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Development of a benthic macroinvertebrate-based multimetric index to quantify riverbed substrate condition in Swedish streams

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ABSTRACT

Human activities have degraded riverbed substrate structure via reduced heterogeneity of substrate particle sizes and increased fine sediment loadings. Despite increasing recognition of the importance of substrate quality in river ecosystems, our ability to assess substrate condition effects on biodiversity and functioning of river ecosystems is at present inadequate resulting in discrepancies between the needs of conservation, restoration, and mitigation of running waters, and water management practice. Several macroinvertebrate-based metrics are currently used to assess the impacts of degraded riverbed substrate structure. However, few metrics confer a direct relationship to the functions that substrates provide independent of interacting and potentially confounding factors (e.g. current velocity, excess nutrients and pesticides) or, based solely on presence-absence of taxa so that they may be implemented regardless of the identification method. Data on riverbed substrates and benthic macroinvertebrates was extracted from several databases. Using stream data on riverbed substrates and macroinvertebrates, we developed a macroinvertebrate-based multimetric index (MMI) for riverbed substrate condition (LISSA) to assess impacts of and recovery from substrate degradation using information on measures of traits. The dataset was explored for correlation between measures of traits to riverbed substrate condition. A substrate quality gradient (SQG) was constructed by combining four components of substrate quality in an index where decreasing substrate quality is defined as an increasing percentage of fine sediment and sand and decreasing substrate diversity and evenness. Significantly correlated candidate metrics were chosen using forward stepwise linear regression models against SQG. Five metrics were included in LISSA: one trait state of aquatic stages (egg), two trait states of reproduction (isolated eggs, cemented + clutches, cemented or fixed and clutches, free + asexual reproduction), one trait state of locomotion and substrate relation (crawler + temporarily attached) and the locomotion trait state (% burrowing/boring). LISSA is a promising metric for stream macroinvertebrate assessments of riverbed substrate condition and monitoring impacts and recovery across Sweden and potentially elsewhere.

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1. Introduction

Riverbed substrate structure is connected to biodiversity and river ecosystem functioning. A wide range of diversity and evenness of substrate sizes occur naturally, and the erosion and deposition of fine sediment are components of natural processes of river systems. However, widespread degradation of substrate condition has resulted from human activities in terms of reduced heterogeneity of substrate particle sizes and increased fine sediment loadings. Human activities that result in riverbed substrate degradation include agriculture (Richards et al. 1996; Benoy et al. 2012; Burdon et al. 2013), forestry activities (Törnlund and Östlund 2006; Johansson et al. 2013) and damming and flow regulation (Wood and Armitage 1999; Crosa et al. 2010). Despite increasing recognition of the importance of substrate quality in river ecosystems, recognition of the ecological consequences of substrate degradation have been late, ignored or in many cases even neglected, compared to other pressures of habitat quality such as organic pollution, nutrients and acidification (e.g. Friberg et al. 2005). Consequently, our ability to assess substrate condition effects on biodiversity and functioning of river ecosystems is inadequate, resulting in discrepancies between the needs of conservation, restoration, and mitigation of running waters, and water management practices. An inability to assess substrate condition may lead to management decisions resulting in continued impairment of the river ecosystem (European Environmental Agency 2012), and conflicts with implementation of the goals of the Water Framework Directive (WFD) and other relevant legislation.

Because of their ubiquity, high diversity and range of sensitivities, benthic macroinvertebrates are used extensively to assess ecological status in freshwaters (Johnson et al. 1993). Benthic macroinvertebrate distribution and colonization are influenced by substratum characteristics, such as particle size (e.g. Pennak and Van Gerpen 1947; Wood and Armitage 1997; Miyake and Nakano 2002), stability (Stanford and Ward 1983), texture (Harman 1972; Lamberti and Resh 1979; Erman and Erman 1984) and heterogeneity (Tolkamp 1980). The majority of benthic macroinvertebrate taxa have specific substrate requirements (Culp et al. 1983; Peckarsky 1991; Williams and Smith 1996; Sarriquet et al. 2007). Detrimental impacts on aquatic invertebrate communities from excessive fine sediments are well documented (e.g. Ellis 1936; Jones et al. 2012). Furthermore, studies have shown that homogeneous substrates harbor lower macroinvertebrate densities and richness than diverse substrates (e.g. Williams 1980; Beisel et al. 2000; Brown 2003; Beauger et al. 2006; Poff et al. 2006; Palmer et al. 2010). Accordingly, benthic macroinvertebrates should be ideal indicators for quantifying the importance of riverbed substrate condition on ecological status.

Most existing macroinvertebrate-based indices in relation to substrate condition have been developed for a single element (e.g. deposition of fine sediment), thus the relationships captured neglect the influence of other potentially important components (e.g. substrate size diversity). Furthermore, most indices have been developed for a specific catchment type (e.g. alpine or lowland) and/or land use (e.g. agricultural or forested). Pressures connected with certain land use could confound relationships between substrate condition and benthic macroinvertebrate taxa, particularly when multiple stressor scenarios exist. For example, substrate degradation in catchments dominated by agricultural land use are often coupled with organic pollution or pesticides (Turley et al. 2016). Additionally, indices have often included metrics where correlations with substrate condition are highly dependent on interactions with other factors, for example; flow velocity, or food resource availability. The inclusion of such metrics may confound direct relationships to substrate condition and potentially explain equivocal responses found in the literature. For example, traits such as functional feeding groups and current velocity preference are often included in indices quantifying substrate condition (Doretto et al. 2018) However, habitats with high substrate size diversity and evenness occur with low or high current velocity, and responses of functional feeding groups depend on not only substrate condition for the availability of food resources but also light (Hill et al. 1995), nutrients (Pocock 2018), allochthonous inputs of leaf litter (Petersen and Cummins 1974), etc.

While many indices have been developed using metrics based solely on taxonomic composition or with a combination of taxonomic composition and traits, the use of multiple biological traits has several advantages over biotic indices based on components of taxonomic composition. Traits have a greater potential for large-scale applicability because functional community descriptions can be compared among regions that differ in their taxonomic composition (Statzner et al. 2001; Horrigan and Baird 2008). The occurrence of trait combinations under a particular environmental condition should reflect the selection pressure (Townsend and Hildrew 1994). In turn, a trait approach would prospectively allow mechanistic predictions to be made regarding the prevalence of certain trait combinations along specific gradients of substrate condition. Indeed, an index based on multiple traits with mechanistic response to a broader than typical characterization of substrate condition (by including elements of substrate size diversity and evenness, and the percentage of fine substrates and sand across sites with various catchment types and land use) and low potential to be confounded by pressures or other factors would help in applied applications such as regional biomonitoring programs (Ofogh et al. 2024).

Lastly, most biotic indices have been developed to assess the ecological quality based on morphological identification of indicator organisms (Birk et al. 2012) using both taxa occurrence and abundance data (Hering et al. 2010). However, eDNA-based approaches are being increasingly used, in part because they are often cost-effective compared to traditional morphological identification, although use of eDNA is often limited in terms of inferring quantitative abundance from metabarcoding data, which is a key component of the most commonly used indices implemented in legal frameworks (e.g. Water Framework Directive). Considering the expected increase in eDNA-based identification methods the development of new biotic indices based solely on presence-absence of taxa that can be implemented regardless of the identification method would be advantageous.

The aim of this study is the development of a benthic macroinvertebrate trait-based multimetric index (MMI) to assess riverbed substratum condition in Swedish streams. In contrast to most earlier work we calibrate an MMI based solely on taxa presence/absence across catchments with a range of catchment types and land use. We hypothesize that reproduction, aquatic life stages, mode of locomotion and substrate preference are traits that will respond strongly to substrate condition regardless of level and type of other pressures. We expect this study to result in a new tool that differentiates biological impact of substrate condition from other stressor impacts.

2. Methods

2.1. Description of data and sites

Benthic macroinvertebrate assemblages and corresponding substrate data were extracted from the datasets of three previous projects resulting in 730 sites. The bulk of data, 583 sites (80%), were extracted from the Swedish national lake and stream survey in 2000 (Wilander et al. 1998; Johnson and Goedkoop 2000). The remaining data included 100 sites extracted from an in-house pilot project survey conducted in 2020 and 2021, and 47 sites extracted from Swedish national monitoring data (https://miljodata.slu.se/mvm/).

The method for sampling benthic macroinvertebrates was generally the same for all 730 sites regardless of the source. Sample collection was in autumn and samples were collected using standardized kick-sampling (European Committee for Standardization 1994) with a hand net (0.5 mm mesh size). A composite sample consisting of five (Swedish national lake and stream survey in 2000 and Swedish national monitoring data) or three (pilot project) kick-samples (each 60 s · 1 m) was taken from each site (one site per stream) and pooled. Benthic samples were usually collected using standardized kick nets (width 25 cm). However, in the pilot project for very small streams (widths <25 cm) we used a modified kick net (width of 15 cm). The size of the sampling area (upstream/downstream length) was the same (i.e. 10 m) whereas the sampling sites covered half of the stream width (stream edges were not sampled).

For the Swedish national lake and stream survey in 2000 and Swedish monitoring data (630 sites) macroinvertebrate samples were sorted at the Department of Environmental Assessment of the Swedish University of Agriculture according to quality control and assurance protocol. For the Swedish national stream survey in 2000 taxonomic identification was carried out as far as possible but at least to a predetermined list of some 500 'operable' taxonomic units (Wilander et al. 1998) For the Swedish monitoring data taxonomic identification was done to the lowest taxonomic unit possible, usually to species or species groups, except for oligochaetes and chironomids. The remaining 100 site samples from the pilot project were analyzed by eDNA according to Buchner et al. (2021). In brief, bulk samples were homogenized without sorting in a common kitchen blender at 25000 rpm for 3 min. Prior to homogenization the samples were cooled to −20 °C. The blender was cleaned with ddH2O and then filled with either 100 ml of ddH2O or a decontamination solution (DIY-DS, 0.6% bleach, 1% NaOH, 1% Alconox, 90 mM sodium bicarbonate). Samples were stored at −20 °C until DNA extraction. DNA extraction was done on 55 µl of sample, all samples were centrifuged at 14000 x *g* for 5 min to pellet the tissue and the pellet was broken up by 30 s of bead-beating in a FastPrep Bead Beater (MP Biomedicals, Eschwege, Germany). Subsequent processing steps were done on a Biomek FX liquid handling workstation (Beckman Coulter, Brea, CA, USA). Duplicate extractions and two-step PCR with unique-twin indexing was done for each sample. The lab achieved >200 reads per sample using MiSeq or HiSeq (depending on the primers we shall use fwhF2 + fwhR2n or BF3 + BR2. Data analysis using JAMP/BOLDigger/TaxonTableTools. Only species occurring in both replicate samples and having at least 0.01% abundance were used in further analyses.

For all 730 sites the presence/absence of identified taxa were harmonized to the taxa lists from ASTERICS software version 8.0 (Schmidt-Kloiber and Hering 2015) (https://www.gewaesser-bewertung-berechnung.de) and Tachet et al. (2010) (Appendix B).

For the 583 sites from Swedish national lake and stream survey in 2000 and the 100 pilot project sites substrate was assessed according to the percent of the total cover of six substratum classes (ranging from fine to boulder) at the same time and in the same area where the benthic macroinvertebrates were sampled. For the 47 Swedish national monitoring data information on the percent of the total cover of six substratum classes was extracted and matched to each site from a separate survey of the stream habitat (https://www.biotopkartering.se/).

In all analyses, we used taxon presence/absence and the percent of substratum classes as the subsequent substrate quality indicators: percent fine sediment, percent sand, substrate diversity (Shannon diversity of all substrate size categories) and substrate evenness (Shannon evenness of all substrate size categories) calculated for each site. Furthermore, other environmental variables including catchment characteristics (size and land use), mean depth, mean width and altitude were compiled to characterize the range of study sites (Table 1).

Table 1. Mean and range of environmental descriptors for the 730 stream study sites.

Environmental descriptor	Mean	Max	Min
% Catchment forest	66.3	99.8	0
% Catchment forested wetland	1.9	26.3	0
% Catchment open wetland	7.5	67.4	0
% Catchment artificial	1.1	26.3	0
% Catchment arable	6.6	91.2	0
% Catchment water	4.7	28.9	0
% Catchment other open land	4.5	31.2	0
% Catchment alpine	5.6	100	0
Catchment size (km2)	126.6	4170	0.3
Altitude m a.s.l.	194.1	805	1
Mean depth (m)	0.47	2.5	0.1
Mean width (m)	5.6	50	0.5
% Fine sediment (<0.063 mm)	14.5	100	0
% Sand (0.063–2 mm)	10.9	75	0
% Gravel (2–63 mm)	11.8	75	0
% Cobble (63–200 mm)	32.1	83.3	0
% Stone (200–4000 mm)	24	84	0
% Boulder (>4000 mm)	6.6	69.8	0
Substrate diversity (Shannon)	1.19	1.75	0
Substrate evenness (Shannon)	0.78	1	0

2.2. Constructing the gradient of substrate quality

A gradient of substrate quality was constructed by combining four components of substrate quality in an index where decreasing substrate quality is defined as an increasing percentage of fine sediment and sand and decreasing substrate diversity and evenness. First, we normalized values of each index component between 0 and 1 based on the lowest and highest values in the dataset (Hering et al. 2006). For the percentage of fine sediment and the percentage of sand we used:

$$Value = \frac{\text{Site value} - \text{Lowest value in dataset}}{\text{Highest value in dataset} - \text{Lowest value in dataset}}$$

and for substrate diversity and substrate evenness we used:

$$Value = 1 - \frac{\text{Site value} - \text{Lowest value in dataset}}{\text{Highest value in dataset} - \text{Lowest value in dataset}}$$

The substrate quality index values for each site were than calculated as the mean of the 0 to 1 scores of each of the four components. Increasing values in the constructed gradient represents decreasing substrate quality and here forth referred to as the Substrate Quality Gradient (SQG).

2.3. Defining the calibration and validation datasets

The validation of a multimetric index requires two independent datasets with comparable gradients of the dependent variable, in this case SQG. One dataset is used to calibrate the index while the second dataset is used to validate that the index is an effective method for evaluating stream substrate conditions, and thus is appropriate for use in future studies measuring the long-term status of streams, and the effectiveness of restoration methods. With a working MMI we expect there to be no significant difference between the calibration and validation datasets in their R-square values and slopes.

In order to create calibration and validation datasets we first sorted the 730 sites into eight 0.1 categories based their SQG values and then made a validation dataset by a random selection of 20% of sites within each SQG category. This resulted in 583 sites for the calibration dataset and 147 sites for the validation dataset each representing a similar SQG gradient and geographic distribution (Figure 1).

2.4. Analyses

Step 1: Calculation and selection of candidate traits

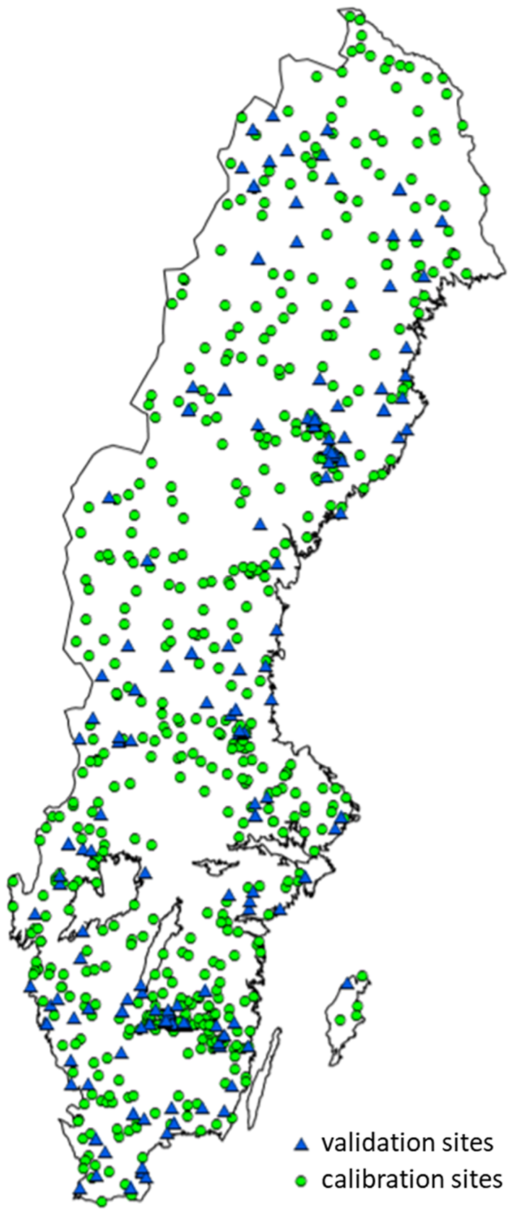

Figure 1. Map of calibration and validation sites across Sweden.

Using the full dataset (730 sites) a large number of traits were calculated from ASTERICS software version 8.0 (Schmidt-Kloiber and Hering 2015) (https://www.gewaesser-bewertung-berechnung.de) (Appendix B) and Tachet et al. (2010) (Appendix A). We then extracted 24 metric values for each site from six selected traits based on expectations of a strong response to substrate condition and a high potential to be robust against potentially interacting factors or the level and type of other confounding pressures. Seven additional component metrics were further calculated as the sum of trait states (e.g. crawler + temporarily attached) within traits (e.g. locomotion and substrate relation) that represent different aspects of a predicted response to the SQG gradient resulting in a total of 31 metrics (Table 2). Using the calibration dataset, each metric was tested for their correlation to the SQG gradient and retained if they correlated significantly (Spearman $\rho < 0.05$) and if the direction of response was as anticipated.

Step 2: Selecting metric combinations

Multiple stepwise regressions, with forward selection, were run using SQG as the dependent and the candidate metrics as the independent variables. Based on Akaike information criterion (AIC), only candidate traits that contributed significant additional information in the regression model were chosen for possible inclusion in the final multimetric index. Furthermore, the possible inclusion of a metric was based on that the metric should not be redundant (e.g. Microhabitat preference - % Type Pel [mud] *and* Substrate preference - mud).

Step 3: Scaling of metrics prior to creating MMI's

Before candidate metrics were combined and used for an MMI, each metric value was normalized between 0 and 1 based on the lowest and highest values in the dataset (Hering et al. 2006). For metrics decreasing with increasing SQG we used:

$$Metric\ value = \frac{\text{Metric result} - \text{Lowest metric result in dataset}}{\text{Highest metric result in dataset} - \text{Lowest metric result in dataset}}$$

and for metrics increasing with increasing SQG we used:

$$Metric\,value = 1 - \frac{\text{Metric result} - \text{Lowest metric result in dataset}}{\text{Highest metric result in dataset} - \text{Lowest metric result in dataset}}$$

MMI's were calculated as the mean of the 0 to 1 scores of all core metrics of each MMI.

Step 4: Performance evaluation of MMI's

Least squares regression of each MMI was carried out separately using SQG as the dependent variable and evaluated by its R-square value.

Step 5: Validation of the MMI

Validation was achieved by comparing the R-square values and slopes between the calibration and validation data sets. With a working MMI we expect there to be no significant difference between the calibration and validation datasets in their R-square values and slopes.

All statistical analyses were done in JMP 14.0.0 (SAS Institute Inc. JMP 2018).

3. Results

3.1. Gradient of substrate quality

Values within each of the four components of the SQG index indicated a range of substrate quality among the 730 sites (Table 3).

Table 2. List of the 31 selected metrics (trait/trait states) extracted from values calculated from ASTERICS and Tachet and the expected response to decreasing substrate condition.

Source	Trait	Trait state	Expected response
ASTERICS	Microhabitat preference	[%] Type Pel [mud (grain size <0.063 mm)]	Positive
		[%] Type Psa [sand (grain size 0.063–2 mm)]	Positive
		[%] Type Lit [coarse gravel, stones, cobbles, boulders, bedrock (grain size >2 cm)]	Negative
		[%] Type Pom [coarse and fine particulate organic matter]	Positive
		[%] Type Pel + Type Psa	Positive
		[%] Type Pel + Type Pom	Positive
		[%] Type Psa + Type Pom	Positive
		[%] Type Pel + Type Psa + Type Pom	Positive
	Locomotion type	[%] Burrowing/boring	Positive
		[%] (Semi)sessil	Negative
Tachet	Aquatic stages	Egg	Negative
	Reproduction	Isolated eggs, cemented	Negative
		Clutches, cemented or fixed	Negative
		Clutches, free	Positive
		Asexual reproduction	Positive
		Isolated eggs, cemented + clutches, cemented or fixed	Negative
		Clutches, free + asexual reproduction	Positive
	Locomotion and substrate relation	Crawler	Negative
		Burrower	Positive
		Temporarily attached	Negative
		Crawler + temporarily attached	Negative
	Substrate preference	Flags/boulders/cobbles/pebbles	Negative
		Gravel	Negative
		Silt	Positive
		Microphytes	Positive
		Mud	Positive
		Flags/boulders/cobbles/pebbles + gravel	Negative
		Silt + microphytes	Positive
		Silt + mud	Positive
		Microphytes + mud	Positive
		Silt + microphytes + mud	Positive

Explanation of trait states can be found at https://www.freshwaterecology.info/.

Table 3. Normalization of index values for the four components included SQG index to values between 0 and 1.

Component of substrate quality	Highest value	Lowest value	Component index value
% fine sediment	100	0	$Value = \frac{\text{Componant result} - 0}{100 - 0}$
% sand	75	0	$Value = \frac{\text{Componant result} - 0}{75 - 0}$
Substrate size diversity	1.75	0	$Value = 1 - \left(\frac{\text{Componant result} - 0}{1.75 - 0}\right)$
Substrate size evenness	1	0	$Value = 1 - \left(\frac{\text{Componant result} - 0}{1 - 0}\right)$

The distribution of values in the SQG index ranged from a minimum of 0.08 to a maximum of 0.75. There were fewer sites with low quality substrates compared to sites with high quality substrates (median SQG = 0.179). Environmental descriptors were similar between calibration and validation datasets (Table 4).

Table 4. Mean and range of environmental descriptors for the calibration and validation datasets.

Environmental descriptor		Calibration ($n = 583$)			Validation ($n = 147$)		
		Mean	Max	Min	Mean	Max	Min
Catchment	% Catchment forest	66.3	99.8	0	66.3	99.6	0
	% Catchment forested wetland	1.9	26.3	0	2.2	14.2	0
	% Catchment open wetland	7.7	67.4	0	6.5	55.4	0
	% Catchment artificial	1	26.3	0	1.1	14.8	0
	% Catchment arable	6.4	91.2	0	7.7	86	0
	% Catchment water	4.6	28.9	0	5.1	26.4	0
	% Catchment other open land	4.3	31.2	0	5.2	28.6	0
	% Catchment alpine	5.8	100	0	5	94.4	0
	Catchment size (km^2)	130	4170	0.9	112.9	3540	0.3
Spatial	Altitude m a.s.l.	198.6	796	1	176.5	805	1
Local	Mean depth (m)	0.5	2	0.1	0.5	2.5	0.1
	Mean width (m)	5.7	50	0.5	5.2	35	0.5
	% fine sediment (<0.063 mm)	14.7	100	0	13.7	100	0
	% sand (0.063–2 mm)	10.8	65.9	0	11.7	75	0
	% gravel (2–63 mm)	11.6	75	0	12.5	69.8	0
	% cobble (63–200 mm)	32.2	83.3	0	31.9	75	0
	% stone (200–4000 mm)	24.1	84	0	23.5	79	0
	% boulder (>4000 mm)	6.6	37.5	0	6.6	69.8	0
	Substrate diversity (Shannon)	1.19	1.75	0	1.2	1.73	0
	Substrate evenness (Shannon)	0.78	1	0	0.78	1	0

Table 5. The 31 candidate metrics representing six trait groups and the direction of response (Spearman ρ), the response expected, and the *p*-value.

TRAIT	TRAIT STATE	Spearman ρ	Expected response	*p*-value
Microhabitat preference	[%] Type Pel [mud (grain size <0.063 mm)]	0.0832	Positive	0.0446
	[%] Type Psa [sand (grain size 0.063–2 mm)]	0.2083	Positive	<.0001
	[%] Type Lit [coarse gravel, stones, cobbles, boulders, bedrock (grain size >2 cm)]	−0.2351	Negative	<.0001
	[%] Type Pom [coarse and fine particulate organic matter]	0.1115	Positive	0.007
	[%] Type Pel + Type Psa	0.1723	Positive	<.0001
	[%] Type Pel + Type Pom	0.1396	Positive	0.0007
	[%] Type Psa + Type Pom	0.2188	Positive	<.0001
	[%] Type Pel + Type Psa + Type Pom	0.2016	Positive	<.0001
Locomotion type	**[%] burrowing/boring**	**0.1677**	**Positive**	**<.0001**
	[%] (semi)sessil	−0.0863	Negative	0.0372
Aquatic stages	**Egg**	**−0.3377**	**Negative**	**<.0001**
Reproduction	Isolated eggs, cemented	−0.2132	Negative	<.0001
	Clutches, cemented or fixed	−0.1585	Negative	0.0001
	Clutches, free	0.2737	Positive	<.0001
	Asexual reproduction	0.1984	Positive	<.0001
	Isolated eggs, cemented + clutches, cemented or fixed	**−0.2918**	**Negative**	**<.0001**
	Clutches, free + asexual reproduction	**0.3306**	**Positive**	**<.0001**
Locomotion and substrate relation	Crawler	−0.2881	Negative	<.0001
	Burrower	0.1813	Positive	<.0001
	Temporarily attached	−0.1781	Negative	<.0001
	Crawler + temporarily attached	**−0.3505**	**Negative**	**<.0001**
Substrate preference	Flags/boulders/cobbles/pebbles	−0.3415	Negative	<.0001
	Gravel	−0.1402	Negative	0.0007
	Silt	0.296	Positive	<.0001
	Microphytes	0.1838	Positive	<.0001
	Mud	0.308	Positive	<.0001
	Flags/boulders/cobbles/pebbles + gravel	−0.3187	Negative	<.0001
	Silt + microphytes	0.305	Positive	<.0001
	Silt + mud	0.326	Positive	<.0001
	Microphytes + mud	0.2989	Positive	<.0001
	Silt + microphytes + mud	0.3204	Positive	<.0001

Values in bold text show the five core metrics (traits) used in the LISSA. TRAIT STATE refers to a subcomponent of TRAIT. Explanation of trait states can be found at https://www.freshwaterecology.info/.

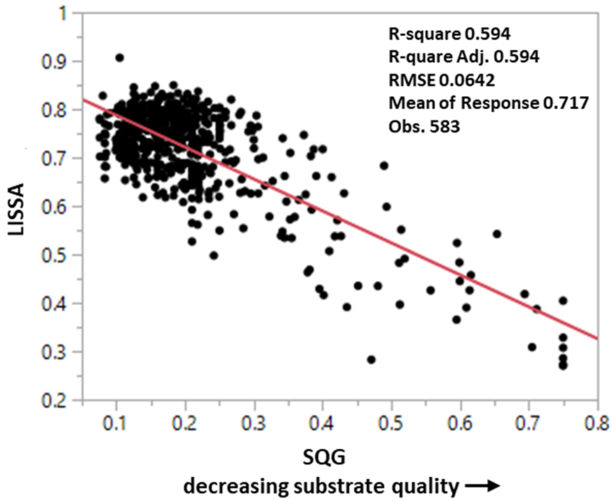

Figure 2. Relationship of LISSA to decreasing substrate quality (SQG) in the calibration dataset.

Table 6. Normalization of index values for the five simple metrics included in the multimetric index to values between 0 and 1.

Source	Trait state/metric	Highest value	Lowest value	Trait/metric index value
Tachet	Aquatic stages – egg	0.454	0.152	$Value = \frac{\text{Metric result} - 0.152}{0.454 - 0.152}$
Tachet	Reproduction – isolated eggs, cemented + clutches, cemented or fixed	0.923	0.12	$Value = \frac{\text{Metric result} - 0.12}{0.923 - 0.12}$
Tachet	Reproduction – clutches, free + asexual reproduction	0.382	0	$Value = 1 - \left(\frac{\text{Metric result} - 0}{0.382 - 0}\right)$
Tachet	Locomotion and substrate relation – crawler + temporarily attached	1	0.191	$Value = \frac{\text{Metric result} - 0.191}{1 - 0.191}$
ASTERICS	Locomotion – [%] burrowing/boring	40	0	$Value = 1 - \left(\frac{\text{Metric result} - 0}{40 - 0}\right)$

3.2. Multimetric index development

Correlation analyses (step 1) indicated that all 31 metrics were significantly correlated ($p < 0.05$) and responded to the SQG as predicted, thus all metrics were included in further analyses (Table 5 and Appendix C).

Multiple stepwise regression (step 2) resulted in 26 combinations of the 31 candidate metrics potentially contributing to the final MMI's. Least squares regression (step 4) indicated that five metrics resulted in the MMI with the highest performance (greatest R-square) (Figure 2, Tables 5 and 6). This is hereafter referred to as the Lotic Index of Substrate and Sediment Assessment (LISSA). The five metrics included in the LISSA index comprised one trait state of aquatic stages (egg), two trait states of reproduction (isolated eggs, cemented + clutches, cemented or fixed and clutches, free + asexual reproduction), one trait state of locomotion and substrate relation (crawler + temporarily attached) and the locomotion trait state (% burrowing/boring) (Table 5).

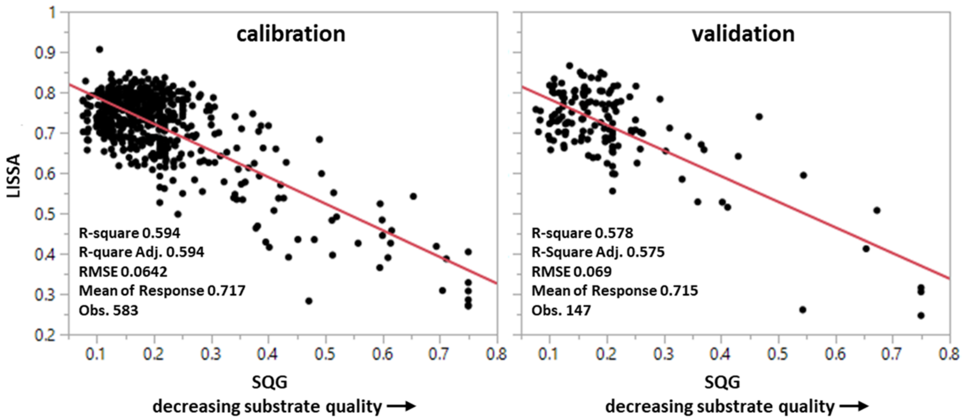

Figure 3. Relationship of LISSA to decreasing substrate quality (SQG) in the calibration and validation datasets.

3.3. Validation

The results of the comparison of LISSA in the calibration and validation datasets (step 5) showed that the R-square values (0.594 and 0.575, respectively) and slopes (0.853 and 0.847, respectively) were similar (Figure 3).

4. Discussion

The absence of a significant difference between the R-square values or slopes between independent calibration and validation datasets provided assurance of a working MMI. LISSA has the advantage to be effectively employed over a broader range of catchment types because it has independence from interacting and potentially confounding factors associated with those developed in specific catchment types such as in agricultural (Turley et al. 2014; Turley et al. 2015; Naden et al. 2016) or in alpine streams (Doretto et al. 2018). LISSA is an improvement over biotic indices that were developed using a single element of substrate condition (e.g. fine sediment deposition) (Turley et al. 2014; Turley et al. 2015; Naden et al. 2016; Doretto et al. 2018) because it permits for the assessment of the influence of multiple components of substrate condition. For example, in addition to assessment of fine sediment deposition, LISSA can assess response to restoration *via* increasing substrate size diversity or can provide insight to how impacts of sediment deposition may differ according to the context of fundamental substrates. Novel to most other biotic indices, LISSA is based solely on traits calculated from the presence-absence of taxa and it can be utilized when samples are missing abundance data, i.e. DNA-based approaches. Because LISSA is exclusively trait based, and traits are expressed in many species, it has a greater potential for large-scale applicability compared to indices that include regionally specific taxonomy-based metrics (Statzner et al. 2001; Horrigan and Baird 2008). However, the uncertainty of LISSA increases with increasing SQG values because there were fewer sites at this end of the gradient, and results in a greater potential for not detecting or falsely detecting changes. In turn, caution is advised in using LISSA until more data are available.

LISSA includes traits related to reproduction, aquatic life stages, and mode of locomotion that provide insights into the potential mechanisms driving community response to substrate condition. In accordance with predictions, streams with little fine sediment and/or sand and high substrate diversity and evenness (low SQG values) correlated with eggs as aquatic stages, isolated eggs, cemented + clutches, cemented or fixed, and crawler + temporarily attached. Streams on the other end of the SQG gradient with increasing substrates dominated by fine sediment and sand and low substrate diversity and evenness were correlated with clutches, free + asexual reproduction, and % burrowing/boring.

LISSA's component traits in relation to reproduction agree with other studies that in habitats dominated by smaller substrate size taxa that reproduce *via* cemented or fixed clutches of eggs are reduced (Mathers et al. 2022), while increases are observed in free clutches (Dolédec et al. 2006; Lange et al. 2014; Mor et al. 2019) and asexual reproduction (Leiva et al. 2022; Magbanua et al. 2013; Stearns 1976; Verberk et al. 2008). Cemented or fixed eggs are groups of eggs are laid down and fixed on appropriate substrate while free egg clutches are groups of eggs are laid down in the water freely and do not require a substrate on which to attach (https://www.freshwaterecology.info/). A reduction in taxa that have cemented or fixed clutches of eggs may result from a lack of specific substrate suitable to oviposit or attach eggs (Lancaster et al. 2010; Heino and Peckarsky 2014), while for taxa that reproduce asexually or *via* free clutches, where the eggs are laid down in groups in the water freely, specific substrates are probably less important.

Furthermore, in habitats dominated by smaller substrate sizes negative effects to macroinvertebrates associated with disturbance increase due to reduced physical stability (Dole-Olivier et al. 1997; Gurtz and Wallace 1984) and a lack of interstitial space as refuge (Dole-Olivier et al. 1997), which can have a tremendous impact on determining lotic macroinvertebrates communities (Alp et al. 2013; Encalada and Peckarsky 2012; Kennedy et al. 2016). In particular, conditions for eggs as aquatic stages are unfavorable as they are more vulnerable to burial than other life stages (Jones et al. 2012) and in systems where sediment loading is high, egg survival may be reduced by abrasion by fine sediment (Kefford et al. 2010). In environments that impair egg development studies have advocated that asexual reproduction can enhance resilience (Dolédec and Statzner 2008; Fenoglio et al. 2016) *via* the ability to reproduce without exposing eggs to harsh environmental conditions (Townsend and Hildrew 1994).

LISSA is consistent with other studies that have found that habitats dominated by fine sediments result in a reduction of crawlers (Bo et al. 2007; Buendia et al. 2013; Mathers et al. 2017; Mathers et al. 2022), while burrowers increase (Mathers et al. 2022). Other studies have demonstrated that taxa that crawl or temporarily attach decrease in relation to unsuitable sites to grasp or attach when substrate surfaces are dominated by fine and less stable sediment (Bass 1998; Ciborowski et al. 1977; Corkum et al. 1977). Furthermore, studies have shown habitats of diverse substrate sizes generally have greater interstitial space compared to habitats dominated by fine sediment (Dubuis and De Cesare 2023) and with increasing sedimentation and embedded substrates motile taxa are often lacking (Gjerløv et al. 2003). For taxa that crawl, decreasing interstitial space may restrict migration into the streambed for refuge during disturbance events and partially explain the correlation between increasingly fewer crawlers and higher SQG values. On the other hand, borrowers benefit from finer sediments associated with higher SQG values, into which they penetrate (Lancaster et al. 1991; Lancaster 1996), potentially reducing their susceptibility to disturbance events.

5. Conclusions

The multimetric LISSA has potential as a reliable tool for quantifying the riverbed substrate condition and monitoring effects of and recovery from substrate degradation in stream ecosystems. Because it is trait-based LISSA does not depend on regional taxa differences and its usefulness may potentially extend beyond Sweden and across the temperate zone especially where boreal regions dominate. However, given the relatively low number of sites in the high end of the SQG caution is advised in using LISSA until more data are available. In particular, fewer sites with high SQG values increases the uncertainty of LISSA within this part of the gradient and increasing the potential for not detecting or falsely detecting changes. The certainty of LISSA could be improved by reassessment when additional data from habitats that fall within the higher end of the SQG gradient become available. Nevertheless, LISSA is a first step toward filling the gap in the tools needed for quantifying hydromorphological impacts and the efficacy of restoration endeavors such as recreating mineral substrate diversity or measures that reduce inputs of fine sediment. To understand the full potential of LISSA, more studies are needed that cover a broader range in riverbed substrate degradation from hydromorphological alterations.

Author contribution

Peter E. Carlson: Conceptualization, Methodology, Formal analysis, Writing - original draft.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The data that support the findings of this study are available from the corresponding author, [PEC], upon reasonable request.

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Appendix A. List of all traits and trait states from Tachet (Tachet et al. 2000) and ID code (CODE).

TACHET_TRAITS		
Trait	Trait state	CODE
Maximal potential size	≤.25 cm	T1
Maximal potential size	>.25–.5 cm	T2
Maximal potential size	>.5–1 cm	T3
Maximal potential size	>1–2 cm	T4
Maximal potential size	>2–4 cm	T5
Maximal potential size	>4–8 cm	T6
Maximal potential size	>8 cm	T7
Life cycle duration	≤1 year	T8
Life cycle duration	>1 year	T9
Potential number of cycles per year	<1	T10
Potential number of cycles per year	1	T11
Potential number of cycles per year	>1	T12
Aquatic stages	Egg	T13
Aquatic stages	Larva	T14
Aquatic stages	Nymph	T15
Aquatic stages	Adult	T16
Reproduction	Ovoviviparity	T17
Reproduction	Isolated eggs, free	T18
Reproduction	Isolated eggs, cemented	T19
Reproduction	Clutches, cemented or fixed	T20
Reproduction	Clutches, free	T21
Reproduction	Clutches, in vegetation	T22
Reproduction	Clutches, terrestrial	T23
Reproduction	Asexual reproduction	T24
Dispersal	Aquatic passive	T25
Dispersal	Aquatic active	T26
Dispersal	Aerial passive	T27
Dispersal	Aerial active	T28
Resistance forms	Eggs, statoblasts	T29
Resistance forms	Cocoons	T30
Resistance forms	Housings against desiccation	T31
Resistance forms	Diapause or dormancy	T32
Resistance forms	None	T33
Respiration	Tegument	T34
Respiration	Gill	T35
Respiration	Plastron	T36
Respiration	Spiracle	T37
Respiration	Hydrostatic vesicle	T38
Locomotion and substrate relation	Flier	T39
Locomotion and substrate relation	Surface swimmer	T40
Locomotion and substrate relation	Full water swimmer	T41
Locomotion and substrate relation	Crawler	T42
Locomotion and substrate relation	Burrower	T43
Locomotion and substrate relation	Interstitial	T44
Locomotion and substrate relation	Temporarily attached	T45
Locomotion and substrate relation	Permanently attached	T46
Food	Microorganisms	T47
Food	Detritus < 1 mm	T48
Food	Dead plant >= 1 mm	T49
Food	Living microphytes	T50
Food	Living macrophytes	T51
Food	Dead animal >= 1 mm	T52
Food	Living microinvertebrates	T53
Food	Living macroinvertebrates	T54
Food	Vertebrates	T55
Feeding habits	Absorber	T56
Feeding habits	Deposit feeder	T57
Feeding habits	Shredder	T58
Feeding habits	Scraper	T59
Feeding habits	Filter-feeder	T60

(*Continued*)

Appendix A. Continued.

TACHET_TRAITS		
Trait	Trait state	CODE
Feeding habits	Piercer	T61
Feeding habits	Predator	T62
Feeding habits	Parasite	T63
Transversal distribution	River channel	T64
Transversal distribution	Banks, connected side-arms	T65
Transversal distribution	Ponds, pools, disconnected side-arms	T66
Transversal distribution	Marshes, peat bogs	T67
Transversal distribution	Temporary waters	T68
Transversal distribution	Lakes	T69
Transversal distribution	Groundwaters	T70
Longitudinal distribution	Crenon	T71
Longitudinal distribution	Epirithron	T72
Longitudinal distribution	Metarithron	T73
Longitudinal distribution	Hyporithron	T74
Longitudinal distribution	Epipotamon	T75
Longitudinal distribution	Metapotamon	T76
Longitudinal distribution	Estuary	T77
Longitudinal distribution	Outside river system	T78
Altitude	Lowlands	T79
Altitude	Piedmont level	T80
Altitude	Alpine level	T81
Substrate (preferendum)	Flags/boulders/cobbles/pebbles	T82
Substrate (preferendum)	Gravel	T83
Substrate (preferendum)	Sand	T84
Substrate (preferendum)	Silt	T85
Substrate (preferendum)	Macrophytes	T86
Substrate (preferendum)	Microphytes	T87
Substrate (preferendum)	Twigs/roots	T88
Substrate (preferendum)	Organic detritus/litter	T89
Substrate (preferendum)	Mud	T90
Current velocity (preferendum)	Null	T91
Current velocity (preferendum)	Slow	T92
Current velocity (preferendum)	Medium	T93
Current velocity (preferendum)	Fast	T94
Trophic status (preferendum)	Oligotrophic	T95
Trophic status (preferendum)	Mesotrophic	T96
Trophic status (preferendum)	Eutrophic	T97
Salinity (preferendum)	Fresh water	T98
Salinity (preferendum)	Brackish water	T99
Temperature	Psychrophilic	T100
Temperature	Thermophilic	T101
Temperature	Eurythermic	T102
Saprobity	Xenosaprobic	T103
Saprobity	Oligosaprobic	T104
Saprobity	b-mesosaprobic	T105
Saprobity	a-mesosaprobic	T106
Saprobity	Polysaprobic	T107
pH (preferendum)	≤4	T108
pH (preferendum)	>4–4.5	T109
pH (preferendum)	>4.5–5	T110
pH (preferendum)	>5–5.5	T111
pH (preferendum)	>5.5–6	T112
pH (preferendum)	>6	T113

Appendix B. List of all metrics and states from ASTRICS PERLODES (https://www.gewaesser-bewertung-berechnung.de) and ID code (CODE).

ASTERICS		
	Metric	CODE
Abundance [ind/m^2]	Abundance [ind/m^2]	A1
Number of Taxa	Number of Taxa	A2
Saprobic Index (Zelinka & Marvan)	Saprobic Index (Zelinka & Marvan)	A3
Saprobic Valence	Xeno [%]	A4
	Oligo [%]	A5
	Betameso [%]	A6
	Alphameso [%]	A7
	Poly [%]	A8
	No data available [%]	A9
	Xeno [%] (scored taxa 100%)	A10
	Oligo [%] (scored taxa 100%)	A11
	Alphameso [%] (scored taxa 100%)	A12
	Xeno [%] (abundance classes) (scored taxa 100%)	A13
	Oligo [%] (abundance classes) (scored taxa 100%)	A14
German Saprobic Index (old version)	German Saprobic Index (old version)	A15
Dispersion	Dispersion	A16
Sum of abundance classes	Sum of abundance classes	A17
Number of indicator taxa	Number of indicator taxa	A18
Water Quality Class	Water Quality Class	A19
German Saprobic Index (new version)	German Saprobic Index (new version)	A20
Dispersion	Dispersion	A21
Sum of abundance classes	Sum of abundance classes	A22
Number of indicator taxa	Number of indicator taxa	A23
Water Quality Class	Water Quality Class	A24
Dutch Saprobic Index	Dutch Saprobic Index	A25
Czech Saprobic Index	Czech Saprobic Index	A26
Romania Saprobic Index	Romania Saprobic Index	A27
Slovakian Saprobic Index	Slovakian Saprobic Index	A28
BMWP Score	BMWP Score	A29
Ntaxa	Ntaxa	A30
Average score per Taxon	Average score per Taxon	A31
BMWP Score (Spanish version)	BMWP Score (Spanish version)	A32
Ntaxa	Ntaxa	A33
BMWP Score (Hungarian version)	BMWP Score (Hungarian version)	A34
Ntaxa	Ntaxa	A35
Average score per Taxon (Hungaria version)	Average score per Taxon (Hungaria version)	A36
BMWP Score (Czech version)	BMWP Score (Czech version)	A37
Ntaxa	Ntaxa	A38
Average score per Taxon (Czech version)	Average score per Taxon (Czech version)	A39
BMWP Score (Polish version)	BMWP Score (Polish version)	A40
Ntaxa	Ntaxa	A41
BMWP Score (Greek version)	BMWP Score (Greek version)	A42
Ntaxa	Ntaxa	A43
DSFI Diversity Groups	DSFI Diversity Groups	A44
DSFI	DSFI	A45
Diversity Groups	Diversity Groups	A46
BBI	BBI	A47
IBE	IBE	A48
Quality Class	Quality Class	A49
Systematic Units	Systematic Units	A50
IBE Aqem	IBE Aqem	A51
Quality Class	Quality Class	A52
MAS	MAS	A53
Integr. Class	Integr. Class	A54
Operational Units	Operational Units	A55
MTS	MTS	A56
Diversity (SimpsonIndex)	Diversity (SimpsonIndex)	A57
Diversity (ShannonWienerIndex)	Diversity (ShannonWienerIndex)	A58

(*Continued*)

Appendix B. Continued.

ASTERICS		
	Metric	CODE
Diversity (Margalef Index)	Diversity (Margalef Index)	A59
Evenness	Evenness	A60
Acid Class (Braukmann) (5class version)	Acid Class (Braukmann) (5class version)	A61
Acid Index (Hendrikson & Medin)	Acid Index (Hendrikson & Medin)	A62
German Fauna Index	German Fauna Index D1	A63
	German Fauna Index D2	A64
	German Fauna Index D3	A65
	German Fauna Index D4	A66
	German Fauna Index D5	A67
	German Fauna Index type 1.1	A68
	Sum of abundance classes	A69
	Number of indicator taxa	A70
	German Fauna Index type 1.2	A71
	Sum of abundance classes	A72
	Number of indicator taxa	A73
	German Fauna Index type 2.1	A74
	Sum of abundance classes	A75
	Number of indicator taxa	A76
	German Fauna Index type 2.2	A77
	Sum of abundance classes	A78
	Number of indicator taxa	A79
	German Fauna Index type 3.1	A80
	Sum of abundance classes	A81
	Number of indicator taxa	A82
	German Fauna Index type 3.2	A83
	Sum of abundance classes	A84
	Number of indicator taxa	A85
	German Fauna Index type 4	A86
	Sum of abundance classes	A87
	Number of indicator taxa	A88
	German Fauna Index type 5	A89
	Sum of abundance classes	A90
	Number of indicator taxa	A91
	German Fauna Index type 9	A92
	Sum of abundance classes	A93
	Number of indicator taxa	A94
	German Fauna Index type 9.1	A95
	Sum of abundance classes	A96
	Number of indicator taxa	A97
	German Fauna Index type 9.2	A98
	Sum of abundance classes	A99
	Number of indicator taxa	A100
	German Fauna Index type 9.1_K additional Metric: Taxa number EPTCBO	A101
	Sum of abundance classes	A102
	Number of indicator taxa	A103
	German Fauna Index type 11/12	A104
	Sum of abundance classes	A105
	Number of indicator taxa	A106
	German Fauna Index type 14/16	A107
	Sum of abundance classes	A108
	Number of indicator taxa	A109
	German Fauna Index type 15/17	A110
	Sum of abundance classes	A111
	Number of indicator taxa	A112
	German Fauna Index type 15.2	A113
	Sum of abundance classes	A114
	Number of indicator taxa	A115
Lake outlet index, quantitativ	Lake outlet index, quantitativ	A116

(*Continued*)

Appendix B. Continued.

ASTERICS		
	Metric	CODE
Potamon Typie Index (describes how strongly an observed benthic invertebrate assemblage deviates from an expected near-natural or minimally disturbed state in large and very large rivers based on taxon-specific indicator values)	Potamon Typie Index	A117
Standard deviation	Standard deviation	A118
Number of samples	Number of samples	A119
Number of scored taxa	Number of scored taxa	A120
Average Number of taxa	Average Number of taxa	A121
Standard deviation of Average Number of taxa	Standard deviation of Average Number of taxa	A122
Minimum Number of scored taxa	Minimum Number of scored taxa	A123
Abundance of scored taxa/all taxa [%]	Abundance of scored taxa/all taxa [%]	A124
Homogeneity criterion [%]	Homogeneity criterion [%]	A126
Standard deviation of Homogeneity criterion [%]	Standard deviation of Homogeneity criterion [%]	A127
rDominance	rDominance	A128
r/K relationship	r/K relationship	A129
Portuguese Index	Portuguese Index	A130
Number of sensitive taxa (Austria)	Number of sensitive taxa (Austria)	A131
Zonation	[%] crenal	A132
	[%] hypocrenal	A133
	[%] epirhithral	A134
	[%] metarhithral	A135
	[%] hyporhithral	A136
	[%] epipotamal	A137
	[%] metapotamal	A138
	[%] hypopotamal	A139
	[%] littoral	A140
	[%] profundal	A141
	[%] littoral + profundal	A142
	[%] no data available	A143
	[%] hypocrenal (scored taxa 100%)	A144
	[%] epirhithral (scored taxa 100%)	A145
	[%] metarhithral (scored taxa 100%)	A146
	[%] hyporhithral (scored taxa 100%)	A147
	[%] epipotamal (scored taxa 100%)	A148
	[%] metapotamal (scored taxa 100%)	A149
	[%] littoral (scored taxa 100%)	A150
Current preference	[%] Type LB	A151
	[%] Type LP	A152
	[%] Type LR	A153
	[%] Type RL	A154
	[%] Type RP	A155
	[%] Type RB	A156
	[%] Type IN	A157
	[%] no data available	A158
	[%] Type RP (scored taxa 100%)	A159
	[%] Type RP (abundance classes) (scored taxa 100%)	A160
Rheoindex (Banning, with abundance)	Rheoindex (Banning, with abundance)	A161
Rheoindex (Banning, with abundance classes)	Rheoindex (Banning, with abundance classes)	A162
Rhithron Typie Index	Rhithron Typie Index	A163

(*Continued*)

Appendix B. Continued.

ASTERICS		
	Metric	CODE
Microhabitat preference	[%] Type Pel	A164
	[%] Type Arg	A165
	[%] Type Psa	A166
	[%] Type Aka	A167
	[%] Type Lit	A168
	[%] Type Phy	A169
	[%] Type Pom	A170
	[%] Type Oth	A171
	[%] No data available	A172
	[%] Type Aka + Lit + Psa	A173
	[%] Type Pel (scored taxa 100%)	A174
	[%] Type Psa (scored taxa 100%)	A175
	[%] Type Aka (scored taxa 100%)	A176
	[%] Type Lit (scored taxa 100%)	A177
	[%] Type Phy (scored taxa 100%)	A178
	[%] Type Aka + Lit + Psa (scored taxa 100%)	A179
Stonedwelling taxa (Braukmann, with abundance classes)	Stonedwelling taxa (Braukmann, with abundance classes)	A180
Feeding types	[%] Grazers and scrapers	A181
	[%] Miners	A182
	[%] Xylophagous Taxa	A183
	[%] Shredders	A184
	[%] Gatherers/Collectors	A185
	[%] Active filter feeders	A186
	[%] Passive filter feeders	A187
	[%] Predators	A188
	[%] Parasites	A189
	[%] Other Feeding types	A190
	[%] (Grazers + Scrapers)/ (GatherersCollectors + FilterFeeders)	A191
	[%] Xyloph. + Shred. + ActFiltFee. + PasFiltFee	A192
	[%] no data available	A193
	[%] Shredders (scored taxa 100%)	A194
	[%] Gatherers/Collectors (scored taxa 100%)	A195
	[%] Active/Passive filter feeders (all taxa)	A196
RETI	RETI	A197
Locomotion type	[%] swimming/skating	A198
	[%] swimming/diving	A199
	[%] burrowing/boring	A200
	[%] sprawling/walking	A201
	[%] (semi)sessil	A202
	[%] others (e.g. climbing)	A203
	[%] no data available	A204
Salinity preference	Freshwater [%] (<0.5)	A205
	Oligohalin [%] (0.5 < 5)	A206
	Mesohalin [%] (5 < 18)	A207
	Polyhalin [%] (18 30)	A208
	Euhalin [%] (>30)	A209
	No data available [%]	A210
	Number of indicator taxa salinity preference	A211
	Freshwater [%] (<0.5) (scored taxa 100%)	A212
	Oligohalin [%] (0.5 < 5) (scored taxa 100%)	A213
	Mesohalin [%] (5 < 18) (scored taxa 100%)	A214
	Polyhalin [%] (18 30) (scored taxa 100%)	A215
	Euhalin [%] (>30) (scored taxa 100%)	A216

(*Continued*)

Appendix B. Continued.

ASTERICS		
	Metric	CODE
Taxonomic group [%]	Porifera [%]	A217
	Coelenterata [%]	A218
	Cestoda [%]	A219
	Trematoda [%]	A220
	Turbellaria [%]	A221
	Nematoda [%]	A222
	Nematomorpha [%]	A223
	Gastropoda [%]	A224
	Bivalvia [%]	A225
	Polychaeta [%]	A226
	Oligochaeta [%]	A227
	Hirudinea [%]	A228
	Crustacea [%]	A229
	Araneae [%]	A230
	Ephemeroptera [%]	A231
	Odonata [%]	A232
	Plecoptera [%]	A233
	Heteroptera [%]	A234
	Planipennia [%]	A235
	Megaloptera [%]	A236
	Trichoptera [%]	A237
	Lepidoptera [%]	A238
	Coleoptera [%]	A239
	Diptera [%]	A240
	Bryozoa [%]	A241
	Hydrachnidia [%]	A242
	Others [%]	A243
	EPTTaxa [%]	A244
	EPT/OL [%]	A245
	EP [%]	A246
	EPind/Totind [%]	A247
	EPT [%] (abundance classes)	A248
Hololimnic [%]	Hololimnic [%]	A249
Taxonomic group (number of taxa)	Porifera	A250
	Coelenterata	A251
	Cestoda	A252
	Trematoda	A253
	Turbellaria	A254
	Nematoda	A255
	Nematomorpha	A256
	Gastropoda	A257
	Bivalvia	A258
	Polychaeta	A259
	Oligochaeta	A260
	Hirudinea	A261
	Crustacea	A262
	Araneae	A263
	Ephemeroptera	A264
	Odonata	A265

(*Continued*)

Appendix B. Continued.

ASTERICS		
	Metric	CODE
	Plecoptera	A266
	Heteroptera	A267
	Planipennia	A268
	Megaloptera	A269
	Trichoptera	A270
	Lepidoptera	A271
	Coleoptera	A272
	Diptera	A273
	Bryozoa	A274
	Hydrachnidia	A275
	Others	A276
	EPTtaxa	A277
	EPT/OL	A278
	EPT/Diptera	A279
	ODTaxa [%] (Austria)	A280
	EPTTaxa [%] (Austria)	A281
	OD/Totaltaxa	A282
	EPtaxa	A283
	EPTCBO (Eph., Ple., Tri., Col., Bivalv., Odo.)	A284
Taxonomic group (abundance)	Porifera	A285
	Coelenterata	A286
	Cestoda	A287
	Trematoda	A288
	Turbellaria	A289
	Nematoda	A290
	Nematomorpha	A291
	Gastropoda	A292
	Bivalvia	A293
	Polychaeta	A294
	Oligochaeta	A295
	Hirudinea	A296
	Crustacea	A297
	Araneae	A298
	Ephemeroptera	A299
	Odonata	A300
	Plecoptera	A301
	Heteroptera	A302
	Planipennia	A303
	Megaloptera	A304
	Trichoptera	A305
	Lepidoptera	A306
	Coleoptera	A307
	Diptera	A308
	Bryozoa	A309
	Hydrachnidia	A310
	Others	A311
Number of Families	Number of Families	A312
Number of Genera	Number of Genera	A313
Index of Biocoenotic Region	Index of Biocoenotic Region	A314
Austria, 100% class and FAA	[%] littoral (scored taxa 100%)	A315
	[%] littoral + profundal (scored taxa 100%)	A316
	RETI	A317
	[%] Gatherers/Collectors	A318
	[%] Shredders	A319

(*Continued*)

Appendix B. Continued.

ASTERICS		
	Metric	CODE
Active filter feeders/passive filter feeders (all taxa)	Active filter feeders/passive filter feeders (all taxa)	A320
Italian metrics	Trichoptera_taxa	A321
	Plecoptera_taxa	A322
	TROPHIC_Sel_Grazers	A323
	Cordulegaster_Dinocras	A324
	Amphinemura_Protonemura	A325
	A.Muticus + N.digitatus	A326
	Sel_Ephemeroptera_GS	A327
	Leptophlebiidae	A328
	Sel_Trichoptera_GS	A329
	DIPTERA_Good_G	A330
	DIPTERA_Bad_SIPH_G	A331
	HABITAT_Argillal	A332
	TROPHIC_Filterer	A333
	BEHAV_Borrowing	A334
	Sel_Ephemeroptera_M	A335
	Sel_Plecoptera_M	A336
	Sel_nonEPTaxa_M	A337
	Dugesia_Lymnaea	A338
	all/Diptera	A339
	Sel_Ephemeroptera_GN	A340
	Sel_Trichoptera	A341
	Leuctra_Calopteryx	A342
	Elmidae	A343
	Lumbricidae	A344
	Tubificidae	A345
	PleTri_taxa	A346
Life Index	Life Index	A347
Portuges GoldIndex	Portuges GoldIndex	A348
sel EPTD	sel EPTD	A349
AWIC Index	AWIC Index	A350
Neozoenanteil	Neozoenanteil	A351
Croatia Saprobic Index HRISSystem	Croatia Saprobic Index HRISSystem	A352
Croatia Saprobic Index WEGLSystem	Croatia Saprobic Index WEGLSystem	A353
German Fauna Index	German Fauna Index type 19	A354
	Sum of abundance classes	A355
	Number of indicator taxa	A356
SPEAR Indizes	SPEAR pesticides	A357
	SPEAR organic	A358
SPEAR [%]	SPEAR [%]	A359
Anzahl Indiaktorarten	Anzahl Indiaktorarten	A360
wärmeliebende Neozoa	wärmeliebende Neozoa	A361

Appendix C. Spearmans correlations of traits/metrics with significant correlation ($p < 0.05$) to SQG.

CODE	Metric	METRIC_STATE	Spearman ρ	Prob>|p|
A1	Number of Taxa (DNA)	Number of Taxa (DNA)	−0.1901	0.0022
A2	Number of Taxa (ASTERICS)	Number of Taxa (ASTERICS)	−0.2329	0.0002
A3	Saprobic Index (Zelinka & Marvan)	Saprobic Index (Zelinka & Marvan)	0.1463	0.0189
A5	Saprobic Valence	Oligo [%]	−0.1425	0.0223
A6	Saprobic Valence	Betameso [%]	−0.1342	0.0315
A7	Saprobic Valence	Alphameso [%]	0.1293	0.0382
A17	German Saprobic Index (old version)	Sum of abundance classes	−0.33	<.0001
A18	German Saprobic Index (old version)	Number of indicator taxa	−0.33	<.0001
A22	German Saprobic Index (new version)	Sum of abundance classes	−0.3788	<.0001

(*Continued*)

Appendix C. Continued.

CODE	Metric	METRIC_STATE	Spearman ρ	Prob>|p|
A23	German Saprobic Index (new version)	Number of indicator taxa	−0.3788	<.0001
A29	BMWP Score	BMWP Score	−0.4513	<.0001
A30	BMWP Score	Ntaxa	−0.4283	<.0001
A31	Average score per Taxon	Average score per Taxon	−0.3298	<.0001
A32	BMWP Score (Spanish version)	BMWP Score (Spanish version)	−0.4597	<.0001
A33	BMWP Score (Spanish version)	NTaxa	−0.4353	<.0001
A34	BMWP Score (Hungarian version)	BMWP Score (Hungarian version)	−0.4514	<.0001
A35	BMWP Score (Hungarian version)	Ntaxa	−0.4396	<.0001
A37	BMWP Score (Czech version)	BMWP Score (Czech version)	−0.4623	<.0001
A38	BMWP Score (Czech version)	Ntaxa	−0.3968	<.0001
A39	Average score per Taxon (Czech version)	Average score per Taxon (Czech version)	−0.4512	<.0001
A40	BMWP Score (Polish version)	BMWP Score (Polish version)	−0.4881	<.0001
A41	BMWP Score (Polish version)	Ntaxa	−0.4708	<.0001
A42	BMWP Score (Greek version)	BMWP Score (Greek version)	−0.4452	<.0001
A43	BMWP Score (Greek version)	Ntaxa	−0.4123	<.0001
A44	DSFI Diversity Groups	DSFI Diversity Groups	−0.1451	0.02
A57	Diversity (Simpson-Index)	Diversity (SimpsonIndex)	−0.4877	<.0001
A58	Diversity (Shannon-Wiener-Index)	Diversity (ShannonWienerIndex)	−0.2473	<.0001
A59	Diversity (Margalef Index)	Diversity (Margalef Index)	−0.2493	<.0001
A60	Evenness	Evenness	−0.4874	<.0001
A62	Acid Index (Hendrikson & Medin)	Acid Index (Hendrikson & Medin)	−0.3209	<.0001
A69	German Fauna Index	Sum of abundance classes	−0.3646	<.0001
A70	German Fauna Index	Number of indicator taxa	−0.3646	<.0001
A72	German Fauna Index	Sum of abundance classes	−0.3533	<.0001
A73	German Fauna Index	Number of indicator taxa	−0.356	<.0001
A75	German Fauna Index	Sum of abundance classes	−0.3375	<.0001
A76	German Fauna Index	Number of indicator taxa	−0.3375	<.0001
A78	German Fauna Index	Sum of abundance classes	−0.2624	<.0001
A79	German Fauna Index	Number of indicator taxa	−0.2643	<.0001
A81	German Fauna Index	Sum of abundance classes	−0.2546	<.0001
A82	German Fauna Index	Number of indicator taxa	−0.2546	<.0001
A84	German Fauna Index	Sum of abundance classes	−0.3715	<.0001
A85	German Fauna Index	Number of indicator taxa	−0.3715	<.0001
A87	German Fauna Index	Sum of abundance classes	−0.343	<.0001
A88	German Fauna Index	Number of indicator taxa	−0.3439	<.0001
A90	German Fauna Index	Sum of abundance classes	−0.3766	<.0001
A91	German Fauna Index	Number of indicator taxa	−0.3766	<.0001
A93	German Fauna Index	Sum of abundance classes	−0.3069	<.0001
A94	German Fauna Index	Number of indicator taxa	−0.3069	<.0001
A96	German Fauna Index	Sum of abundance classes	−0.3091	<.0001
A97	German Fauna Index	Number of indicator taxa	−0.3091	<.0001
A99	German Fauna Index	Sum of abundance classes	−0.3158	<.0001
A100	German Fauna Index	Number of indicator taxa	−0.3158	<.0001
A102	German Fauna Index	Sum of abundance classes	−0.3167	<.0001
A103	German Fauna Index	Number of indicator taxa	−0.3167	<.0001
A105	German Fauna Index	Sum of abundance classes	−0.2791	<.0001
A106	German Fauna Index	Number of indicator taxa	−0.2791	<.0001
A108	German Fauna Index	Sum of abundance classes	−0.2542	<.0001
A109	German Fauna Index	Number of indicator taxa	−0.2574	<.0001
A111	German Fauna Index	Sum of abundance classes	−0.3056	<.0001
A112	German Fauna Index	Number of indicator taxa	−0.3084	<.0001
A114	German Fauna Index	Sum of abundance classes	−0.4308	<.0001
A115	German Fauna Index	Number of indicator taxa	−0.4351	<.0001
A120	Potamon Typie Index	Number of scored taxa	−0.3688	<.0001
A123	Potamon Typie Index	Minimum number of scored taxa	−0.3523	<.0001
A124	Potamon Typie Index	Abundance of scored taxa/all taxa [%]	−0.2154	0.0005
A128	Potamon Typie Index	rDominance	−0.1347	0.0308
A129	r/K relationship	r/K relationship	−0.1547	0.013
A130	Portuguese Index	Portuguese Index	−0.3341	<.0001
A131	Number of sensitive taxa (Austria)	Number of sensitive taxa (Austria)	−0.4414	<.0001

(*Continued*)

Appendix C. Continued.

CODE	Metric	METRIC_STATE	Spearman ρ	Prob>|p|
A132	Zonation	[%] crenal	0.167	0.0073
A134	Zonation	[%] epirhithral	−0.2355	0.0001
A135	Zonation	[%] metarhithral	−0.3989	<.0001
A136	Zonation	[%] hyporhithral	−0.3183	<.0001
A139	Zonation	[%] hypopotamal	0.1311	0.0357
A140	Zonation	[%] littoral	0.3429	<.0001
A141	Zonation	[%] profundal	0.3113	<.0001
A142	Zonation	[%] littoral + profundal	0.38	<.0001
A145	Zonation	[%] epirhithral (scored taxa = 100%)	−0.2283	0.0002
A146	Zonation	[%] metarhithral (scored taxa = 100%)	−0.4252	<.0001
A147	Zonation	[%] hyporhithral (scored taxa = 100%)	−0.3504	<.0001
A150	Zonation	[%] littoral (scored taxa = 100%)	0.3576	<.0001
A152	Current preference	[%] Type LP	0.2674	<.0001
A153	Current preference	[%] Type LR	0.3636	<.0001
A155	Current preference	[%] Type RP	−0.3887	<.0001
A156	Current preference	[%] Type RB	−0.2495	<.0001
A158	Current preference	[%] no data available	0.1993	0.0013
A159	Current preference	[%] Type RP (scored taxa = 100%)	−0.3796	<.0001
A160	Current preference	[%] Type RP (abundance classes) (scored taxa = 100%)	−0.3738	<.0001
A164	Microhabitat preference	[%] Type Pel	0.1767	0.0045
A166	Microhabitat preference	[%] Type Psa	0.2967	<.0001
A168	Microhabitat preference	[%] Type Lit	−0.4608	<.0001
A173	Microhabitat preference	[%] Type Aka + Lit + Psa	−0.3241	<.0001
A174	Microhabitat preference	[%] Type Pel (scored taxa = 100%)	0.2265	0.0003
A175	Microhabitat preference	[%] Type Psa (scored taxa = 100%)	0.3536	<.0001
A177	Microhabitat preference	[%] Type Lit (scored taxa = 100%)	−0.4845	<.0001
A179	Microhabitat preference	[%] Type Aka + Lit + Psa (scored taxa = 100%)	−0.3299	<.0001
A181	Feeding types	[%] Grazers and scrapers	−0.3728	<.0001
A185	Feeding types	[%] Gatherers/Collectors	0.1856	0.0028
A187	Feeding types	[%] Passive filter feeders	−0.2062	0.0009
A191	Feeding types	[%] (Grazers + Scrapers)/ (GatherersCollectors + FilterFeeders)	−0.3675	<.0001
A193	Feeding types	[%] no data available	0.1759	0.0047
A195	Feeding types	[%] Gatherers/Collectors (scored taxa = 100%)	0.2117	0.0006
A197	RETI	RETI	−0.3118	<.0001
A198	Locomotion type	[%] swimming/skating	−0.1411	0.0236
A200	Locomotion type	[%] burrowing/boring	0.166	0.0077
A202	Locomotion type	[%] (semi)sessil	−0.1946	0.0017
A205	Salinity preference	freshwater [%] (<0.5)	0.2769	<.0001
A206	Salinity preference	oligohalin [%] (0.5 < 5)	0.2807	<.0001
A210	Salinity preference	no data available [%]	−0.3212	<.0001
A212	Salinity preference	freshwater [%] (<0.5) (scored taxa = 100%)	−0.1611	0.0097
A213	Salinity preference	oligohalin [%] (0.5 < 5) (scored taxa = 100%)	0.1866	0.0027
A217	Taxonomic group [%taxa]	Porifera [%taxa]	−0.211	0.0007
A221	Taxonomic group [%taxa]	Turbellaria [%taxa]	−0.1276	0.041
A227	Taxonomic group [%taxa]	Oligochaeta [%taxa]	0.311	<.0001
A229	Taxonomic group [%taxa]	Crustacea [%taxa]	0.1831	0.0032
A231	Taxonomic group [%taxa]	Ephemeroptera [%taxa]	−0.3096	<.0001
A233	Taxonomic group [%taxa]	Plecoptera [%taxa]	−0.2828	<.0001
A236	Taxonomic group [%taxa]	Megaloptera [%taxa]	0.1418	0.023
A237	Taxonomic group [%taxa]	Trichoptera [%taxa]	−0.3373	<.0001
A239	Taxonomic group [%taxa]	Coleoptera [%taxa]	−0.1917	0.002
A240	Taxonomic group [%taxa]	Diptera [%taxa]	0.2806	<.0001
A244	Taxonomic group [%taxa]	EPTTaxa [%taxa]	−0.483	<.0001
A246	Taxonomic group [%taxa]	EP [%taxa]	−0.3679	<.0001

(*Continued*)

Appendix C. Continued.

CODE	Metric	METRIC_STATE	Spearman ρ	Prob>|p|
A247	Taxonomic group [%taxa]	EPind/Totind [%taxa]	−0.3679	<.0001
A248	Taxonomic group [%taxa]	EPT [%taxa] (abundance classes)	−0.4698	<.0001
A249	Taxonomic group [%taxa]	hololimnic [%taxa]	0.304	<.0001
A250	Taxonomic group (number of taxa)	Porifera	−0.2103	0.0007
A254	Taxonomic group (number of taxa)	Turbellaria	−0.1265	0.0428
A258	Taxonomic group (number of taxa)	Bivalvia	−0.1497	0.0163
A260	Taxonomic group (number of taxa)	Oligochaeta	0.1662	0.0076
A264	Taxonomic group (number of taxa)	Ephemeroptera	−0.3857	<.0001
A266	Taxonomic group (number of taxa)	Plecoptera	−0.3073	<.0001
A269	Taxonomic group (number of taxa)	Megaloptera	0.1318	0.0348
A270	Taxonomic group (number of taxa)	Trichoptera	−0.3436	<.0001
A272	Taxonomic group (number of taxa)	Coleoptera	−0.2455	<.0001
A277	Taxonomic group (number of taxa)	EPTTaxa	−0.4409	<.0001
A280	Taxonomic group (number of taxa)	ODTaxa [%] (Austria)	0.3805	<.0001
A281	Taxonomic group (number of taxa)	EPTTaxa [%] (Austria)	−0.4712	<.0001
A282	Taxonomic group (number of taxa)	OD/TotalTaxa	0.3805	<.0001
A283	Taxonomic group (number of taxa)	EPTaxa	−0.406	<.0001
A284	Taxonomic group (number of taxa)	EPTCBO (Eph., Ple., Tri., Col., Bivalv., Odo.)	−0.4171	<.0001
A285	Taxonomic group (number of taxa DNA)	Porifera	−0.2103	0.0007
A289	Taxonomic group (number of taxa DNA)	Turbellaria	−0.1265	0.0428
A293	Taxonomic group (number of taxa DNA)	Bivalvia	−0.1497	0.0163
A295	Taxonomic group (number of taxa DNA)	Oligochaeta	0.181	0.0036
A297	Taxonomic group (number of taxa DNA)	Crustacea	0.1408	0.0239
A299	Taxonomic group (number of taxa DNA)	Ephemeroptera	−0.3688	<.0001
A301	Taxonomic group (number of taxa DNA)	Plecoptera	−0.3073	<.0001
A304	Taxonomic group (number of taxa DNA)	Megaloptera	0.1318	0.0348
A305	Taxonomic group (number of taxa DNA)	Trichoptera	−0.3363	<.0001
A307	Taxonomic group (number of taxa DNA)	Coleoptera	−0.2345	0.0001
A312	Number of Families	Number of Families	−0.3957	<.0001
A313	Number of Genera	Number of Genera	−0.2518	<.0001
A317	Austria, 100% class and FAA	RETI	−0.2967	<.0001
A318	Austria, 100% class and FAA	[%] Gatherers/Collectors	0.1813	0.0035
A321	Italian metrics	Trichoptera_taxa	−0.3436	<.0001
A322	Italian metrics	Plecoptera_taxa	−0.3073	<.0001
A323	Italian metrics	TROPHIC_Sel_Grazers	−0.3133	<.0001
A325	Italian metrics	Amphinemura_Protonemura	−0.2492	<.0001
A326	Italian metrics	A.Muticus + N.digitatus	−0.167	0.0073
A328	Italian metrics	Leptophlebiidae	−0.2078	0.0008
A329	Italian metrics	Sel_Trichoptera_GS	−0.1357	0.0297
A330	Italian metrics	DIPTERA_Good_G	−0.1676	0.0071
A335	Italian metrics	Sel_Ephemeroptera_M	−0.4245	<.0001
A336	Italian metrics	Sel_Plecoptera_M	−0.2446	<.0001
A341	Italian metrics	Sel_Trichoptera	−0.1932	0.0019
A342	Italian metrics	Leuctra_Calopteryx	−0.2295	0.0002
A343	Italian metrics	Elmidae	−0.305	<.0001
A344	Italian metrics	Lumbricidae	0.2104	0.0007
A345	Italian metrics	Tubificidae	0.3045	<.0001
A346	Italian metrics	PleTri_taxa	−0.4025	<.0001
A348	Portuges Gold-Index	Portuges GoldIndex	−0.4149	<.0001
A349	sel EPTD	sel EPTD	−0.2735	<.0001
A350	AWIC Index	AWIC Index	0.1783	0.0041
A355	German Fauna Index	Sum of abundance classes	−0.2999	<.0001

(*Continued*)

Appendix C. Continued.

CODE	Metric	METRIC_STATE	Spearman ρ	Prob>|p|
A356	German Fauna Index	Number of indicator taxa	−0.3026	<.0001
A357	SPEAR Indizes	SPEAR pesticides	−0.4037	<.0001
A358	SPEAR Indizes	SPEAR organic	−0.2724	<.0001
A359	SPEAR [%]	SPEAR [%]	−0.3937	<.0001
A360	SPEAR [%]	Anzahl Indiaktorarten	−0.2329	0.0002
T3	Maximal potential size	>.5–1 cm	−0.1719	0.0057
T6	Maximal potential size	>4–8 cm	0.1649	0.0081
T8	Life cycle duration	≤1 year	−0.3097	<.0001
T9	Life cycle duration	>1 year	0.1832	0.0032
T11	Potential number of cycles per year	1	−0.4423	<.0001
T12	Potential number of cycles per year	>1	0.2984	<.0001
T13	Aquatic stages	Egg	−0.5007	<.0001
T15	Aquatic stages	Nymph	0.2021	0.0011
T16	Aquatic stages	Adult	0.1825	0.0033
T19	Reproduction	Isolated eggs, cemented	−0.3602	<.0001
T20	Reproduction	Clutches, cemented or fixed	−0.2949	<.0001
T21	Reproduction	Clutches, free	0.3546	<.0001
T24	Reproduction	Asexual reproduction	0.1736	0.0053
T25	Dispersal	Aquatic passive	0.1337	0.0321
T26	Dispersal	Aquatic active	−0.2821	<.0001
T28	Dispersal	Aerial active	−0.2221	0.0003
T29	Resistance forms	Eggs, statoblasts	−0.4817	<.0001
T30	Resistance forms	Cocoons	0.1909	0.0021
T35	Respiration	Gill	−0.1287	0.0392
T36	Respiration	Plastron	−0.2562	<.0001
T38	Respiration	Hydrostatic vesicle	0.4173	<.0001
T39	Locomotion and substrate relation	Flier	−0.1441	0.0209
T41	Locomotion and substrate relation	Full water swimmer	0.2218	0.0003
T42	Locomotion and substrate relation	Crawler	−0.3439	<.0001
T43	Locomotion and substrate relation	Burrower	0.1539	0.0135
T44	Locomotion and substrate relation	Interstitial	0.2465	<.0001
T45	Locomotion and substrate relation	Temporarily attached	−0.3037	<.0001
T47	Food	Microorganisms	0.229	0.0002
T49	Food	Dead plant >= 1 mm	−0.1715	0.0058
T50	Food	Living microphytes	−0.2055	0.0009
T51	Food	Living macrophytes	−0.2666	<.0001
T52	Food	Dead animal >= 1 mm	0.1451	0.0199
T53	Food	Living microinvertebrates	0.2436	<.0001
T56	Feeding habits	Absorber	0.2145	0.0005
T57	Feeding habits	Deposit feeder	0.1888	0.0024
T58	Feeding habits	Shredder	−0.1717	0.0058
T59	Feeding habits	Scraper	−0.1872	0.0026
T60	Feeding habits	Filter-feeder	−0.1607	0.0099
T63	Feeding habits	Parasite	0.1393	0.0255
T64	Transversal distribution	River channel	−0.5175	<.0001
T65	Transversal distribution	Banks, connected side-arms	−0.1309	0.0359
T66	Transversal distribution	Ponds, pools, disconnected side-arms	0.2949	<.0001
T68	Transversal distribution	Temporary waters	0.264	<.0001
T69	Transversal distribution	Lakes	0.29	<.0001
T72	Longitudinal distribution	Epirithron	−0.3321	<.0001
T73	Longitudinal distribution	Metarithron	−0.4377	<.0001
T74	Longitudinal distribution	Hyporithron	−0.4554	<.0001
T76	Longitudinal distribution	Metapotamon	0.241	<.0001
T77	Longitudinal distribution	Estuary	0.1325	0.0337
T78	Longitudinal distribution	Outside river system	0.2512	<.0001
T82	Substrate (preferendum)	Flags/boulders/cobbles/pebbles	−0.4761	<.0001
T83	Substrate (preferendum)	Gravel	−0.2179	0.0004
T85	Substrate (preferendum)	Silt	0.3684	<.0001
T87	Substrate (preferendum)	Microphytes	0.3398	<.0001
T88	Substrate (preferendum)	Twigs/roots	−0.4541	<.0001
T90	Substrate (preferendum)	Mud	0.4638	<.0001

(*Continued*)

Appendix C. Continued.

CODE	Metric	METRIC_STATE	Spearman ρ	Prob>|p|
T91	Current velocity (preferendum)	Null	0.2053	0.0009
T93	Current velocity (preferendum)	Medium	−0.3402	<.0001
T94	Current velocity (preferendum)	Fast	−0.137	0.0281
T95	Trophic status (preferendum)	Oligotrophic	−0.3263	<.0001
T97	Trophic status (preferendum)	Eutrophic	0.3335	<.0001
T98	Salinity (preferendum)	Fresh water	−0.1636	0.0086
T99	Salinity (preferendum)	Brackish water	0.2781	<.0001
T102	Temperature	Eurythermic	−0.1764	0.0046
T103	Saprobity	Xenosaprobic	−0.2815	<.0001
T104	Saprobity	Oligosaprobic	−0.2195	0.0004
T106	Saprobity	a-mesosaprobic	0.2398	0.0001
T107	Saprobity	Polysaprobic	0.3108	<.0001
T108	pH (preferendum)	≤4	0.4938	<.0001
T109	pH (preferendum)	>4–4.5	0.4517	<.0001
T110	pH (preferendum)	>4.5–5	0.1953	0.0017
T111	pH (preferendum)	>5–5.5	−0.1965	0.0016
T112	pH (preferendum)	>5.5–6	−0.3794	<.0001
T113	pH (preferendum)	>6	−0.3254	<.0001