey's watersheds and surface waters is one part of the requirements for membership set by the EU. According to the EU Water Framework Directive (WFD, 2000), all surface waters need to be of at least a good ecological quality, which includes their good physical and chemical quality as well as a good status of the biological quality components, which usually requires a good to medium hydromorphological quality. In addition, a monitoring program is required to analyse and document the ecological status of all surface water bodies. The development of water quality assessment systems and monitoring programmes according to the EU-WFD (2000) has taken more than ten years in Central Europe, and an extraordinary amount of research effort has been invested in it (e.g. Hering et al., 2004a, 2004b, 2006a; Böhmer et al., 2004a, 2004b; Haase et al., 2004; Schaumburg et al., 2004; Schmidt-Kloiber et al., 2006). To support a similar development in Turkey, many steps are required. Although the EU membership process is delayed by the political developments in the country, Turkey has been preparing management plans for the protection of its catchment areas since 2008. To date, management plans have been published for 19 of the 25 main catchment areas. For the Euphrates und Tigris, a management plan will be drawn up within the next years (Ministry of agriculture and forestry of Turkey; General directorate of water management, 2019).

In Turkey, in addition to natural influences (e.g. geology), anthropogenic influences such as domestic sewage, industrial wastewater and water withdrawal for irrigation affect the water quality (Akbulut et al., 2009). Distorted urbanisation and unplanned industrialisation have resulted in the severe pollution of streams and rivers in Turkey. Generally, the lower catchment areas in Turkey are more at risk than the upper catchment areas because of their growing population and more intense agriculture (National strategy for the management of catchment areas of Turkey, 2014). In contrast to German rivers, Turkish rivers are not used as waterways for transportation. For this reason, there are only moderate morphological changes in rivers, mainly related to reservoirs, hydropower stations and the infrastructure of cities (Sekercioglu et al., 2011; National strategy for the management of catchment areas of Turkey, 2014). Numerous dams and hydroelectric power stations (HES) are planned in Turkey (Directorate general for state hydraulic works of Turkey (DSI), 2017), and the disadvantages these plans may have for the environment are currently a subject of debate (Islar & Boda, 2014).

For sustainable environmental conservation, the first step is to analyse potential impact factors. The German assessment system PERLODES divides the main stressors of surface waters into the three modules: organic pollution, general degradation and acidification (Meier et al., 2006). Two of them, organic pollution and general degradation, also affect the upper Euphrates tributaries. The first stressor, organic pollution is expected in some tributaries of Erzurum and Erzincan due to districts or villages (wastewater). The second stressor, general degradation, refers to stressors resulting from the land-use effects of watersheds, such as the input of pesticides or hormone equivalents and the degradation of stream morphology. The stressors listed above are to be expected in the upper Euphrates River mainly due to some degradation and agricultural use of the catchment area. The importance of the third main stressor, acidification, in the upper Euphrates is not known yet.

To detect the effect of disturbances on surface-water systems, assessment methods are required. In most of the EU member states, water quality monitoring was based on chemical and physical parameters until the early nineties of the last century (Hering et al., 2003). However, because benthic invertabrates react to environmental changes, they are particularly well suited for assessment and quality indication systems. For example, the saprobic index (Friedrich & Herbst, 2004) serves as an indicator of the impairment of a water body based on easily degradable organic substances, mostly as a result of deficits in wastewater treatment. A high concentration of these substances results in an oxygen deficit within the water body caused by their oxidative degradation. The saprobic value assigned to a taxon represents its ability to tolerate oxygen shortages.

Although management plans have been completed for most catchment areas, there is a lack of methods for assessing environmental impacts in Turkey (National strategy for the management of catchment areas of Turkey, 2014). In Europe, on the other hand, country-specific biological indication methods for surface-water quality based on benthic invertebrates have been developed for more than one hundred years. Well known examples are the German Saprobic Index (Kolkwitz & Marsson, 1909; Liebmann, 1951, Pantle & Buck, 1955; Rolauffs et al., 2003), the Belgian Biotic Index (De Pauw and Vanhooren, 1983), the Biological Monitoring Working Party (BMWP) for the UK (Armitage et al., 1983), the German Fauna Index (Lorenz et al., 2004; Meier et al., 2006) and PERLODES in Germany (Meier et al., 2004).

Since the beginning of the quality assessment of surface water based on benthic invertebrates in Turkey, European methods have been used (e.g. Kalyoncu and Zeybek, 2009, 2011; Kazanci et al., 2010a, 2010b; Zeybek et al., 2014; Arslan et al., 2016; Zeybek, 2017). The first regional adaptation was conducted based on the BMWP score system only recently (Kazanci et al., 2016), and this adaptation has not yet been validated by other scientists. Thanks to many faunistic studies in Turkey, several detailed taxa lists exist for specific regions in Turkey (e.g. Kiyak, 2000; Kazanci, 2009a, 2009b, 2001; Kalkman et al., 2003; Darilmaz & Salur, 2015; Darilmaz et al., 2016; Salur et al., 2016; Kalyoncu & Salur, 2018). However, many basic characteristics of stream invertebrate communities and species traits are unknown (Kazanci & Dügel, 2000). This information is required to parameterise the assessment tool and to interpret the absence or presence of certain species in relation to the existing natural environmental conditions and anthropogenic pressures. Therefore, the development of assessment methods requires a detailed knowledge of species traits such as their function in the food chain, environmental preferences, habitat use as well as morphological and physiological traits.

To determine the ecological quality of a certain stream according to the EU-WFD (2000), the community composition and abundance of benthic invertebrates, fish and benthic algae or macrophytes are compared to a reference condition, which represents the ecological condition of that same stream type without recent anthropogenic impact. Therefore, the reference conditions have to be defined before an assessment tool can be developed. Autecological information such as the habitat preferences of the organisms in the reference streams serves as a basis, which can later be used to explain the lack of taxa in anthropogenically impacted streams. It is only after basic autecological information has been obtained from the reference streams that an existing biotic index can be adapted to a specific region. The existing modified BMWP for Turkey is based on many years of taxonomic work and not on statistical analyses (Kazanci et al., 2016). Although expert knowledge is certainly very valuable, adaptation requires a mathematical explanation. The biotic score of Hindu Kush-Himalaya (HKHbios; Ofenböck et al., 2010) seemed to be the most suitable candidate for adaptation because the clearly documented calculation method of the HKHbios is based on data from extensive benthic invertebrate sampling. This index offers scientists the possibility to use it in different ecoregions of the world by creating a specific score list. Additionally, the HKHbios score list is not only based on the family level; all identified taxa – independently of their taxonomic level (family, genus or species) – can be used in the index. Due to the extension of the taxa list, it is possible to increase the preciseness of assessment.

A multi-metric index are composed of several different metrics, which are combined to indicate the ecological status of a surface water. Each metric has a special focus that is reasonably associated with a specific environmental impact. For this reason, combining different metrics is described as a more reliable method for assessing ecological stream quality than assessment methods based on single metrics (Hering et al., 2006b).Therefore, in addition to an adaptation of the HKHbios, further biological indices such as habitat preferences or the proportion of habitat specialists may be considered. Habitat characteristics and their spatial and temporal variability have a strong influence on the occurrence of specific benthic invertebrates (e.g. Southwood, 1977, 1988; Townsend, 1989; Townsend and Hildrew, 1994). For many European freshwater organisms, basic information about habitat use is available (e.g. Schmedtje & Colling, 1996; AQEM consortium, 2002; Graf et al., 2008). Despite the available information about European organisms, it is important to gather own autecological information on the specific fauna in Eastern Turkey. In addition, in order to establish the proportion of habitat specialists as an index, a better knowledge of habitat preference characteristics of stream invertebrates is necessary.

The aim of this PhD thesis was to contribute to stream conservation in Turkey by supporting the development of a quality assessment system of water based on benthic invertebrates. As a first step, it was necessary to acquire autecological information about benthic invertebrates and test some possible indices of ecological stream quality. For this purpose, 17 streams of the same type showing anthropogenic impacts of different intensity were selected in the upper Euphrates basin in Eastern Turkey. The first step towards developing a stream quality assessment system was the characterisation of the reference streams (**Study 1**). Therefore, I selected five streams without visible anthropogenic influences and analysed the seasonal differences in their community structure and the most probable primary basis for benthic secondary production. Several biological indices were determined to characterise the natural status of the streams.

As a second step, possible indices for the assessment of ecological stream quality in Euphrates tributaries were analysed (**Study 2**). Firstly, I analysed the effect of stream degradation on the benthic communities by comparing the community structures of 17 streams. After that, I determined indicator taxa for different ecological quality classes and verified the quality classes using physical and chemical measures as well benthic community indices. Thereafter, I adapted the HKHbios calculation method to the upper Euphrates catchment area by creating a specific scoring list and compared my own results with comparable existing indices. To propose a second potential index, I analysed the habitat preference of benthic invertebrates in the six most natural streams and showed the negative effect of stream degradation on benthic community composition based on the proportion of habitat specialists.

Study 1

Characterisation of natural streams using community indices and basal resources of macroinvertebrates in the upper Euphrates Basin

Reprinted from

Zuhal Gültekin, Wolfram Remmers, Rahmi Aydin, Carola Winkelmann, Claudia Hellmann (2017), *Limnologica,* 65:34–37. Doi: 10.1016/j.limno.2017.04.008

Summary

The characterisation of natural stream conditions is the first important step to analyse ecological quality of streams in the Euphrates basin. We found that the community indices correspond to very good ecological conditions in five natural streams of that region. The macroinvertebrates composition differed significantly between September and May. Number of taxa and Shannon index were significantly higher in autumn than in spring. FPOM and biofilm were the most relevant basal resources of benthic invertebrates.

Introduction and Methods

Turkey still lacks a suitable method for the assessment of ecological quality of streams (Kalyoncu and Zeybek, 2011). The complex and diverse geographical structures (Kazanci, 2011a), lack of knowledge about water organisms and the low level of public concern regarding the protection of aquatic ecosystems hinder the development of Turkish water quality assessment. European indices are therefore often used to evaluate the ecological quality of freshwaters (e.g. Kalyoncu and Zeybek, 2009; Kazanci et al., 2010a). These indices, however, are not adequately parameterized for this geographical region and require extensive ecological information about the macroinvertebrate taxa used as indicator taxa. Although many faunistic studies have compiled detailed taxa lists and collected information about the distribution of species throughout Turkey (e.g. Kalkman et al., 2003; Kazanci, 2001; Kiyak, 2000), autecological information with specific reference to Turkey is often lacking. Therefore, we assessed and compared the macroinvertebrate community structure and important community indices in two seasons in undisturbed tributaries of the Euphrates (Eastern Turkey). To characterise the relevant basal resources for the benthic invertebrates, we used stable isotope analyses (SIA) and determined the abundance of functional feeding types.

We surveyed the macroinvertebrate community in five streams without visible anthropogenic use (settlements, agriculture, livestock farming; Corine, 2017) in the catchment areas which were located in the Upper Euphrates Basin near the cities of Erzincan and Tunceli in Eastern Turkey (Appendix 1 in supplementary material). In each stream, we sampled one site (50 m length) in two seasons (spring, autumn). Sampling sites, which was located between epirithral and metarhithral zones at 1000 m above sea level (Appendix 2 in supplementary material). The streams showed similar environmental conditions and had low nutrient concentrations (Appendix 2 in supplementary material).

The benthic community was collected with a multi-habitat-sampling methods (net area: 0.0625 m^2, 1 mm mesh; Hering et al., 2004a) in autumn (September) 2013 and spring (May) 2014. Twenty subsamples were taken from each stream site with a total sampling area of 1.25 m^2. All macroinvertebrates were identified to the lowest feasible taxonomic level and counted. To estimate the composition of functional feeding groups (FG's), the taxa abundance of one feeding type relative to the total taxa density is given. The classification to feeding types was based on Schmedtje and Colling (1996), whereas a taxon was classified to the most represented feeding group, indicated by more than 50%. If a taxon represented different feeding types to similar parts, its abundance was assigned to the "combined FG's". To describe the community structure different indices were calculated from the data (e.g. EPT-Abundance (%), German Saprobian Index (GSI), Rithron Feeding Type Index (RETI); Table 1).

These indices were compared between the two seasons using paired t-test and Wilcoxon test. Differences of the benthic community composition was visualised using non-metric multi-dimensional scaling (nMDS) based on the Bray-Curtis distances of the abundance data (square root transformed) and analysed with a one-factorial analysis of similarities (Anosim). A similarity percentage analysis (Simper) was performed to identify the taxa that contributed most to the differences between seasons. All multivariate analyses were performed with the software Primer 6.

For stable isotope analyses (SIA), the dominant macroinvertebrates were collected with a hand net from all five streams in the autumn of 2013. Basal resources – coarse particulate organic matter (CPOM), macroalgae and moss – were picked by hand from the sediment. Macroalgae encompassed filamentous and gelatinous algae and were almost exclusively epilithic. Fine particulate organic matter (FPOM) samples were carefully collected from patches where it had accumulated using a beaker. Biofilm was scraped from stones with a brush and washed into a beaker where the deposit was extracted with a pipette after sedimentation. All isotope samples were frozen in liquid nitrogen until further processing.
After identifying the taxon, eliminating the guts from predatory taxa and cleaning the specimens in the laboratory, all samples were dried at 60 °C for at least twelve hours and then ground up. Thereafter, 0.5-1 mg of animal tissue and 3-5 mg of resources were weighed into tin capsules (5x9 mm) with a microbalance (precision: 0.01 mg). To prevent the high content of inorganic carbon in some resources from altering the organic carbon signatures, inorganic carbon was removed from all resource samples (Harris et al., 2001; Mazumder et al., 2010). Three replicates of each invertebrate taxon and five replicates of each resource were analysed using a Delta V™ Advantage isotope ratio mass spectrometer connected with a Flash HT elemental analyser (Thermo Finnigan, Bremen/Germany) at the Institute for Environmental Sciences (University Koblenz-Landau). The stable isotope signatures of carbon and nitrogen (X) are expressed in a δ notation relative to the international standard (carbon: Vienna Peedee Belemnite, nitrogen: N_2) in per mill units: δX [‰]=(R_{sample} / $R_{standard}$ – 1) * 1000, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample and in the standard. The precision of the isotope values was 0.12 ‰ for carbon and 0.05 ‰ for nitrogen.

Results and Discussion

In total, 10,781 individuals and 45 taxa were observed in the five streams. Ephemeroptera, Plecoptera and Trichoptera (EPT) comprised approximately 80% of the specimens and about 50% of the total number of taxa found in each season (Table 1). The Shannon Index and the Evenness of the community indicated a high species diversity and a homogenous taxa distribution in both seasons, although the number of taxa and the Shannon Index were significantly higher in autumn than in spring (Table 1). Further, the number of EPTCOB also tended to be higher in autumn, whereas the abundance of Ephemeroptera [%] was significantly higher in spring. GSI, Biological Monitoring Working Party (BMWP) score, Average Score Per Taxon (ASPT) and RETI indicated for a very good ecological quality of the streams (Table 1).

Table 1: Community Indices (means ± sd) of the five streams in autumn and spring and the results of paired t-test or wilcoxon test (marked with *). Significant p values are bold typed. Number of EPTCOB: taxa number of Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Odonata, Bivalvia. For the taxa that were most important for the seasonal differences in the benthic community, the abundances in individuals m² of benthic taxa (means ± sd, n = 5) and their contribution to the difference [%] are given. In addition, the mean abundance (± sd) of different functional feeding groups (FG's) to the total invertebrate abundance is given.

	Autumn	**Spring**	**Statistic results**	
Community Indices				
Total Number of Individuals	1496 (± 1124)	660 (± 517)	t=1867.0	p=0.135
Total Number of Taxa	39 (± 6)	27 (± 10)	t=2.89	**p=0.045**
Number of EPT-Taxa	17 (± 2)	13 (± 4)	t=1.82	p=0.142
Number of EPTCOB	27 (± 5)	19 (± 7)	t=2.16	p=0.097
EPT [Taxa %]	44 (± 4)	49 (± 10)	t=-1.75	p=0.155
EPT [Abundance %]	64 (± 12)	78 (± 18)	t=-1.41	p=0.233
Odonota [Abundance]	2.66 (± 3)	0.74 (± 1)	V=12*	p=0.313
Ephemeroptera [Abundance]	32 (± 21)	62 (± 13)	t=-3.48	**p=0.025**
EPT/Diptera [Abundance]	3.39 (± 1)	21 (± 33)	t=-1.19	p=0.302
EPT/Diptera [Taxa]	1.87 (± 0.23)	3.29 (± 3.23)	t=-1.19	p=0.302
Shannon-Index [H]	2.67 (± 0.30)	2.18 (± 0.37)	t=3.96	**p=0.017**
Pielues Eveness [J']	0.73 (± 0.08)	0.67 (± 0.06)	t=2.06	p=0.108
German Saprobian Index	1.61 (± 0.09)	1.78 (± 0.16)	t=-2.2087	p=0.092
BMWP score	156 (± 15.65)	112.20 (± 26.33)	V=14*	p=0.125
Average Score Per Taxon	7.18 (± 0.12)	6.79 (± 0.30)	t=1.8994	p=0.130
RETI	0.59 (± 0.11)	0.60 (± 1.14)	t=20.26	p=0.849
Abundances [Ind m^{-2}]				**Contribution**
Baetis spp.	183 (± 172)	193 (± 163)		6.1 %
Leuctra spp.	68 (± 15)	2.1 (± 4.2)		5.9 %
Epeorus spp.	137 (± 205)	21 (± 36)		5.3 %
Rhithrogena sp.	118 (± 186)	5.4 (± 4)		4.5 %
Simulium spp.	94 (± 112)	12.2 (± 16)		4.0 %

Hydropsyche instabilis-gr.	110 (± 70)	34 (± 22)	3.4 %
Elmidae	78 (± 106)	12 (± 18)	3.4 %
Epeorus caucasicus	0.0 (± 0)	26 (± 23)	3.2 %
Ecdyonurus starmachi	38 (± 28)	15 (± 25)	3.1 %
Epeorus zaitzevi	0.0 (± 0)	27 (± 28)	3.0 %
Abundance of FG's [%]			
Grazers	46.6 (± 20.8)	67.0 (± 16.1)	
Predators	11.3 (± 7.4)	5.6 (± 1.8)	
Gatherers	9.0 (± 4.1)	15.9 (± 10.4)	
Shredders	7.6 (± 5.2)	4.8 (± 4.4)	
Filter feeder	5.1 (± 5.2)	3.5 (± 5.3)	
Combination of FG's	14.5 (± 10.9)	1.7 (± 1.2)	
Others	5.9 (± 4.8)	1.6 (± 1.3)	

The benthic community composition differed significantly between autumn and spring (Anosim, Global R = 0.408, p = 0.024), indicated by two distinct clusters representing the two seasons in the nMDS (Fig. 2). Although similarity between the stream sites was relatively low within one season, it was even lower for the specific sites between the two seasons represented by the high distances to each other (Fig. 2). The mean dissimilarity of the community composition between the seasons was 59.94% and *Baetis* spp., *Leuctra* spp. and *Epeorus* spp. contributed to it with more than 5% each. *Ecdyonurus starmachi*, *Epeorus* spp., Elmidae, *Hydropsyche instabilis*-group, *Leuctra* spp., *Rhithrogena* sp. and *Simulium* spp. seemed to occur in higher mean densities in autumn, while *Baetis* spp., *Epeorus caucasicus* and *Epeorus zaitzevi* showed somewhat higher mean abundances in spring (Table 1).

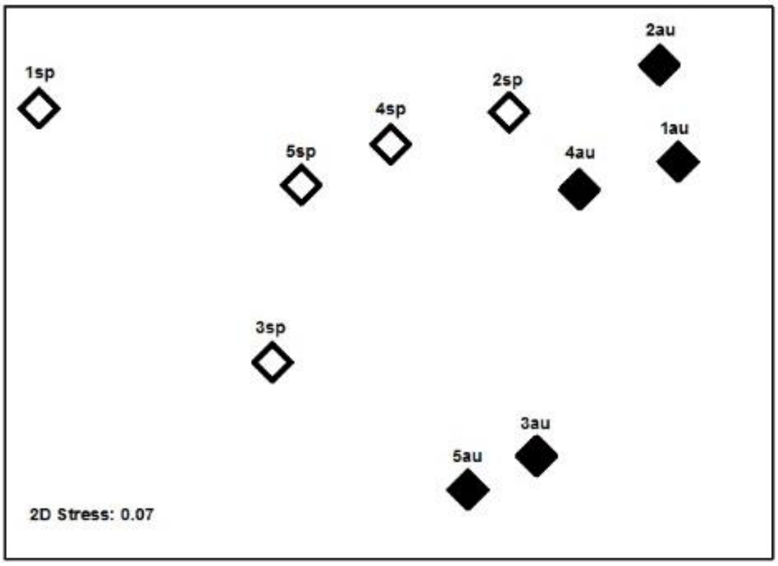

Fig. 2: Non-metric MDS of the benthic community composition in the five streams in both seasons [au: autumn 2013, black symbols; sp: spring, white symbols], based on the taxa abundances (square root transformed) and the Bray-Curtis similarity.

The stable isotope signatures of the basal resources varied greatly between the five streams and within each stream (Fig. 3). Biofilm and FPOM showed the smallest ranges in mean carbon and nitrogen signatures. The consumers in all streams together showed a range for $\delta^{15}N$ from -1.76 to 3.82 ‰ and for $\delta^{13}C$ from -35.78 to -23.98 ‰ in 95% of the data. Correcting the consumer signatures with the trophic discrimination factors ($\Delta\delta^{15}N$ = 3.4 ‰ and $\Delta\delta^{13}C$ = 0.4 ‰; Post, 2002) revealed that biofilm and FPOM signatures overlapped the most with the consumer signatures (Fig. 3). Therefore they were most probable relevant resources for benthic invertebrates. Macroalgae, moss and CPOM seemed to be of minor relevance as resources for the consumers.

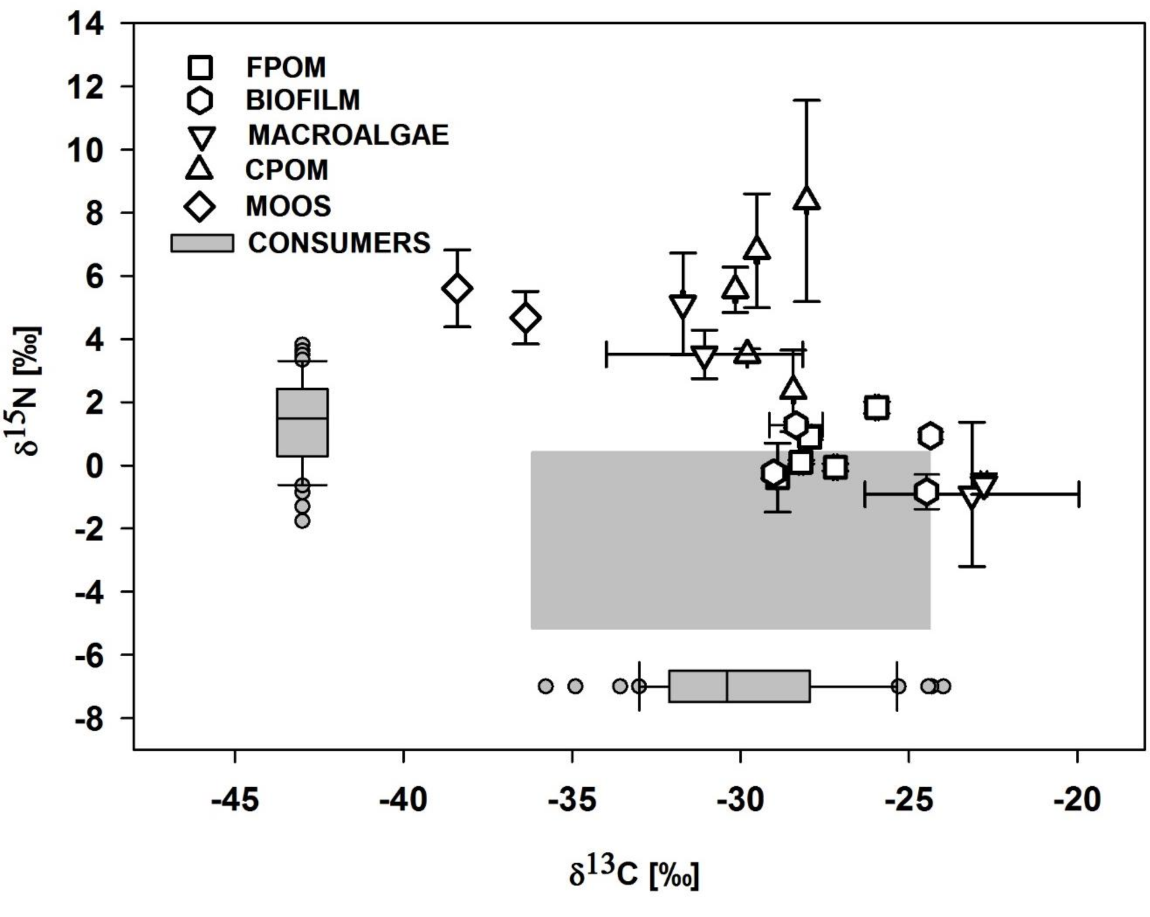

Fig. 3: Carbon and nitrogen isotope signatures of the basal resources in the five streams [means ± sd, n = 5, white symbols] and ranges of the isotope signatures for all consumers, separately shown for carbon (in direction of x-axis) and nitrogen (in direction of y-axis; grey Box-Whisker plots, median, quartiles, each outliner shown). The grey area shows the area of resource signatures that was covered by the consumer signatures, including the trophic discrimination factors ($\Delta\delta^{13}C$ = 0.4 ‰, $\Delta\delta^{15}N$ = 3.4 ‰; Post, 2002).

The aim of this study was to characterise natural mountain streams in the Euphrates Basin in Eastern Turkey with regard to benthic community structure and important basal resources for benthic invertebrates. Community indices show values largely similar to or slightly better than those of roughly comparable stream types in Europe representing good ecological quality (e.g. Böhmer et al., 2004a; Moog et al., 2004). Shannon Index and the mean taxa number in our study were similar to values of alpine streams with a very good ecological quality used as reference conditions in Germany (3.2 and 17, respectively, Böhmer et al., 2004a).The proportions of EPT abundance in the streams in Turkey indicated a very high insect diversity. Values were similar to streams of very good ecological quality in the Limestone Alps (64 - 75% EPT abundance, Meier et al., 2006) or the Crystalline Alps in Austria (55%, Moog et al., 2004). In the Aksu River, a mostly undisturbed river in Northern Turkey, comparable values of Shannon Index, Evenness and percentage of EPT to our study were found (Kazanci et al., 2010a). Ecological indices like GSI, BMWP score, ASPT and RETI support this assessment and indicate a good ecological quality in this streams. However, these metrics are not parametrised for Turkey and should be used carefully. Because, our results support our assumption that the studied streams have a very natural state, we propose that they might be used to define reference conditions for further assessment of the ecological quality of mountain streams in Turkey.

For ecological quality assessment of the benthic community, the time of sampling has high relevance for obtaining representative results. Based on our study, we assume that early autumn (September/October) might be a better sampling time than spring for the small mountain streams in the Upper Euphrates Basin. As expected, the community composition differed between spring and autumn. This was mainly due to Plecoptera and Ephemeroptera (especially Heptageniidae), which showed mostly higher abundances in autumn. This seems to be very important, because species from these taxonomic groups are widely known to be sensitive to water and habitat quality and can react to multiple anthropogenic stressors (Hering et al., 2004a). Supporting this result, the community indices considering the occurrence of sensitive insects (e.g. EPT taxa number, Odonata abundances) tended to be higher in autumn than in spring. However, the variability of most of the indices between the streams was high and no clear seasonal effect was revealed. The tendency of lower scores and high variability of several indices in spring compared to autumn can likely be explained by the high flow fluctuations in spring caused by snowmelt, while flow was generally very low during autumn sampling due to summer aridity. Different flow conditions and temperatures can have strong effects on the invertebrate community (Avlyush et al., 2013; Winterbottom et al., 1997). We assume that the occurrence of invertebrate taxa and the community composition are more strongly affected by stochastic changes of abiotic environmental factors shortly after winter than by the more constant conditions after a dry summer. The community structure could therefore vary more in springtime between years, depending on the time and magnitude of floods, than in autumn. Summer seems not favorable for macroinvertebrate sampling, because most of the insect taxa (e.g. mayflies, stoneflies) are emerging in the early summer. Consequently we propose to sample stream invertebrates for ecological quality assessment in early autumn when environmental conditions are more stable and larval stages of insect taxa re-occur in the water.

Our results show that the most probable basal resources for the consumers in the studied natural streams are autochthonous biofilm and FPOM. In Central Europe, the importance of autochthonous primary production for benthic invertebrates usually increases with higher stream orders (Vannote et al., 1980), and CPOM is the main basis of food webs in second order mountain streams. Leaf litter seems to be of minor relevance in the five streams, probably due to a general lack of trees near the riverbanks. Our results concur with studies from Alpine streams, which clearly demonstrate the importance of biofilm and filamentous algae for consumers at higher altitudes (1768-2159 m, Zäh et al., 2001) and a use of FPOM of about 60% in streams both below and above the tree line (Füreder et al., 2003). Because the prevalence of basal resources determines the species composition of the benthic community (Cummins and Klug, 1979), grazers can be assumed to dominate the community in the studied streams of the Euphrates basin due to the high importance of biofilm. Indeed, grazers was accounted for nearly 50% of benthic abundance in autumn and two third of the benthic abundance in spring. These values correspond well to the feeding type composition in natural streams (Schweder, 1992). Gatherers and filter feeders together, as FPOM users, represented about 15 % of the benthic abundance, also supporting the importance of FPOM as resource. Especially grazers as most important feeding group in our streams, represent sensitive taxa such as heptageniids. Therefore we assume that the composition of the feeding groups might provide a useful indicator to evaluate the ecological quality of mountain streams in Turkey. To develop such an indicator, the identification of the main basal resources in different streams and stream types is necessary to establish a clear picture of the natural resource use of macroinvertebrate assemblages in Eastern Turkey.

Study 2

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

Manuscript authored by

Zuhal Gültekin, Claudia Hellmann, Rahmi Aydin, Carola Winkelmann

Summary

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; Ofenböck et al., 2010) to the Euphrates catchment by establishing a new and ecoregion-specific score list. The new biotic scoring list for the Euphrates (Euph-Scores) was calculated for 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. A comparison of the different ASPT values showed that the weighted ASPT, named the Euphrates Biotic Score (EUPHbios), was the most useful value because the weighting enabled a sharper differentiation between the quality classes. The EUPHbios was compared to the ASPT values of the HKH scores ($ASPT_{HKH)}$, the original Biological Monitoring Working Party (BMWP) ($ASPT_{OR}$) and the Turkish scores ($ASPT_{TR}$). These ASPT values were significantly lower than the EUPHbios and did not differ significantly between the quality classes, although the $ASPT_{HKH}$ value was closest to our results.

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.

Introduction

Due to the rapid economic development in Turkey, there is high anthropogenic pressure on aquatic habitats, in the form of construction of dams, artificial embankments and channel straightening (Sekercioglu et al., 2011). In addition, intensification of land use has increased the input of nutrients and toxins (e.g. Varol & Sen, 2012; Alkan et al., 2013). The first step to protect the ecological quality of streams is to objectively monitor the development of ecological quality and to show the negative consequences of the existing anthropogenic impact. It is generally assumed that the hydromorphological characteristics of a stream, such as the substrate structure of the stream bed or the development of the riparian zone, directly relate to habitat diversity (Feld, 2004). A reduction in habitat diversity is a major contributor towards a decrease in invertebrate diversity (Beisel et al., 2000). Aquatic ecosystems with increased nutrient enrichment are especially affected, which has a further impact, for example on drinking water and food quality (Carpenter et al., 1998). Especially increased nitrogen and phosphorus concentrations often cause eutrophication. Extrapolating from experiences in Western Europe, we assume that this combination of anthropogenic impacts will result in a dramatic loss of natural stream habitats and a reduction of water quality, which consequently will negatively affect the streams typical benthic communities and reduce aquatic biodiversity. It is therefore of very high importance to retain the natural habitats in Turkey, because the recovery of benthic fauna after restoration takes a long time at best or does not occur at worst, as studies of restoration projects in Western Europe have shown (e.g. Tullos et al., 2006; Lorenz et al., 2018). To support the protection of ecological stream quality in Turkey, a reliable and sensitive assessment of streams ecological status is necessary.

Benthic invertebrates are the most commonly used biological indicators for assessing the ecological quality of running waters (Rosenberg & Resh, 1993) and for estimating the intensity of anthropogenic impacts. The analysis is based on their differences in environmental preferences and tolerances. For instance, while Chironomidae tend to have a higher tolerance limit regarding oxygen shortage, many Ephemeroptera taxa show lower tolerance limits (Connolly et al., 2004). In several European countries, different assessment methods based on benthic invertebrate indicator taxa are used to monitor the ecological quality of streams and lakes on a regular basis. Although the general methods of benthic invertebrate bioindication are 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 through 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 England on the basis of the in-depth knowledge of experts on the environmental requirements of taxa (Armitage et al., 1983). Later, BMWP was modified in several countries such as Canada (Barton & Metcalfe-Smith, 1992), Spain (Zamora-Munoz & Alba-Tercedor, 1996) or Poland (Czerniawska-Kusza, 2005) and has been used in Turkey (e.g. Kazancı et al., 1997, 2010a, 2010b, 2011b, 2013; Duran et al., 2003; Kalyoncu and Zeybek, 2011; Zeybek et al., 2014). A comparative study of the various BMWP values using the different national ASPT conducted by Zeybek et al. (2014) showed that the transfer of these country-specific indices to Turkey produces inaccurate results.

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 more 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 the Euphrates region.

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., 2004b; Hering et al., 2004a, 2004b). Biological indices used for this purpose are, for instance, feeding type or microhabitat preference (Schweder, 1992; Böhmer et al., 1999, 2004b; Hering et al., 2004b; Meier et al., 2006). Besides the ASPT, which might be included in such a multi-metric approach in Turkey, the proportion of habitat specialists is a potential indicator. 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 & 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 & Türkmen 2012; Darilmaz & 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 and 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). The most common impacts at the sampling sites were wastewater input, water abstraction for irrigation in agriculture, intensive livestock farming and hydromorphological degradation due to channelizing. 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) located in the Upper Euphrates Basin near the cities of Erzincan, Erzurum and Tunceli in Eastern Anatolia (Turkey, Fig. 4).

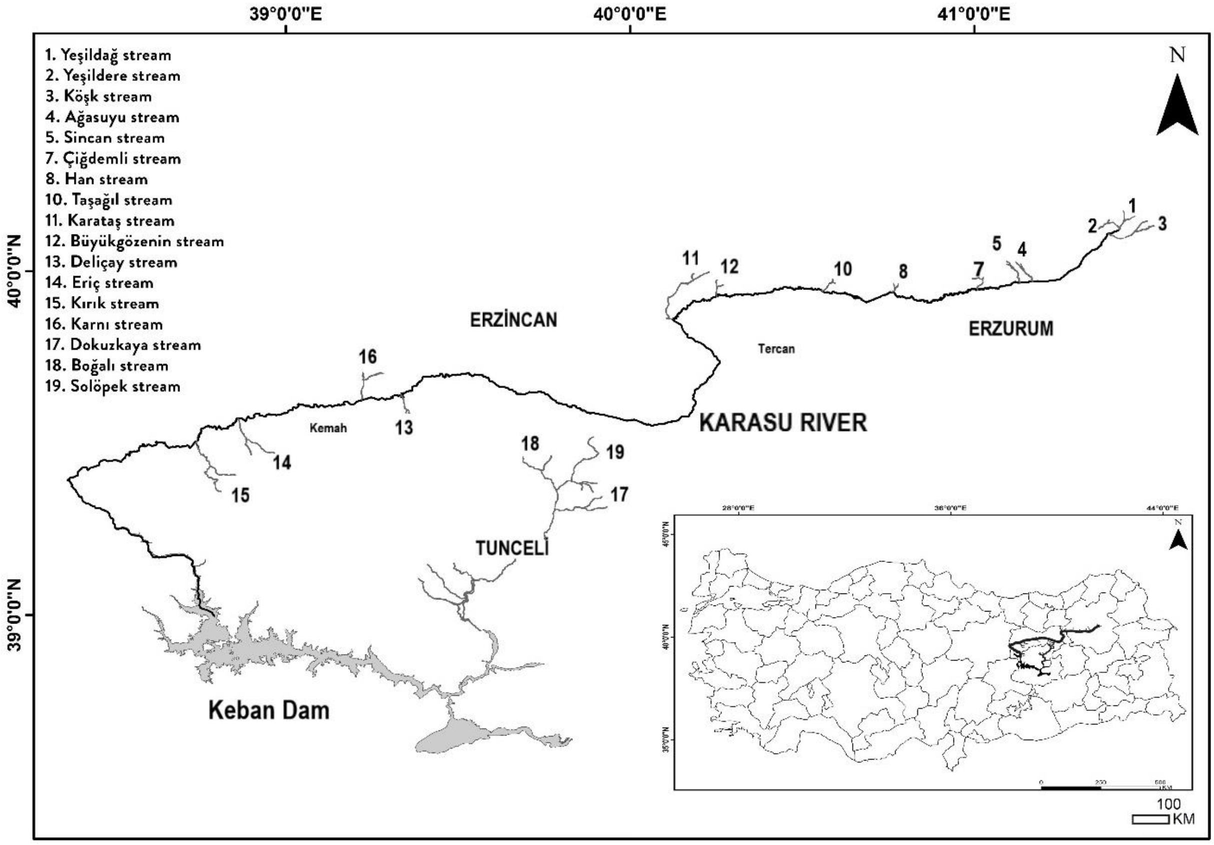

Fig. 4: Location of stream sites.

Eastern Anatolia has a continental climate characterised by warm, dry summers and cold, snowy winters (Sensoy et al., 2008). All sampling sites were located in the epirhithral or metarhithral zones of the streams at about 970 to 1940 m above sea level. The streams differed slightly regarding discharge and size of their catchment area (Table 2). 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 reports of the provinces Erzincan and Erzurum, 2016). The sampling sites represent different levels of habitat diversity and different levels of water pollution and structural degradation (Table 3).

Table 2: Stream characteristics of studied streams. The measurement of discharge Q [$m^3 * s^{-1}$] was based on Carufel (1980). Mean width [m]: n = 3.

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

Table 3: Anthropogenic stressors of studied streams. **X** = impact, **XX** = slight impact, **XXX** = moderately strong impact.

Anthropogenic stressors							
Stream	Agriculture	Allotment	Banks fixed/Riverbed straightened	Extraction (chrome & 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							

Field sampling

We sampled all 17 streams at one site per stream in two different seasons. One sampling was conducted in autumn (September 26th to October 5th 2013) and one in spring (May 25th to May 31th 2014). At each site, the benthic community of each stream 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 at 5% intervals. Thereafter, 20 individual samples, each representing 5% of substrate coverage, were taken by kick sampling in front of a 25 x 25 cm dip net (1 mm meshes, surface area 0.0625 m^2) 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. This resulted in a different number of habitat-specific subsamples per sampling site. After washing out the coarse inorganic material at the sampling site, all subsamples were stored in 96% ethanol, which was replaced by 70% ethanol in the laboratory.

In addition, the most important environmental factors were measured to characterise the stream sites. Discharge ($m^3 * s^{-1}$) was determined by estimating the sectional stream area and current velocity. To measure the flow velocity, a velocity head rod was positioned on the streambed with its sharpened side pointed towards the flow direction to measure water depth. Next, the rod was turned 180 degrees. As a result, the flat side pointed upstream, resulting in an increase of the water level because of the impingement of water against the broad edge of the rod. The resulting difference h (velocity head) formed the basis for calculating the flow velocity (Carufel, 1980). The sectional stream area was calculated using the water depth, which was measured at intervals of 1 m or 0.5 m (small streams up to a width of 5 m) perpendicular to the flow direction. Depending on the stream width, the number of measurements varied between four and nine.

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], O_2-content [$mg \cdot L^{-1}$], O_2-saturation [%], pH and conductivity [µS/cm] were measured in September/October 2013 using a YSI Professional Plus Multiparameter Probe (YSI, Ohio/USA) and in May 2014 with the WTV Oxi 330 (WTV GmbH, Weilheim/Germany) and WalkLAB TI 9000 (Trans Instruments Pte Ltd, Petro Centre/Singapore). To analyse the NO_2, NO_3, NH_4 and PO_4 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, Göttigen/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 NO_2, NO_3, NH_4 and PO_4 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). The lack of available determination key for macroinvertebrates in Turkey often hampered identification of the species. To verify the results, specimens with an unclear identity were sent to experts for the respective groups (Ephemeroptera: Dr. Caner Aydinli, Anadolu University; Coleoptera: Dr. Hans Fery, Berlin, Dr. André Skale, Natural History Museum Erfurt and Dr. Mustafa Darilmaz, Aksaray University; Trichoptera: Prof. Wolfram Graf, University of Natural Resources and Life Sciences Vienna).

Data analysis

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

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. The highest indicator value (v = 1) means that the taxon occurred in all samples of one group. All taxa that were characterised as indicator taxa in a quality class by our analysis and with an indicator value > 0.5 were presented. To verify the indicator taxa identified by the “indval” function, nonmetric multidimensional scaling (nMDS: Anderson, 2001) was performed individually for each season.

nMDS was used to show the distances between the invertebrate community compositions of different water quality classes based on Bray-Curtis distances and to highlight the most representative taxa for a specific quality level. For this purpose, only the taxa that were identified at least to the genus level were included in this analysis, and only the indicator taxa based on the function “indval” in nMDS were presented. Multivariate statistics were calculated using the R package “vegan” (Oksanen, 2018).

Nutrient concentrations [mg L^{-1}] of nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NO_4-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 Nemathoda, 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. With the reduction to family level, the mean values of the occurring taxa in a family were calculated. To distinguish between the two groups, they were named as follows: 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 (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:

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

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 samples from both campaigns (autumn 2013 and spring 2014) were used, the maximum number of samples in a quality group was 12: 10 and 6 for Qc I (natural streams), Qc II (slightly polluted streams) and Qc III (moderately polluted streams). Because the obtained guide scores differ 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 ($ASPT_{W}$ = 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: 101-1,000; Class 4: 1,001-10,000; 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, Psychodoidae 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 (distribution of habitats in Appendix 4 in supplementary material) 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 4, Step A). Next, the relative abundance of each taxon for each habitat was calculated (percentage of total abundance for the stream, Table 4, 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).

As one other metric for the assessment procedure, the proportions of specialists and generalists in each stream were calculated, and each sampling campaign and quality class were compared using a one-way ANOVA. Generalist and specialist taxa were separated based on the 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 (Table 9). 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.

Table 4: 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 ∑
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 ∑	**267.86**		**24.40**		**7.74**	**300**
Mean ∑	(267.86 * 100)/300 = **89.29**		(24.40 * 100)/300 = **8.13**		(7.74 * 100)/300 = **2.58**	
Habitat Score	**9**		**1**		**+**	

Results

Indicator taxa of different ecological quality classes

The cluster analysis resulted in three groups of stream communities (Fig. 5) 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 the anthropogenic pressure on the studied streams (Table 3). 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.

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', Table 5). A visualization of the 23 potential indicator taxa with nMDS completely supported the class assignment for 17 taxa because they were located in the middle of the respective quality class (Fig. 6). The other five taxa showed a less clear fit because they were grouped between Qc II and III in the nMDS *(Baetis rhodani*, *Epeorus zaitzevi*, *Baetis* spp., *Electrogena* sp. *and Potamopyrgus* sp.; Fig. 6) and were therefore not considered as indicator taxa. Most of the taxa indicated by indval and supported by nMDS were assigned to Qc III, among others *Batracobdella* sp., *Eiseniella* sp. and *Serratella ignita.* The only taxa assigned to Qc II were the *Hydropsyche instabilis* group and the *Baetis lutheri* group. *Perlodes* sp., *Protonemura* sp. and *Atherix* sp. were assigned to Qc I. The quality classification of *Leuctra* sp. varied between seasons; it was classified Qc I in autumn and Qc III in spring (Table 5), possibly reflecting the ecological requirements of different species. Therefore, *Leuctra* sp. could not be used as an indicator taxon (Fig. 6).

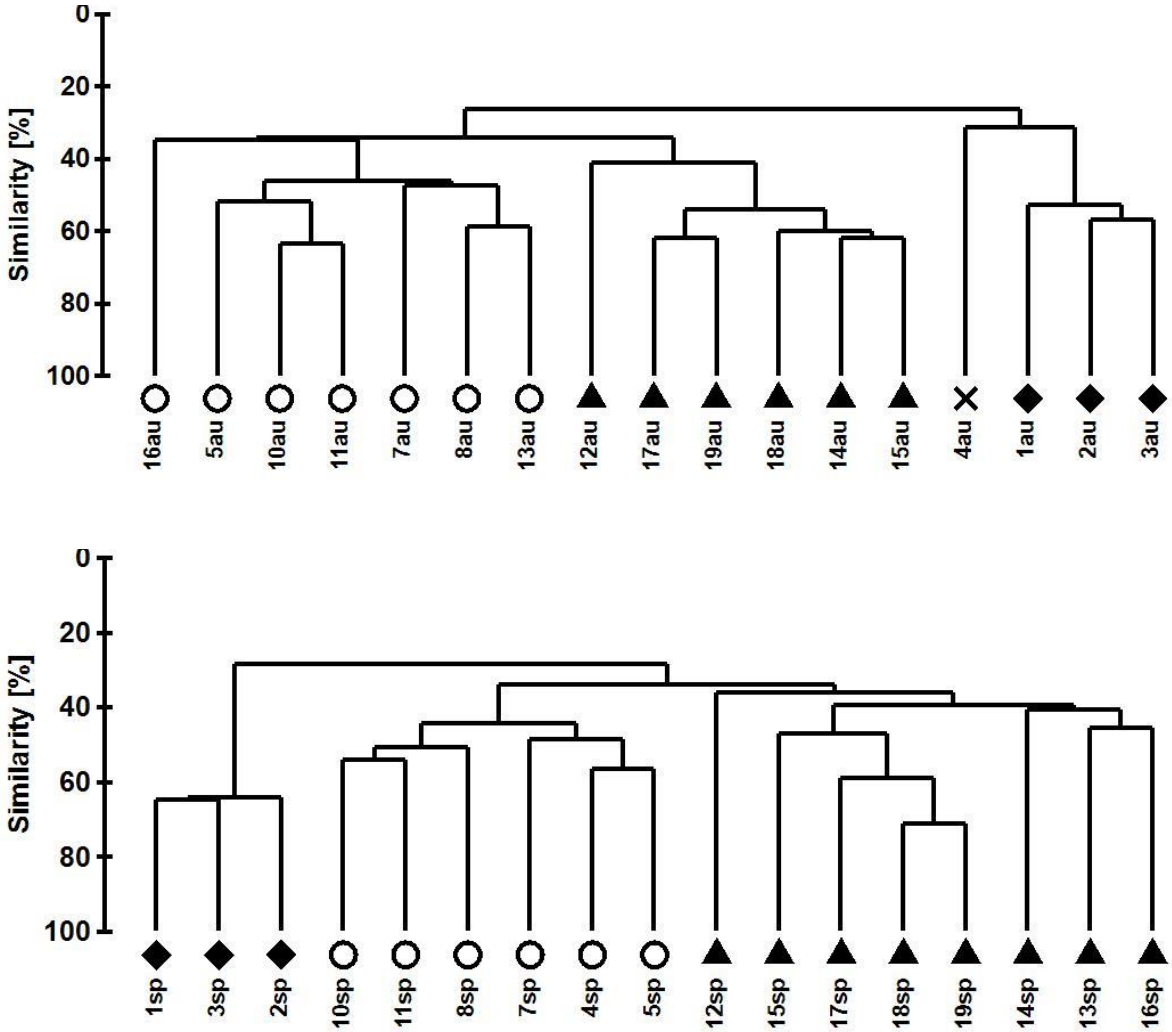

Fig. 5: Cluster analyses of benthic community of all the sites from both seasons based on Bray-Curtis similarity: autumn (au) 2013 and spring (sp) 2014. Quality class I = ▲, Quality class II = ○, Quality class III = ◆, **X** = own group because of 35% similarity to the other three groups.

Table 5: 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) and from assignment to a quality class resulting from the nMDS. Written in bold, taxon represent a clear quality class. Freq is the number of times the species was present among the samples (not abundance).

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

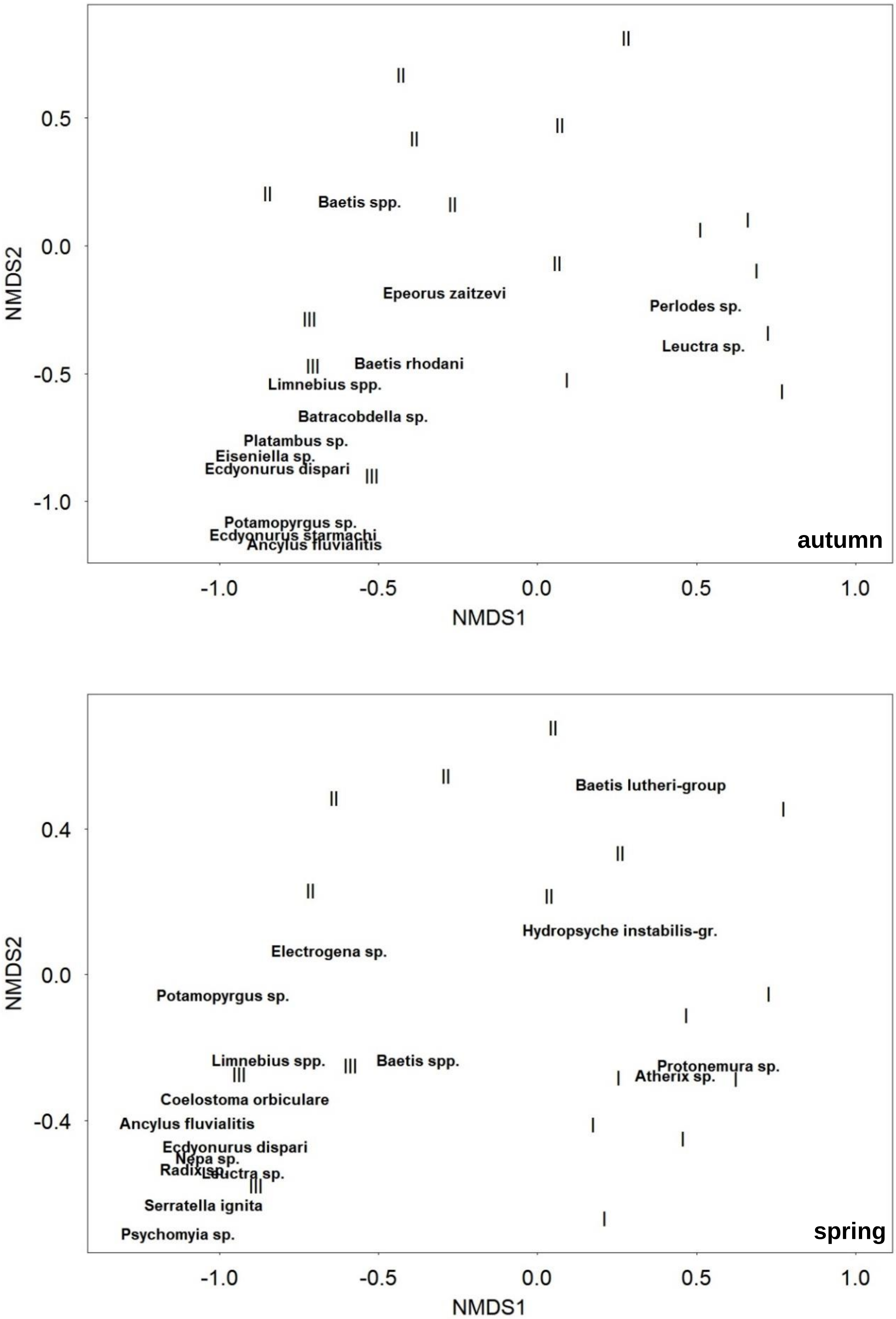

Fig. 6: Multidimensional scaling of benthic community composition of the sampling sites (based on Bray-Curtis similarity calculated from abundance data) with an indication of the three quality classes after separating the data with cluster analyses; indicator taxa were additionally shown for their main assignment to quality classes for both seasons: autumn (2013) and spring (2014).

Abiotic characteristics and community indices

Independent of their quality class assignment, the streams were characterised by high oxygen concentrations and alkaline pH values (Appendix 3 in supplementary material). 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. Conductivity [µS/cm] were high in the streams of Qc I (12, 14, 15, 17, 18, 19).

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 & 10; spring: Qc II streams no. 7 & 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 & 5). The ammonium concentration of most streams was very high in autumn (up to max. 2.32 $mg \cdot L^{-1}$). The phosphate concentrations were below the detection limit of the analysis (< 0.01 $mg \cdot L^{-1}$ in autumn and < 0.003 $mg \cdot L^{-1}$ in spring) in both seasons, except in Qc III (spring: streams no.1, 2, 3).

In four of the six calculated community indices, the three quality classes differed significantly (Table 6, Fig. 7). The total abundance of Ephemeroptera, Plecoptera and Trichoptera (EPT) and EPT including Coleoptera, Bivalvia and Odonata (EPTCBO) was highest in Qc I and differed significantly from Qc II. The highest evenness score was recorded in Qc I; it 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 ($n = 12$) x Qc III ($n = 6$), $Q = 3.83$, $p < 0.05$, Dunn's method, Qc I ($n = 12$) x Qc II ($n = 10$), $Q = 2.82$, $p < 0.05$). The quality classes did not differ regarding the number of taxa and Shannon diversity.

Table 6: Comparison of the biological indices in three quality classes (Qc). EPTCBO = Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata.

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

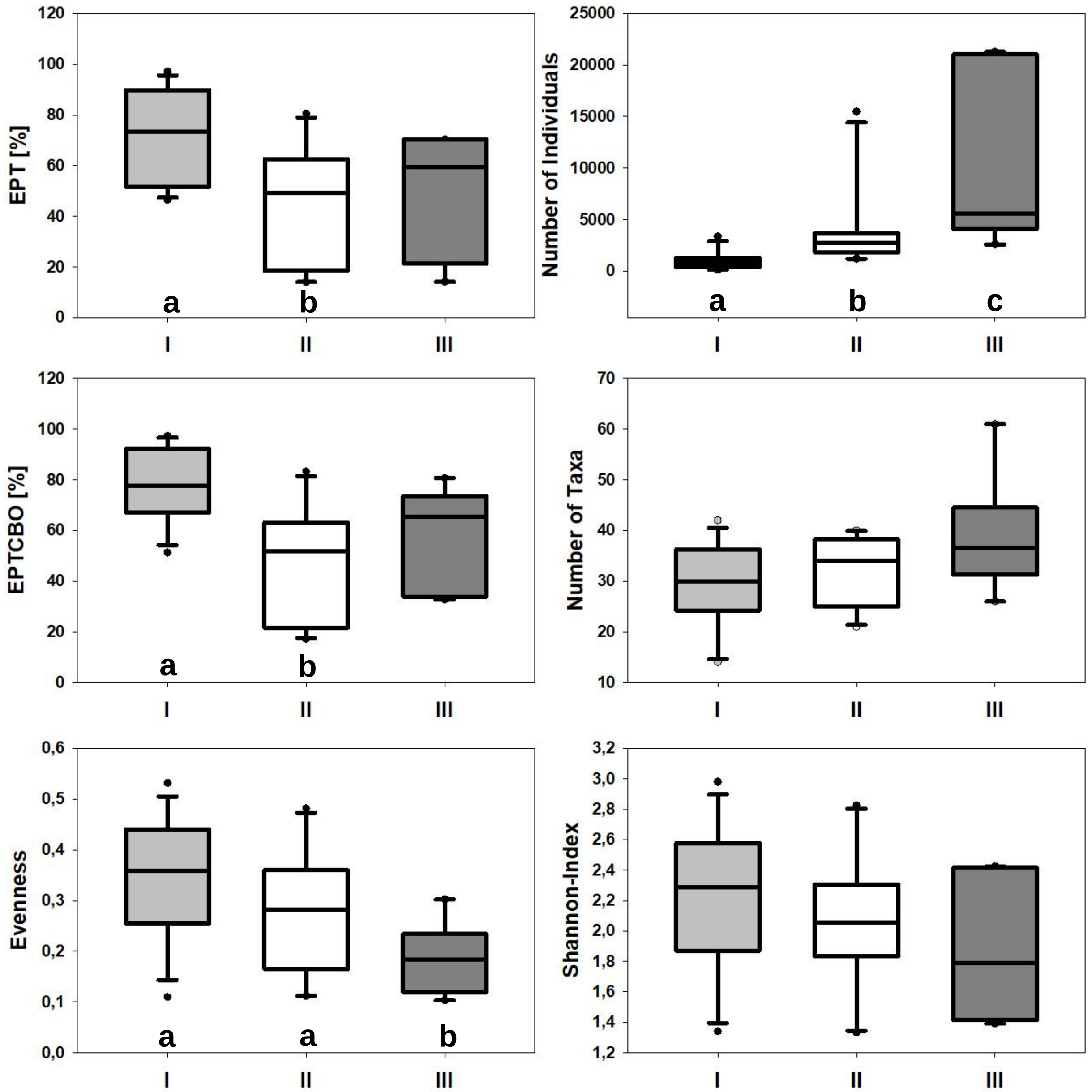

Fig. 7: Proportion of community indices in the three different quality classes (I, II, III) visualized by Box-Whisker plots (median, quartiles, 5th and 95th percentiles, outliers). EPTCBO = Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata. “a”, “b” and “c” showed significant differences between the plots.

Euphrates biotic score

The Euph-Scores of 93 taxa, their respective weights (Table 7) and abundance classes were used to calculate several different versions of ASPT values. However, as assumed, EUPHbios showed the sharpest separation among the investigated indices, indicated by higher differences between the means of the quality classes than other scores (Fig 5). A comparison of the EUPHbios indices to the other ASPT values of the Euph-Scores revealed significant interaction between the quality classes and the index (two-way ANOVA, quality x index, $p < 0.001$, Table 8), showing that the effect of the quality class depends on the selected index. Weighting of the Euph-Scores of selected 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, Table 8, Fig. 8). On the other hand, including abundance in the weighting ($ASPT_{WA}$) did not improve the separation, because the abundance values did not differ significantly from those of the EUPHbios (Table 8, and Fig. 8). Similarly, the ASPT values without extreme taxa (ASPT without extremes) did not differ from the ASPT with extreme taxa (ASPT, Table 8 and Fig. 8).

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 those of the EUPHbios (Table 8, Fig 8). For this reason, they did not seem to be suitable for assessment in this study.

Table 7: Euph-Score list of 93 taxa. Taxa written in bold were considered to be "extreme" based on the calculations of the ASPT-EUPHbios.

Order	Familly	Taxon	Score	Weight
Acari	HYDRACHNIDIAE	Gen. spp.	8	1
Amphipoda	GAMMARIDAE	Gen. spp.	8	
Amphipoda	Gammaridae	*Gammarus* spp.	8	1
Bivalvia	SPHAERIIDAE	Gen. spp.	7	
Bivalvia	Sphaeriidae	*Pisidium* spp.	7	3
Coleoptera	DYTISCIDAE	Gen. spp.	8	
Coleoptera	Dytiscidae	*Platambus lunulatus*	9	3
Coleoptera	Dytiscidae	*Platambus* sp.	7	3
Coleoptera	Dytiscidae	*Nebrioporus stearinus*	9	3
Coleoptera	ELMIDAE	Gen. spp.	9	3
Coleoptera	Elmidae	*Esolus* sp.	8	1
Coleoptera	Elmidae	*Grouvellinus caucasicus*	10	5
Coleoptera	Elmidae	*Limnius* sp.	9	3
Coleoptera	Elmidae	*Normandia nitens*	10	5
Coleoptera	Elmidae	*Riolus* sp.	9	3
Coleoptera	GYRINIDAE	Gen. sp.	8	
Coleoptera	Gyrinidae	*Gyrinus* sp.	8	1
Coleoptera	HELODIDAE	Gen. sp.	7	1
Coleoptera	HYDRAENIDAE	Gen. spp.	8	
Coleoptera	Hydraenidae	*Hydraena* spp.	9	3
Coleoptera	Hydraenidae	*Limnebius* spp.	7	3
Coleoptera	Hydraenidae	*Ochthebius* spp.	9	3
Coleoptera	HYDROPHILIDAE	Gen. spp.	8	
Coleoptera	Hydrophilidae	*Helophorus* spp.	8	1
Coleoptera	Hydrophilidae	Hydrophilidae	7	1
Coleoptera	Hydrophilidae	*Laccobius* sp.	8	1
Diptera	ATHERICIDAE	Gen. sp.	8	
Diptera	Athericidae	*Atherix* 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	*Prosimilium* sp.	8	1
Diptera	Simuliidae	*Simulium* spp.	8	1
Diptera	STRATIOMYOIDAE	Gen. sp.	10	
Diptera	Stratiomyoidae	*Stratiomys* sp.	10	3
Diptera	TABANIDAE	Gen. sp.	8	1

Diptera	TIPULIDAE	Gen. spp.	8	1
Diptera	Tipulidae	*Hexatoma* sp.	7	1
Ephemeroptera	BAETIDAE	Gen. spp.	8	
Ephemeroptera	Baetidae	*Baetis lutheri*-group	8	1
Ephemeroptera	Baetidae	*Baetis rhodani*	8	1
Ephemeroptera	Baetidae	*Baetis* spp.	8	1
Ephemeroptera	CAENIDAE	Gen. spp.	8	
Ephemeroptera	Caenidae	*Caenis macrura*	8	1
Ephemeroptera	Caenidae	*Caenis* sp.	8	1
Ephemeroptera	EPHEMERELLIDAE	Gen. spp.	8	
Ephemeroptera	Ephemerellidae	*Ephemerella* sp.	8	1
Ephemeroptera	Ephemerellidae	*Serratella ignita*	6	3
Ephemeroptera	EPHEMERIDAE	Gen. sp.	8	
Ephemeroptera	Ephemeridae	*Ephemera* spp.	10	5
Ephemeroptera	HEPTAGENIIDAE	Gen. spp.	8	
Ephemeroptera	Heptageniidae	*Ecdyonurus dispari*	6	5
Ephemeroptera	Heptageniidae	*Ecdyonurus macani*	8	1
Ephemeroptera	Heptageniidae	*Ecdyonurus* sp.	8	1
Ephemeroptera	Heptageniidae	*Ecdyonurus starmachi*	8	1
Ephemeroptera	Heptageniidae	*Electrogena* sp.	7	1
Ephemeroptera	Heptageniidae	*Epeorus caucasicus*	9	3
Ephemeroptera	Heptageniidae	*Epeorus zaitzevi*	8	1
Ephemeroptera	Heptageniidae	*Heptagenia* sp.	7	1
Ephemeroptera	Heptageniidae	*Rhithrogena puytoraci*	8	1
Ephemeroptera	Heptageniidae	*Rhithrogena* sp.	8	1
Ephemeroptera	SIPHLONURIDAE	Gen. sp.	6	5
Gastropoda	HYDROBIIDAE	Gen. sp.	6	3
Gastropoda	LYMNAEIDAE	Gen. sp.	6	
Gastropoda	Lymnaeidae	Radix sp.	6	3
Gastropoda	PLANORBIDAE	Gen. spp.	6	
Gastropoda	Planorbidae	*Ancylus fluvialitis*	6	5
Gastropoda	Planorbidae	*Gyraulus* sp.	7	1
Gastropoda	TATEIDAE	Gen. sp.	6	
Gastropoda	Tateidae	*Potamopyrgus* sp.	6	3
Heteroptera/Hemiptera	NEPIDAE	Gen. sp.	6	
Heteroptera/Hemiptera	Nepidae	*Nepa* sp.	6	3
Hirudinea	ERPOBDELLIDAE	Gen. sp.	7	
Hirudinea	Erpobdellidae	*Erpobdella* sp.	7	3
Hirudinea	GLOSSIPHONIIDAE	Gen. sp.	7	
Hirudinea	Glossiphoniidae	*Batracobdella* sp.	7	3
Nematoda	**NEMATODA**	**Gen. spp.**	**8**	**1**
Odonata	AESHNIDAE	Gen. spp.	9	
Odonata	Aeshnidae	*Caliaeshna microstigma*	9	3
Odonata	CALOPTERYGIDAE	Gen. sp.	8	
Odonata	Calopterygidae	*Calopteryx splendens*	8	1
Odonata	GOMPHIDAE	Gen. spp.	8	
Odonata	Gomphidae	*Onychogomphus* spp.	8	1

Odonata	Gomphidae	*Ophiogomphus* sp.	8	1
Oligochaeta	OLIGOCHAETA	Gen. sp.	6	
Oligochaeta	**Oligochaeta**	***Eiseniella* sp.**	**6**	**3**
Plathelminthes	TURBELLARIA	Gen. spp.	7	1
Plecoptera	CHLOROPERLIDAE	Gen. spp.	7	
Plecoptera	Chloroperlidae	*Chloroperla* sp.	8	1
Plecoptera	Chloroperlidae	*Siphonoperla* sp.	6	3
Plecoptera	LEUCTRIDAE	Gen. sp.	8	
Plecoptera	Leuctridae	*Leuctra* sp.	8	1
Plecoptera	NEMOURIDAE	Gen. spp.	9	
Plecoptera	Nemouridae	*Amphinemura* sp.	9	3
Plecoptera	Nemouridae	*Protonemura* sp.	9	3
Plecoptera	PERLIDAE	Gen. sp.	8	
Plecoptera	Perlidae	Perla sp.	8	1
Plecoptera	PERLODIDAE	Gen. spp.	9	
Plecoptera	Perlodidae	*Isoperla* sp.	9	3
Plecoptera	Perlodidae	*Perlodes* sp.	9	3
Trichoptera	BRACHYCENTRIDAE	Gen. sp.	10	
Trichoptera	Brachycentridae	*Micrasema* sp.	10	5
Trichoptera	GLOSSOSOMATIDAE	Gen. sp.	8	
Trichoptera	Glossosomatidae	*Glossosoma* sp.	8	1
Trichoptera	HYDROPSYCHIDAE	Gen. spp.	9	
Trichoptera	Hydropsychidae	*Hydropsyche instabilis*-gr.	9	3
Trichoptera	Hydropsychidae	*Hydropsyche* spp.	9	3
Trichoptera	HYDROPTILIDAE	Gen. sp.	8	1
Trichoptera	LEPIDOSTOMATIDAE	Gen. sp.	7	3
Trichoptera	LEPTOCERIDAE	Gen. spp.	10	
Trichoptera	Leptoceridae	*Adicella* sp.	10	5
Trichoptera	Leptoceridae	*Ceraclea* sp.	10	3
Trichoptera	LIMNEPHILIDAE	Gen. sp.	8	1
Trichoptera	PSYCHOMYIIDAE	Gen. sp.	7	
Trichoptera	Psychomyiidae	*Psychomyia* sp.	7	3
Trichoptera	RHYACOPHILIDAE	Gen. sp.	9	
Trichoptera	Rhyacophilidae	*Rhyacophila* sp.	9	3
Trichoptera	SERICOSTOMATIDAE	Gen. spp.	9	
Trichoptera	Sericostomatidae	*Schizopelex* sp.	10	5
Trichoptera	Sericostomatidae	*Sericostoma* sp.	8	1

Table 8: Comparison of the ASPT values via two-way ANOVA. Abbreviations mean GS=Genus/Species, FAM = Family, W = weighted, WA = weighted and abundance-classed, TR = Turkey, OR = Original. Bold values indicate significant values (P < 0.05).

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 x 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 x Index	2	1.11	0.56	10.13	**<0.001**
	Residual	50	2.74	0.05		
EUPHbios * $\mathbf{ASPT_{WA}}$	Quality	2	38.42	19.21	294.68	**<0.001**
	Index	1	0.02	0.02	0.31	0.58
	Quality x Index	2	0.00	0.00	0.01	0.99
	Residual	50	3.26	0.07		
EUPHbios * $\mathbf{ASPT_{FAM}}$	Quality	2	21.30	10.65	206.97	**<0.001**
	Index	1	0.00	0.00	0.00	0.99
	Quality x Index	2	2.59	1.29	25.14	**<0.001**
	Residual	50	2.57	0.05		
EUPHbios * $\mathbf{ASPT_{FAM}}$ without extremes	Quality	2	19.55	9.78	190.13	**<0.001**
	Index	1	0.00	0.00	0.00	0.979
	Quality x 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 x 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 x Index	2	6.77	3.38	48.12	**<0.001**
	Residual	50	3.52	0.07		
EUPHbios * $\mathbf{ASPT_{TR}}$	Quality	2	0.05	0.02	35.29	**<0.001**
	Index	1	0.22	0.22	324.044	**<0.001**
	Quality x Index	2	0.02	0.01	15.43	**<0.001**
	Residual	50	0.03	0.00		
EUPHbios * $\mathbf{ASPT_{OR}}$	Quality	2	14.40	7.20	46.66	**<0.001**
	Index	1	30.30	30.30	196.34	**<0.001**
	Quality x Index	2	6.73	3.36	21.80	**<0.001**
	Residual	50	7.72	0.15		

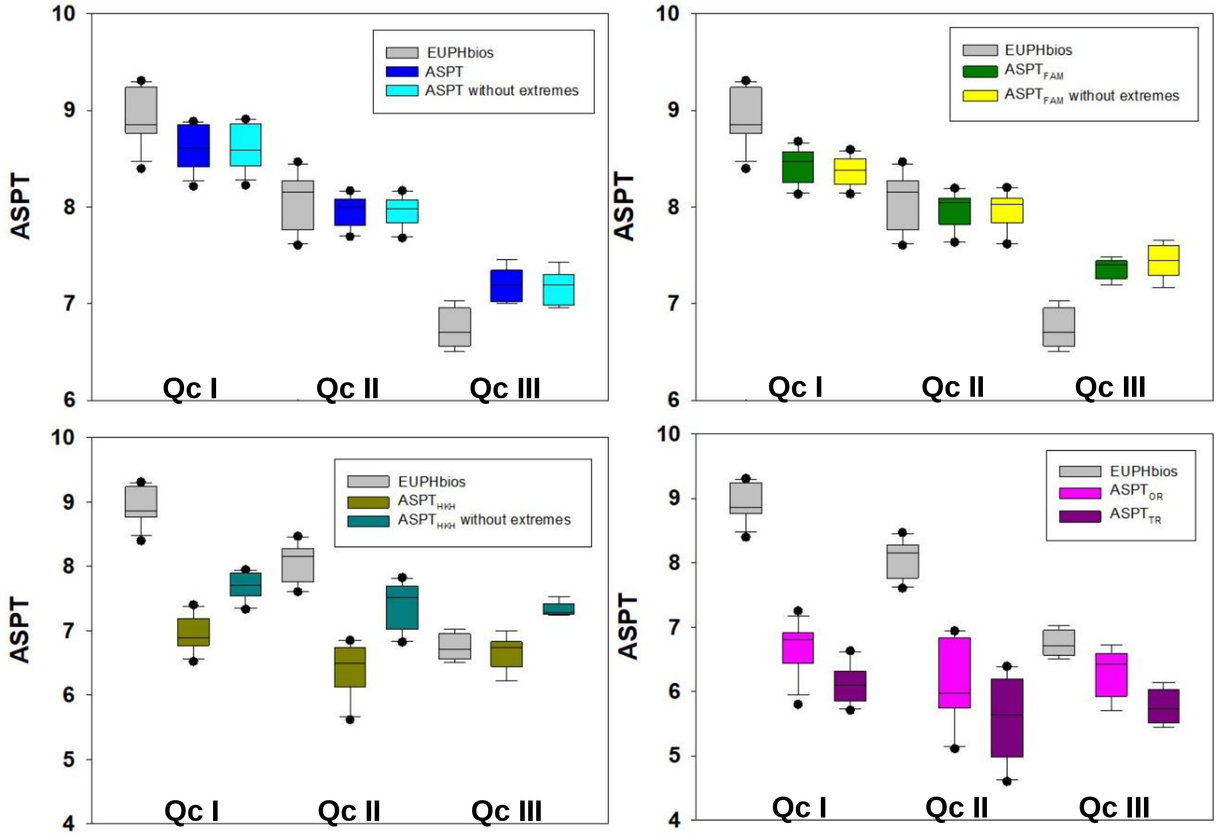

Fig. 8: Box-Whisker plots (median, quartiles, 5th and 95th percentiles, outliers) of the ASPT values in the three different quality classes (I, II, III). ASPT = Average Score per Taxon, EUPHbios = weighted ASPT ($ASPT_W$)**,** ASPT without extremes = ASPT values without extreme taxa**,** $ASPT_{FAM}$ = ASPT values of family level**,** $ASPT_{FAM}$ without extremes = ASPT values of family level 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.

Habitat specialisation as a biotic index

We were able to describe the habitat use of 20 taxa sampled in the streams of Qc I (Table 9). 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 (Table 9). 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 (Appendix 4 in supplementary material), only few taxa seemed to prefer these habitat types specifically or even use them at a moderate level. (Table 9, Appendix 5 in supplementary material).

To analyse the potential effect of habitat degradation on benthic community composition and answer the question of whether habitat specialists might be used as indicator taxa for ecological quality, we compared the proportion of habitat specialists in the different quality classes. Based on the habitat score (score ≥ 4 in one of the habitats, Table 9), the following taxa were considered to be specialists: *Epeorus* sp., *Epeorus caucasicus, Epeorus zaitzevi, Ephemerella* 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, Table 9). The proportion of specialists differed significantly between the three quality classes (ANOVA, F = 3.69, p = 0.039, Fig. 9). The habitat specialists were tendentially more abundant 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, Fig. 9).

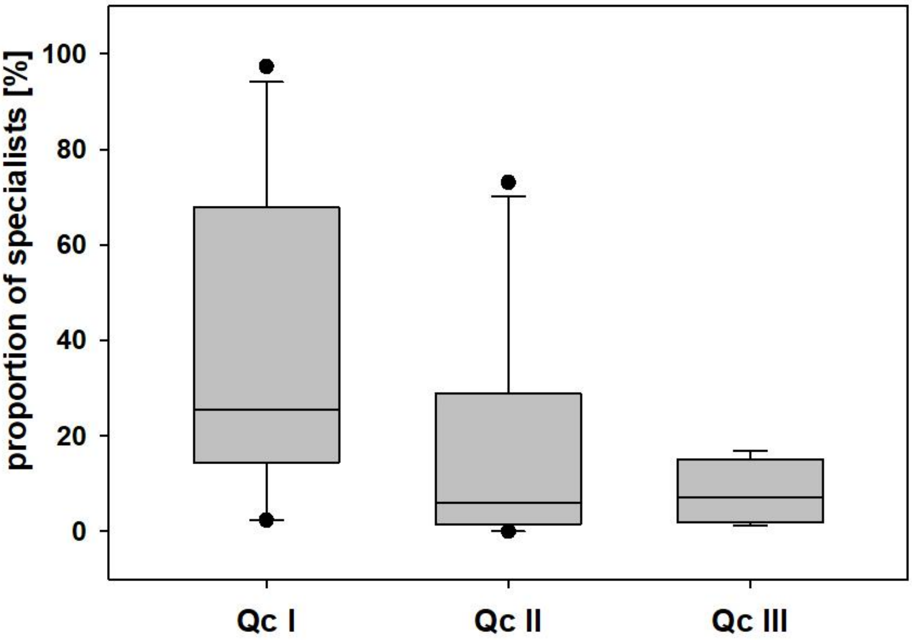

Fig. 9: Box-Whisker plots (median, quartiles, 5th and 95th percentiles, outliers) of the proportion of specialists in the different streams (Quality classes: Qc I; n = 12, Qc II; n = 10, Qc III; n = 6) in the three different quality classes.

Table 9: Habitat use by macroinvertebrate taxa in the studied streams in the Euphrates River Basin based on the percentage of abundances in a specific habitat. 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.

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

Discussion

The aim of this work was to support the development of methods for the assessment of ecological stream quality in Turkey. We have shown that the EUPHbios and the proportion of habitat specialists are promising indices. In our opinion, they can be used as part of a multi-metric index for a Turkish assessment programme. Furthermore, this study is the first adaptation of the HKHbios in the Middle East and clearly confirms the applicability and adaptability of this biotic score.

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 using weighted values, because weighting increased the Qc I scores and decreased the Qc III scores significantly, thereby sharpening the results. There are two advantages of this biotic score 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, much 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 & Shah, 2012; Sharma et al., 2015), and it has already been adapted to Ethiopia (ETHbios, Aschalew & Moog, 2015).

The currently used indicator in Turkey (TR-BMWP) is also calibrated for Turkey, although this calibration is based on expert knowledge alone and includes only the family level. The $ASPT_{TR}$ values resulting from the TR-BMWP are lower than the original ASPT values ($ASPT_{OR}$ without any calibration for Turkey) and do not differentiate between quality classes clearly. The fact that both the original and the adapted BMWP yield significantly lower values 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. After nationwide 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 ecological stream quality in Turkey because it reacted clearly to degradation or pollution in the streams studied here. 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 an important habitat 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%, Appendix 4 in supplementary material).

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. At the moment, we are only able to compare our data with Central European literature. For instance, *Hydropsyche instabilis* is a habitat generalist (Graf et al., 2008), which is supported by our results. On the other hand, *Rhyacophila* sp. was found to be a generalist in our study but is considered a specialist of lithal habitats in Central Europe (Graf et al., 2008). Comparisons of Ephemeroptera taxa show that the *Epeorus* species is also a lithal specialist; at the same time, the *Baetis* species is more a generalist (Buffagni et al., 2009) which is supported by our analyses. Although the *Rhithrogena* species is considered as a lithal specialist (Buffagni et al., 2009), we identified the species in four different lithal habitats (megalithal, macrolithal, mesolithal, microlithal). Because *Rhithrogena* sp. was equally distributed among the four lithal habitats in this study, we assigned it to the habitat generalists. This example shows that, although the available autecological information from Europe is very useful, an ecoregion-specific review is necessary. We are aware that our study alone is not sufficient to determine the habitat requirements of these organisms. However, by obtaining similar information for other streams in Eastern Turkey, the list can be extended, especially if the same calculation method is used.

The anthropogenic impact on the streams seemed more or less comparable and was mainly caused by a considerable input of wastewater, agriculture and livestock farming. The anthropogenic stressors seem to have a less negative effect than expected in this study. Benthic community composition can clearly be divided into two groups. Qc II seemed to tolerate the anthropogenic stress better due to their self-cleaning mechanism than Qc III streams. Indeed, 80% of the land area in the province of Erzurum, where eight (streams no.: 1, 2, 3, 4, 5, 7, 8, 10) of 17 sampling stations were located, is used for livestock farming and agriculture (Environmental report of province Erzurum, 2016). In Erzincan (streams no.: 11, 12, 13, 14, 15, 16) the land is used for similar activities (53%), and the percentage of land used for livestock farming and agriculture close to the Tunceli streams (no.: 17, 18, 19) is 15.53% (Environmental reports of province Erzincan and Tunceli, 2016). Another serious anthropogenic impact in the province of Erzurum is soil pollution caused by industrial and agricultural activities without water treatment plants, resulting in high nitrate values in surface- and groundwater (Environmental report of province Erzurum, 2016).

The environmental conditions of the studied streams were observed only on two occasions and therefore cannot be used to assess the water quality. However, regular recordings are meaningful and required by WFD (2000) as a component for the assessment of water quality. On the other hand, 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. 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 & Rosenberg, 1978; Rygg, 1985). Consequently, a drawback of our analysis of indicator values is that taxa such as *Epallage fatime or Epeorus znojkoi,* 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, this pilot project can be conducted in a similar way in other catchment areas of Turkey. The methods, including the explained calculation methods, could be applied in each ecoregion and can be considered useful for assessing the ecological stream/river quality.

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5 General Discussion

The development of water management in Turkey has strongly accelerated in the last 10 years. It appears that the Turkish government wants to comply with the EU Water Framework Directive (WFD, 2000) regarding topics such as flood protection, wastewater treatment or international water management. Indeed, since 2014, about 70 master's theses focusing on sustainable water management have been written within the framework of cooperation between the General Directorate of Water Management and the universities (Ministry of agriculture and forestry of Turkey; General Directorate of Water Management, 2019). As an indispensable part of water management, assessments of ecological status are necessary. However, the development of such assessment systems requires many years of limnological research. For instance, in Europe, biological methods have been developed for more than 150 years to assess the quality of surface waters (Kolenati, 1848; Hassal, 1850; Cohn, 1853). A milestone in the history of limnology in Europe is the WFD. The WFD (2000) requires advanced assessment systems for biological quality components (fish, benthic invertebrates, macrophytes, benthic algae and phytoplankton). After the enactment of the WFD in 2000, a new assessment system was needed to monitor the ecological quality of biological quality components in Europe. To develop such an assessment system, the first step was to differentiate between the relevant types of surface waters based on their abiotic characteristics and to assess the validity of the individual types regarding their communities. As a next step, stream-type-specific reference conditions had to be defined with regard to biological, hydrological, morphological and chemical/physical components (WFD 2000). Thereafter, the actual state of the biological components had to be determined and compared with the reference streams. In the final step, assessment of ecological status was performed in accordance with Appendix V of the WFD. For this purpose, each of the biological quality components of the surface water is assessed with a specifically developed system for the respective component. The status/potential of the surface water is determined based on the lowest classification it receives among the assessed biological components.

As limnological researchers have only recently started assessing ecological stream quality in Turkey, these steps are currently being taken one by one. The classification of the stream typology of surface waters in Turkey is being carried out by the Turkish authorities in cooperation with scientists (Ministry of agriculture and forestry of Turkey; General Directorate of Water Management, 2019). At the same time, eight scientific pilot projects are planned by the national authorities to develop a biotic index for Turkey, including one in the Lower Euphrates Basin (Erkan, 2014). However, these projects are progressing very slowly due to the administrative expenses and lack of experts, so that so far very few results have been produced. With the implementation of the planned steps above, data can be gathered to enable further progress in the limnological research of Turkey.

To make a contribution to stream protection and to support sustainable water management in Turkey, the main aim of my PhD thesis was to provide basic autecological information about organisms and to support the development of methods for the assessment of ecological stream quality based on benthic invertebrates. The proposed methods contribute to the development of assessment methods in Turkey and can be applied in other ecoregions of the country. Information on the indicator taxa of different ecological quality classes, community indices as well as the habitat preferences of benthic invertebrates in the studied streams provide comparative data for the mountain streams of Turkey, especially for North-East Turkey. However, a comparison is only possible if the streams have a similar surface water type and geographical conditions. The assessment methods of my thesis are based on simple and ecoregion-specific formulas, which can be verified by government agencies as well as by scientists in Turkey. These approaches can be used to develop a country-specific multi-metric index.

All work was conducted at a set of 17 streams in the Upper Euphrates catchment area in Turkey. The selection of these streams was crucial to enable me to analyse the reference conditions as well as a gradient of increasing anthropogenic stress. Second-order streams with catchment areas < 100 km^2 were preselected on the basis of Geographical Information System (GIS) maps. These streams were individually pre-examined in terms of their environmental conditions such as turbidity, algae biomass and odour nuisance in the field and regarding their typology (e.g. width, depth, altitude) compared to each other. Based on own observations as well as interviews with people from the villages in the area, the anthropogenic impacts were defined. Streams without any pollution were selected as reference streams.

Fig. 10: An example of a reference stream (no. 18, Tunceli/Turkey).

In the **first study,** I characterized the reference conditions (e.g. Fig. 10) by showing baseline values for typical indices of the benthic invertebrate community structure, such as the proportion of specific taxonomic groups, species diversity or distribution and general benthic densities. The results are broadly similar to the values published for roughly comparable stream types in Europe; some of the values reflect an even better ecological quality (e.g. EPT abundance; Moog et al., 2004; Meier et al., 2006). The values for Shannon diversity and taxa numbers observed in this study are similar to the reference values in the calcareous foot-hill streams in Germany (Böhmer et al., 2004; 2.8 and 18). In general, the indicator values characterising the benthic community support my assumption that the studied streams have a very natural state and can be used as reference streams for assessment of the ecological quality of mountain streams in Turkey.

To monitor the benthic community for the purpose of an ecological quality assessment, it is common to take samples with the lowest necessary workload to enable the assessment of as many sites as possible. For example, in Germany, the sampling method, the number of the minimum determining organisms and the time of sampling are defined via AQEM/STAR protocol. The sampling time for larger streams and rivers with a catchment size > 100 km^2 is in June or July; for smaller surface waters with a catchment size < 100 km^2 it is in March or April (Haase et al., 2004). The timing of sampling is essential for achieving accurate results. Therefore, the general season-specific pattern of the community structure has to be determined in order to identify the best probable timing for sampling in all streams of the same size and type in the same study region. This includes, for instance, benthic density, the number of taxa, the number of EPTCBO taxa and the Shannon diversity. In addition, logistics and practical aspects such as flood probability, accessibility and working conditions have to be considered. Based on these varying results, the optimal time for benthic invertebrate sampling can vary for each stream or for different regions of river basins.

On the basis of my results, I assumed that early autumn (end of September / early October) might be a suitable sampling time for the tributaries of the Upper Euphrates River. The community composition differed between spring and autumn; community indices such as the number of taxa, the number of EPTCOB taxa and the Shannon diversity seemed to be higher in autumn than in spring. In addition, the benthic density showed the same tendency. The tendency towards lower scores of several indices in spring compared to autumn can be explained by the high flow fluctuations in spring because of snowmelt, whereas the flow during autumn is low due to summer aridity. Therefore, spring seems unsuitable for sampling in the Upper Euphrates River due to stochastic changes of abiotic environmental factors shortly after winter. In contrast, early autumn provides more stable environmental conditions for sampling than spring in the Upper Euphrates Basin.

A further important reason for choosing a sampling time in autumn is the occurrence of more sensitive taxa, which are suitable as indicators of high water quality and habitat quality. My analyses show that some important taxa were present in higher densities in autumn than in spring. The reason for the strong seasonal pattern of these taxa is assumed to be their specific and partially synchronous life cycles (Dobrin and Giberson, 2003; Hellmann et al., 2011; Avlyush et al., 2013). This was the case for the stonefly *Leuctra* sp., which is known to be an indicator of good to very good water quality due to its high demand for oxygen supply and cold temperatures (Thomsen and Friberg 2002; Bottova et al., 2013). Furthermore, some Odonata larvae, such as *Caliaeschna microstigma* and *Ophiogomphus* sp., were also more abundant in autumn. Damselflies and dragonflies are assumed to respond very sensitively to changes in habitat quality and anthropogenic use and are good indicators of a very good ecological status (Clausnitzer et al., 2009). Moreover, abundance of the Heptageniidae *Epeorus* spp., *Rhithrogena* sp. and *Ecdyonurus starmachi* families were significantly higher in autumn than in the spring.

However, different studies have shown varying results regarding the time of sampling. For instance, Duran (2006) found higher abundances of benthic invertebrates in spring and lower abundances in summer in the Behzat stream in Northern Turkey. Callanan et al. (2008) examined the headwaters of Ireland and found that the proportion of EPT taxa of the whole benthic community was also higher in spring than in summer. Similar to my results, Carlson et al. (2013) showed that the mean abundances of several insect taxa in South-Central Sweden were much lower in the spring than in the autumn samples.

An additional aim of the first study was to identify the most-used basal resources in the Upper Euphrates River Basin. The data obtained serve as literature values for comparative purposes. My results show that the primary resources, which might be the most important basis for secondary production in the epi- to metarhithral of natural streams during autumn, were autochthonous primary producers, such as biofilm attached to stone surfaces (epilithon) and fine particulate organic material (FPOM) which covered parts of the stream bottom and organic structures. In the studied streams, leaf litter seemed to be of minor relevance, probably due to a general lack of trees on the riverbanks. Although, for most streams, allochthonous resources such as leaf litter are the main resource (Tank et al., 2010), aquatic primary producers have also been observed to be important in Alpine streams. This clearly shows the importance of epilithon and filamentous algae for consumers at high altitudes between 1768-2159 m (Zäh et al., 2001) and demonstrates a use of FPOM of about 60 % in streams (Füreder et al., 2003). Therefore, comparison of data from North-East Turkey with the Alps seems to be useful, although the selection of stream/river type and section of surface-water is very important.

The prevalence of basal resources has a strong influence on the species composition of the benthic community and on the relative abundance of different functional feeding groups (Cummins and Klug, 1979). The natural dominance structure of functional feeding groups can be useful for evaluating ecological quality, because it can serve as an indicator. In fact, using the functional feeding composition of benthic invertebrates to determine ecological quality has been discussed in Europe since the 1990s. Moog (1992) provided a description of the main autecological information, such as feeding type, habitat preferences, locomotion type and biocoenotic regions, for many taxa in Austria and suggested using this information to develop a community-level index for the purpose of environmental quality assessment. There have been some attempts such as the Rhithron Feeding Index RETI from benthic invertebrate feeding typology (Schweder, 1992) or bio-assessment based on autecological information (Rawer-Jost et al., 2000). Likewise, Ofenböck et al. (2004) tried to use various metrics in Austria, including the composition of feeding types, to determine water quality. In the studied streams of the Euphrates Basin, grazers feeding on biofilm were the dominant feeding group (nearly 50% of benthic abundance in autumn and two-thirds of benthic abundance in spring). This feeding group primarily consists of taxa that are sensitive to changing environmental conditions. In contrast, gatherers and filter feeders (FPOM users), which are known to tolerate more pollution (Barbour et al., 1996), accounted for only 15 % of benthic abundance. Predators made up an average of 8.5 % (± 6.4 %) of the total number of individuals, including mostly taxa of the orders Plecoptera, Coleoptera and Trichoptera, which are generally expected to be particularly sensitive. Therefore, the investigated streams might be very sensitive to pollution and habitat degradation.

Aiming to support the development of ecological stream assessment in the Upper Euphrates **(Study 2)**, I tested two possibilities for evaluating ecological stream quality in Turkey. The first possiblity was the adaptation of the HKHbios (Ofenböck et al., 2010) to the Upper Euphrates Basin, renamed as the Euphrates biotic sore (EUPHbios) for this catchment area. This biological index has proved to be a very suitable assessment method for stream quality, because its application is easy and transparent due to its clearly described calculation method. An important factor in favour of this index is that the taxa list is ecoregion specific. Furthermore, the taxa list can be extended from species level to order level, which offers high precision and flexibility for assessing stream quality. Additionally, weighting of the ASPT values allows for a sharper differentiation between the quality classes.

In fact, the HKHbios has already been successfully applied in several countries such as India (Singh et al., 2017), Nepal (Sharma et al., 2015), Central Himalaya (Shah & Shah, 2012) and Ethiopia (Aschalew & Moog, 2015). This work is the first application in the Middle East and introduces a new possibility for assessing ecological steam quality in this region. Comparative studies would be useful and necessary to verify my results. In the process, it would make sense to involve the surface water bodies of quality classes IV and V in the results. To assess the whole stream quality of a surface water, it has to be compared with different river sections of the same surface water types. Therefore, I recommend that the Turkish authorities test a similar process to the approach proposed here in further pilot projects. The EUPHbios might even be suitable for the Upper Tigris Basin due to its similar geographical and climatic conditions.

Additionally, I recommend using the proportion of habitat specialists, which is identified using the new habitat score, as a biotic index. My study shows that habitat specialists, especially those that prefer lithal and xylal/root habitats, are significantly more abundant in natural streams than in impacted streams (e.g. Fig. 11). Therefore, I can clearly deduce from my results that a decrease in xylal/root and lithal habitats would lead to a reduction of biodiversity due to the loss of several habitat specialists.

Fig. 11: An example of an impacted stream (no. 5, Erzurum/Turkey).

In conclusion, examples from Europe show that projects for developing biotic indices for a specific country require large-scale planning, specialist staff and financial resources. Several projects that have been funded by the EU (e.g. Hering et al., 2003, 2004a, 2006a, 2006b; Stubauer et al., 2010) analysed large gradients and developed a large number of potential metrics for several biological quality components. In comparison to those large projects, the scope of my work is spatially very limited and the range of investigated biological indices focuses only on benthic invertebrates. Nevertheless, the knowledge gained in this study is very useful for the catchment area and for the continuing development of assessment in Turkey. Therefore, I suggest using the new habitat score as well as the EUPHbios as part of a multi-metric index for a water quality assessment programme in Turkey.

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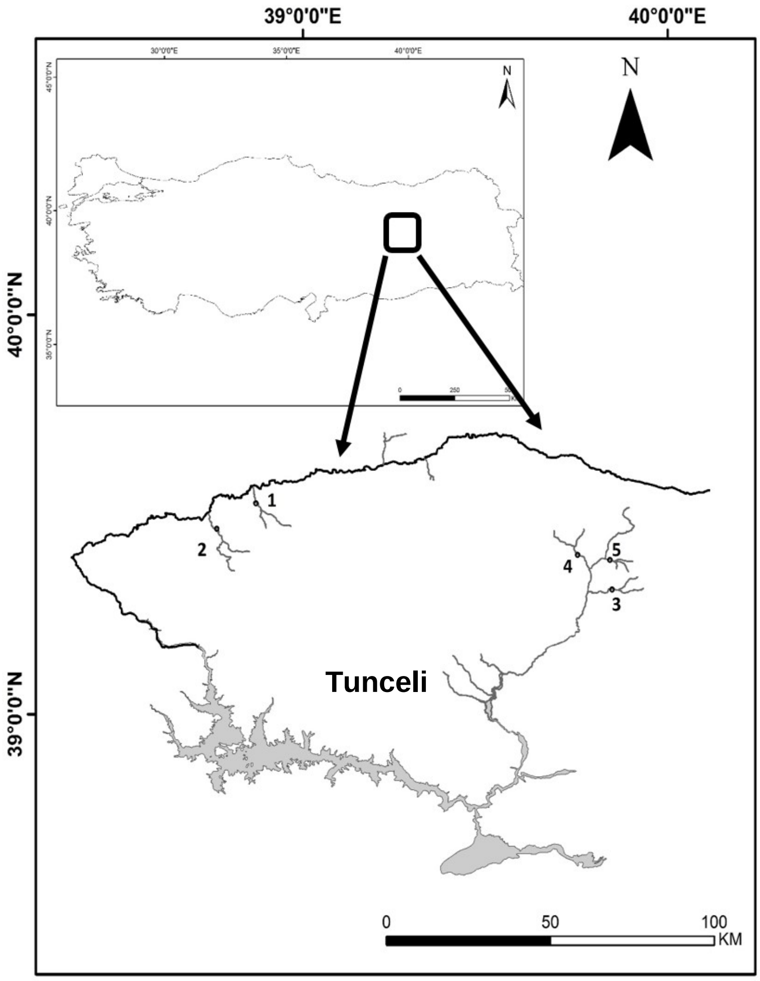
8 Supplementary Material

Appendix 1: Location of the study area in Turkey and the sampling sites (1-5) in the Euphrates basin.



Appendix 2: UTMS-coordinates, and geological and hydro-morphological characterisation of the five stream sites. Physico-chemical conditions and nutrient concentrations [$mg \cdot L^{-1}$] in the studied streams on the sampling days in both seasons (autumn 2013 and spring 2014).

Streams	**1**	**2**	**3**	**4**	**5**
Latitude (N)	39°30’35.98“	39°29’22.37“	39°18’23.52“	39°24’34.15“	39°23’21.88“
Longitude (E)	38°53’13.75“	38°44’36.53“	39°46’59.07“	39°44’41.08“	39°49’45.32“
Altitude [m]	1195	976	1122	1350	1238
Stream order	2	2	2	3	2
Draining into	Euphrates River	Euphrates River	Pülümür River	Pülümür River	Pülümür River
Mean width [m]	4.37	3.53	4.53	9.83	5.2
Catchment Area [km^2]	107.8	206.4	53.3	112.5	85.5
Discharge Q [$m_2 * s^{-1}$]	1.96	1.9	0.7	5.68	1.21
Catchment geology	Calcareous	Calcareous	Calcareous	Calcareous	Calcareous
Autumn					
Temperature [°C]	13.6	12.1	11.1	13.0	18.6
O_2-content [$mg \cdot L^{-1}$]	10.10	10.52	10.27	9.40	8.28
O_2-saturation [%]	97	98	93	90	89
pH	9.10	8.78	8.39	8.57	8.48
Conductivity [µS/cm]	503	400	419	700	1044
NO_3-N	2.56	1.38	0.32	0.35	0.48
PO_4	<0.01	<0.01	<0.01	<0.01	<0.01
Spring					
Temperature [°C]	13.6	12.1	18.1	11.4	19.8
O_2-content [$mg \cdot L^{-1}$]	8.33	8.08	12.70	11.20	11.80
O_2-saturation [%]	90	84	157	122	146
pH	8.36	8.30	8.15	8.22	8.01
Redox potential [mV]	-81	-70	-68	-69	-60
NO_3-N	0.86	0.43	0.29	1.01	0.47
PO_4	<0.003	<0.003	<0.003	<0.003	<0.003

Appendix 3: Physico -chemical conditions and nutrient concentrations [mg·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-content [mg·L⁻¹]	O_2-saturation [%]	pH	Conductivity [µS/cm]	NO_2-N	NO_3-N	NH_4-N	PO_4
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

	Quality classes of LAWA						
Nutriens [mg·L^{-1}]	**I**	**I-II**	**II**	**II-III**	**III**	**III-IV**	**V**
PO_4	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 4: Number of samples in the specific habitats of natural streams (Qc I).au = autumn and sp = spring.

Streams	Megalithal	Macrolithal	Mesolithal	Microlithal	Akal	Psammal	Algae	Macrophytes	Roots	Xylal	CPOM	FPOM
12au	5	5	3	3	1				2			1
14au	4	6	6	1	1						1	1
15au	3	4	3	3	1		1	3	2			
17au	3	3	7				2			1		4
18au	4	6	3	2	1	1	1			1	1	
19au	5	5	3	1		2			2		1	1
12sp	7	6	3	2					2			
14sp	4	7	7	1								1
15sp	3	4	3	1			1	4	4			
17sp	4	6	6							2		2
18sp	5	5	3	1		3			3			
19sp	6	5	3			3			1		1	1
Median	**4.42**	**5.17**	**4.17**	**1.67**	**1.00**	**2.25**	**1.25**	**3.50**	**2.29**	**1.33**	**1.00**	**1.57**

Appendix 5: The relative abundance of each taxon [%] in a habitat. n shows the number of sampling events in autumn and spring together when the taxon was found.

		Megalithal	**Macrolithal**	**Mesolithal**	**Microlithal**	**Akal**	**Psammal**	**Algae**	**Macrophytes**	**Roots**	**Xylal**	**CPOM**	**FPOM**	**n**
Coleoptera	*Elmis* sp.	8.7	25.4	10.1	17.0	2.4	0.5	8.1	7.6	0.9	12.4	4.0	2.7	**4**
	Hydraena sp.	18.7	0.0	13.7	0.0	0.0	0.0	10.8	1.1	24.6	30.0	0.0	0.9	**5**
Diptera	*Atherix ibis*	11.6	12.0	5.6	8.1	2.8	0.0	0.4	0.0	29.2	17.8	11.5	0.9	**5**
	Chironomidae	9.7	8.1	9.7	10.4	1.6	0.3	7.3	0.6	19.9	6.7	16.4	9.3	**7**
	Psychoda sp.	5.1	5.5	2.7	19.4	5.1	0.0	23.3	9.2	22.2	7.5	0.0	0.0	**4**
	Limoniidae	10.1	3.6	3.7	9.8	42.5	5.6	7.0	4.2	0.0	0.0	4.2	9.3	**3**
	Simulium spp.	16.6	10.3	8.1	10.6	0.0	1.6	12.5	7.1	11.3	17.3	1.6	3.1	**6**
	Stratiomys sp.	1.3	1.4	1.3	1.2	1.6	1.3	0.0	-	34.8	43.1	9.6	4.3	**4**
Ephemeroptera	*Baetis* spp.	12.9	13.1	12.2	17.6	1.4	0.1	6.5	0.0	27.2	6.6	0.4	2.0	**6**
	Epeorus spp.	21.3	24.2	51.7	2.8	-	0.0	0.0	0.0	0.0	-	0.0	0.0	**3**
	Epeorus caucasicus	31.1	47.6	15.6	5.7	-	0.0		-	0.0	-	0.0	0.0	**4**
	Epeorus zaitzevi	35.3	21.9	11.5	28.6	-	0.0	0.7	0.0	1.9	0.0	0.0	0.0	**4**
	Ephemerella sp.	3.7	35.6	1.5	2.2	-	0.4	13.5	0.0	43.0	-	0.0	0.0	**3**
	Rhithrogena sp.	28.5	16.6	1.2	29.8	23.8	0.0	0.0		0.0	0.0	0.0	0.0	**3**
Plecoptera	*Leuctra* spp.	4.4	9.2	7.7	16.9	1.3	0.0	11.4	0.4	23.9	20.7	1.1	3.1	**6**
	Perla spp.	35.2	15.2	17.9	19.3	-	0.0	8.6	0.0	0.0	2.6	0.0	1.3	**4**
	Protonemura sp.	5.5	17.3	6.2	2.3	0.0	0.0	16.6	3.6	31.1	15.8	0.0	1.5	**8**
Trichoptera	*Hydropsyche instabilis*-group	14.3	24.6	13.1	17.1	0.0	4.3	5.3	1.0	14.5	3.2	0.9	1.5	**11**
	Hydropsyche spp.	17.4	17.3	24.1	8.2	0.3	8.1	0.5	1.3	14.9	5.8	0.4	1.6	**7**
	Rhyacophila s.str.	10.3	16.5	21.0	11.2	0.0	6.6	3.1	1.0	3.1	23.2	0.0	4.1	**6**