

Anthropogenic Pressures and Ecological Responses of Benthic Communities in the River Jhelum: A Critical Review

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Abstract

Benthic macroinvertebrates are powerful indicators of river health because of their limited movement, tolerance to a range of pollution levels, and life cycles spanning several months. This critical review assesses how indicative of anthropogenic effects Jhelum River benthic communities are and how a defensible bi monitoring program can bolster river restoration and management. Studies specific to the Jhelum River provide a good foundation. Many studies have documented changes in the richness, diversity, and EPT values of benthic communities, as well as the effects of human activities on benthic communities, in different sections of the river and through sampling that spans multiple seasons. In addition to evaluating traditional indices of diversity, this review will also consider the EPT and other multimetric approaches; point score systems such as BMWP and ASPT; trait-based approaches to assessing community health; and the innovative DNA-based metabarcoding approaches using recent international examples. With respect to the Kashmir Himalaya, there will not be a one-size fits all answer; the natural variability of seasonal flow and unaltered biogeographic variation across the region are likely to mimic or confound the disturbance responses. Given the state of local knowledge and material resources, traditional monitoring will continue to be essential as will, and if rigorously implemented, improvements in the resolution and confidence of DNA-based metabarcoding approaches. Therefore, the Jhelum River biomonitoring and management program will include kick sampling, physicochemical assessments, and multimetric approaches. This program will determine the ecological status of the river to facilitate the management of sewage inputs, water discharges, and restoration efforts of the habitat and of the watershed.

Keywords: River Jhelum; Biomonitoring; Benthic Macroinvertebrates; EPT; BMWP; ASPT; DNA Metabarcoding; Ecological Status.

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Introduction

Biomonitoring has a unique perspective when compared to routine water chemistry. Chemistry provides a snapshot of what is happening at the time of the sampling event while benthic assemblages show the integrated impacts of those events over time. This technique is important when evaluating pulses of pollutants, when there are multiple stressors, or when habitat and contaminant concentration may be equally important. Macroinvertebrates include taxa that range from more pollution-sensitive Ephemeroptera, Plecoptera and Trichoptera to groups that are more tolerant to sedimentation or organically enriched conditions, making it possible to evaluate the community and not rely on a single sentinel species (Vourka et al., 2023; Alberdi et al., 2018; Altermatt et al., 2023; Bagley et al., 2019; Baloglu et al., 2021).

The Jhelum Basin is amenable to biomonitoring but also has some issues with calibration. At a basin scale, studies have shown seasonal and longitudinal variations in sediment assemblages. A subsequent study looked at the structure of communities based on riffles, runs, glides and pools. This implies that a monitoring score could be adversely impacted due to a shift in sampling effort among habitats or seasons. Structures of communities based on the four habitat types have been described and established a basis for regional calibration of the environmental gradients and local taxa and habitats (Sabha et al., 2020; Sabha et al., 2023; Sofi et al., 2020).

This review's main concern is not if benthic macroinvertebrates can indicate disturbance, because they can, but how to construct an index and a sampling methodology that generate stakeholder-relevant, comparable results of river management in the Himalayan River. Recent global assessments indicate that countries adopt different approaches to constructing methods and that lack of consensus is a major challenge to comparability (Wang et al., 2021; Weigand et al., 2019; Simaika et al., 2024; Macher et al., 2025). The Jhelum can avoid this problem by establishing the first field, laboratory, and data-quality and taxonomic rules.

Scope and Critical Review Approach

The review prefers DOI-bearing Jhelum and Kashmir Himalayan studies and studies on river bioassessment that focus on the more recent methods of river bioassessment, multimetric indices and DNA-based assessment. Older references are included only to present firsthand definitions of concepts of BMWP/ASPT, quality assurance or flow ecology. The evidence is interpreted within the framework consisting of five interrelated elements: sampling, identification, selection, classification and management. References that totalled 100 were counted in the audit. Every reference cited has a verified DOI against scholarly metadata. Evidence beyond the Himalayas is considered as methodological evidence.

Benthic biomonitoring inference chain

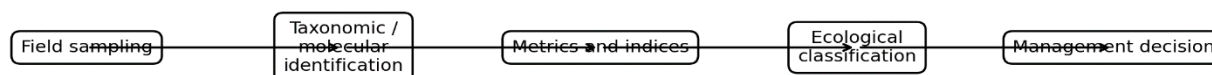


Figure 1. Benthic biomonitoring inference chain from field sampling to management decision.

Why Benthic Communities Matter in River-Health Assessment

Benthic macroinvertebrates offer both breadth and consistency of place. Larval aquatic insects can remain in an environment for weeks or months and are able to integrate changes in temperature, oxygen, nutrients, and flow that may occur multiple times. Therefore, changes within a biological community may show evidence of chronic or recurrent stress that annual water sample collections may not find. Their ecological roles as leaf litter consumers and periphyton grazers, particle filters and consumers of fine detritus and as prey provide a variety of ecological functions and collaboratively help assess changes in ecosystem processes (Sofi et al., 2024; Berry et al., 2021; Blackman et al., 2019; Boyer et al., 2016; Brantschen et al., 2021).

Indicator value is context based. For example, limited macroinvertebrate richness may indicate disturbance, but it may also reflect natural harshness in headwater streams, seasonal high flows or low colonist availability. Total richness may conceal the loss of sensitive taxonomic groups and may also be attributed to invasion by tolerant species. River typology and biodiversity studies reveal that assessments may benefit from explicitly modeling expected natural variation (Edegbene et al., 2023; Esmaeili Ofogh et al., 2023; Wang et al., 2023). In the Jhelum, models of reference conditions prior to the assignment of ecological quality classes should incorporate elevation, stream size, substrate, and season.

Benthic Biodiversity and Habitat Structure in the Jhelum System

The most comprehensive biomonitoring evidence for the Jhelum is provided by sampling across many watersheds and surveys of large spatial extent. Sabha and colleagues found evidence of longitudinal and seasonal structure, resulting in reduced richness, Shannon diversity, density and EPT metrics in the downstream direction. Analysis of habitats across 59 sites and multiple seasons showed that riffles, runs, glides and pools differed in taxonomic richness, density and beta diversity of communities (Sabha et al. 2020; Sabha et al. 2023). These studies have two important implications. The first is that the basin contains enough biological signal for a multimetric assessment. The second is that if sampling is not stratified by habitats or focused on a specific defined habitat, changes in habitat structure and/or composition could be incorrectly interpreted as changes in ecological quality.

The natural hydrological cycle also defines the Jhelum macroinvertebrate baseline. Assemblages in the summer are affected by discharge and temperature of water, while other seasons are more defined by the sand and pH in the substrate. Thus, for an offensive local index, the impact of anthropogenic activities on the natural system should be accounted for, and metrics should be selected that have a compatible correlation with stressors (Sabha et al. 2020; Khanday et al. 2021; Sofi et al. 2020).

Sensitive and Tolerant Taxa

5.1 Ephemeroptera, Plecoptera and Trichoptera

EPT richness and percentage are commonly used, as many taxa of these orders prefer cool, oxygenated, flowing water with coarse substrates. Observations of lower EPT representation in the Jhelum basin, or any downstream position, are therefore useful and constructive. Nevertheless, EPT should not be treated as a single tolerance class, as there are different family and species level responses. For example, some mayflies can actually tolerate a certain degree of water enrichment or the presence of fine sediment. Further advances in eDNA studies could enhance our ability to detect EPT diversity, but the recovery of such data are often highly dependent on the selection of the primers used in the study. Consequently, the values of such metrics are also dependent on primer selection (Bagley et al., 2019; Goldberg et al., 2013; Haase et al., 2010; Jeunen et al., 2022; Ji et al., 2022).

5.2 Diptera, Chironomidae and Oligochaeta

Chironomidae and Oligochaeta are typically observed to increase in organically enriched or depositional environments. Therefore, they are also useful taxa in determining tolerant biota. However, Chironomidae and Oligochaeta are taxonomically and ecologically broad, and therefore, a broad group level scoring could mask sensitive taxa. The same can be said of Mollusca and other abiotic benthos. It is therefore appropriate to develop an index of the Jhelum to test the empirical response curves of families or genera, as opposed to using unvalidated tolerance values (Wang et al., 2021; Weigand et al., 2019; Simaika et al., 2024; Macher et al., 2025; Iwasaki et al., 2024).

Conceptual response of indicator groups along increasing disturbance

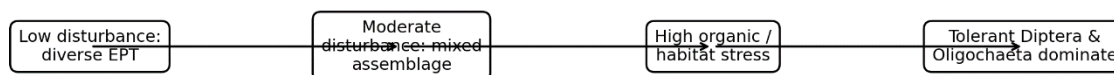


Figure 2. Conceptual shift from EPT-rich assemblages toward tolerant Diptera/Oligochaeta dominance along an increasing disturbance gradient.

Conventional Metrics and Biological Indices

Indices such as the Shannon and Simpson summarize community structure and are weak diagnostics because they combine richness and evenness without indicating which taxa were either lost or gained. The Margalef richness index is also sensitive to sampling effort. Though EPT and percent EPT offer ecologically interpretable results, they still require an adequate taxonomic resolution and a season. BMWP and ASPT systems compensate by weighting taxa based on the sensitivity of the loss of those taxa to pollution. Multiple metrics systems integrate several independent signals (Uchida et al, 2020; Wang et al, 2021; Weigand et al, 2019; Simaika et al, 2024; Macher et al, 2025).

The historical applications of the BMWP retain significant value, and direct transcription of scores to new sites is possible. However, the design of multimetric studies suggests that modern approaches to construction of metrics should begin with pressure gradients, performance tests, and screening for redundancy and be independently validated. Seasonal variability, along with classification of rivers, has been shown to impact the expected limits of reference systems (Simaika et al, 2024; Macher et al, 2025; Sripanya et al, 2023; Edegbene et al, 2023; Esmaeili Ofogh et al, 2023). For the Jhelum, an ASPT-type metric may be useful, but the scores for the tolerances should be tested against local pressure gradients instead of being assumed to be general.

Spatial and Seasonal Design

Samples collected from various points along a river provide information on longitudinal, spatial, and temporal variation. The selected study reaches represent a range of impacts due to human activities. Habitat will be sampled in the same way from headwaters, urban areas, tributary confluences, moderately disturbed reaches, and reaches affected by alteration of flow. Because of the strong evidence for seasonal variations in the local studies, two sampling periods will be selected that represent two opposing hydrological conditions (e.g., selection of spring and winter). If resources permit, sampling would be conducted to improve understanding of seasonal variations. From other basins, it has been reported that seasons of base-flow offer greater discrimination and this could be the case, but should be verified (Sabha et al., 2020; Sabha et al., 2023; Macher et al., 2025).

Factors such as sampling effort, and the taxonomic resolution of the sampling, also contribute uncertainty to the results. An example of an under sampled environment would be the choice for a taxonomic group of higher order, for example from order to class. In the absence of a taxonomic design, it would be prudent to collect several samples from the same habitat and document the sampling in detail, and also maintain a collection of specimens (voucher specimens) that would be available to review the taxonomic design periodically. Historically, auditing biomonitoring programs has shown that the reduction of assessment precision is due, in large part, to the identification of organisms (Fernández et al., 2018; Fernández et al., 2019; Wang et al., 2021; Koyuncuoğlu et al., 2023).

DNA Metabarcoding and eDNA: Advances and Limitations

There are three major advantages of DNA-based techniques in the biomonitoring of the Jhelum river. These include higher taxonomic resolution of morphologically challenging organisms, the availability of standard identification frameworks, and the ability to survey a greater range of organisms beyond what routine family-level sorting allows. Several studies have shown that eDNA or bulk sample metabarcoding can reproduce large scale ecological gradients and, upon intercalibration, can offer congruent classifications of ecological status that are comparable to morphology-based classification methods (Altermatt et al., 2023; Djurhuus et al., 2017; Dowle et al., 2016; Goldberg et al., 2013; Taberlet et al., 2012; Turner et al., 2015). The 2025 Water Research evaluation is unique because it focuses on intercalibration as opposed to simply documenting correlation among different methods (Taberlet et al., 2012). DNA-based methods also have method-specific features. For example, different steps in the process can affect the outcomes of detection, including pore size, ratio of sampled water volume to extraction volume, chemistry of extraction, primers, and the number of PCR replications and the following sequencing and bioinformatics steps. Water eDNA can integrate the upstream transport of DNA, and therefore, can overestimate the local occurrence of species. On the other hand, both bulk tissue and preservative ethanol can represent the local assemblage, but these samples still have biases of biomass and primers (Blackman et al., 2019; Brantschen et al., 2022; Buchner et al., 2022; Carraro et al., 2020; Doi et al., 2021; Dowle et al., 2016; Erdozain et al., 2019; Ficetola et al., 2021; Hajibabaei et al., 2019; Hupalo et al., 2022; Keck et al., 2022; Krehenwinkel et al., 2019; Macher et al., 2018; Mächler et al., 2016; Majaneva et al., 2018; Martins et al., 2021; Meyer et al., 2021; Pawlowski et al., 2018; Persaud et al., 2021; Reinholdt Jensen et al., 2021; Renshaw et al., 2015; Ruppert et al., 2019; Sakata et al., 2021; Seymour et al., 2021; Shirazi et al., 2021). The most reasonable approach for the monitoring of the Jhelum would be a hybrid program: continue standardized kick-net sampling for the abundance estimate and local habitat assessment and preserve voucher material, and in a stepwise approach add bulk DNA or eDNA where the intercomparison would indicate equivalence for the management classification.

Hybrid morphology-DNA biomonitoring framework

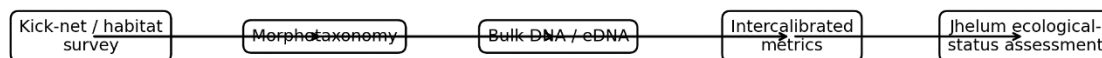


Figure 3. Hybrid morphology–DNA framework proposed for calibrated Jhelum biomonitoring.

Integrating Physicochemical and Biological Monitoring

Biological assessment should not be a substitute for water chemistry. Biological assessments answer some but not all questions and should be used along with water chemistry. [SH] Benthic metrics measure the overall ecological condition of the habitat while chemistry can help identify stressor sources and possible management options. For this reason, macroinvertebrate sampling should be done along with temperature, dissolved oxygen, pH, conductivity, nutrients, turbidity, flow velocity, depth, substrate, and riparian condition. Additionally, where mining, road runoff, waste-water or agricultural contamination is expected, sampling for metals, pesticides, or other organic micropollutants should be included. The proposed design is more for diagnosis of stressors instead of classification of an ecological system (Khanday et al., 2021; Sofi et al., 2020; Edegbene et al., 2023; Lim et al., 2024; Iwasaki et al., 2024; Hertika et al., 2024; Lukhabi et al., 2024).

Proposed Jhelum Multimetric Biomonitoring Framework

The framework consists of five main stages. Stage 1 relates to the classification of rivers and the reference conditions of the least impacted rivers based on elevation, order of streams, slope of land, and hydrology and the nature of riverbeds. Stage 2 standardizes sampling of the benthic habitat and enhances the quality of taxonomy during the sampling. Stage 3 evaluates candidate metrics across the domains of taxonomy, tolerance, function, and composition. Stage 4 evaluates the ecological quality standards and the consistency of seasonal assessments. Stage 5 links status classes to appropriate management actions and monitors the duration of the assessment to review the progress. This design shadows recent advancements in multimetric systems and improve the integrative gaps that international assessments have evaluated (Wang et al., 2021; Weigand et al., 2019; Simaika et al., 2024; Macher et al., 2025; Sripanya et al., 2023; Edegbene et al., 2023).

Table 1. Candidate components of a Jhelum multimetric index

Metric domain	Candidate metric	Interpretation
Taxonomic richness	Total families/genera or standardized OTUs/ASVs	Broad biodiversity response; effort-sensitive
EPT richness / %EPT	Ephemeroptera + Plecoptera + Trichoptera	Sensitive to oxygen, habitat and flow; calibrate seasonally
Tolerance metric	Locally calibrated ASPT/BMWP-like score	Requires regional tolerance validation
Tolerant-group proportion	Selected	Organic/depositional stress;

Metric domain	Candidate metric	Interpretation
	Chironomidae/Oligochaeta or validated families	avoid coarse assumptions
Functional metric	Feeding groups or flow/attachment traits	Improves mechanism diagnosis
Composition metric	Ordination distance or modelled expected/observed similarity	Detects taxon replacement missed by richness

Proposed tiered Jhelum benthic monitoring network

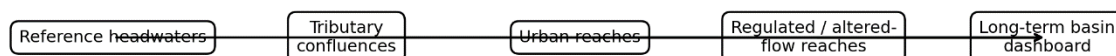


Figure 4. Proposed tiered Jhelum benthic monitoring network feeding a basin-scale ecological-status dashboard.

Biomonitoring for Restoration and Management

Benthic Monitoring can serve as a basis for management when status class thresholds are crossed. In urban reaches, monitoring should focus on sewage collection and treatment systems for low EPT and high proportions of tolerant taxa and elevated nutrient or organic load. In farming sections, riparian buffers and the control of sediment and nutrient inputs should be the main focus of monitoring efforts. Sectioned reaches with flow-dependent trait loss should be managed with the goals of environmental flow and hydropeaking. Benthic monitoring should be conducted using a before-after control-impact design on reaches where physical restoration is undertaken. Restoration effects can be observed quickly, after a long time, or not at all depending on whether the limiting process is restored (Stubington et al. 2024; Leitner et al. 2021; Jupke et al. 2022; Li et al. 2021; Wieringa et al. 2022).

Limitations and Research Gaps

Table 2. Major limitations to operational Jhelum biomonitoring

Limitation	Risk	Recommended response
Local tolerance values	Imported BMWP/ASPT scores may not reflect Himalayan taxa.	Calibrate taxa against a quantified Jhelum pressure gradient.
Reference condition	Pristine sites may be scarce or naturally dissimilar.	Use least-disturbed, type-specific references and predictive models.
Seasonal variability	Assemblages change strongly through the year.	Define standard index seasons or seasonal correction factors.

Limitation	Risk	Recommended response
Taxonomic QA	Morphological error reduces precision.	Voucher collection, audit, reference images and DNA barcodes.
Molecular intercalibration	eDNA and bulk DNA may detect different taxon sets.	Run paired morphology–DNA surveys before regulatory adoption.
Management linkage	Monitoring can remain descriptive.	Predefine status-triggered actions and restoration success criteria.

There is a large opportunity to combine conventional monitoring and establish a regional barcode reference library. One recognized challenge of aquatic assessments is the gap of DNA barcoding. The reliability of metabarcoding is only as good as the taxonomic databases used for assignment (Hering et al., 2018; Sakata et al., 2021). A Kashmir reference library coupled with curated voucher specimens would enhance morphological identification and training.

Conclusion

Benthic macroinvertebrates have the potential to be used as an ecological condition tool in the River Jhelum, but only if locally specific monitoring is applied. Previous studies on the River Jhelum provide the structure of longitudinal and seasonal data, as well as habitat specific data, to construct an assessment framework. The index should not be an import BMWP or EPT threshold, as it should be based on local assessment of impacts, evaluation of stress metrics, defined reference expectations, and assessment of boundaries for ecological quality. The use of morphology and molecular techniques should be considered complementary. Morphology has advantages of abundance and direct links to habitat, whereas molecular techniques are able to improve taxonomy and the identification of close species. A quality assured, seasonal and multi-metric approach would enable the Jhelum to progress from repeatability surveys of the ecological status to assessment of restoration and management of the Catchment.

Table 3. Comparison of major benthic assessment approaches

Approach	Basis	Strength	Principal limitation
BMWP-type score	Family tolerance sum	Simple, historically widespread	Tolerance values may not transfer geographically
ASPT	BMWP score divided by number of scoring taxa	Less sample-size dependent than raw BMWP	Still inherits tolerance-score assumptions
EPT richness	Number of EPT taxa	Ecologically interpretable, widely used	Season/habitat and taxonomic resolution sensitive
Shannon diversity	Richness + evenness	General community summary	Weak stressor diagnosis
Multimetric index	Combination of screened metrics	Integrates several response dimensions	Requires reference sites and validation
DNA metabarcoding metrics	Taxon/sequence-based analogues	High resolution and reproducibility potential	Primer, database and abundance biases

Table 4. Selected verified studies underpinning the biomonitoring synthesis

Study	Year	Focus	Source	DOI
Sabha et al.	2020	Longitudinal and temporal assemblage patterns of benthic macroinvertebra	Ecohydrology	10.1002/eco.2236

Study	Year	Focus	Source	DOI
Khanday et al.	2021	Identifying lithogenic and anthropogenic factors responsible for spatio-	Catena	10.1016/j.catena.2020.104853
Sabha et al.	2023	Habitat preferences and biomonitoring of aquatic macroinvertebrates along	International Journal of Environmental Science and Technology	10.1007/s13762-022-04523-y
Sofi et al.	2020	The natural flow regime: A master variable for maintaining river ecosystem	Ecohydrology	10.1002/eco.2247
Sofi et al.	2024	Navigating a broken river: Understanding the ecological consequences of	Ecohydrology	10.1002/eco.2575
Rather et al.	2016	Massive land system changes impact water quality of the Jhelum River in	Environmental Monitoring and Assessment	10.1007/s10661-016-5190-x
Vourka et al.	2023	River benthic macroinvertebrates and environmental DNA metabarcoding: A	Biodiversity and Conservation	10.1007/s10531-023-02710-y
Alberdi et al.	2018	Scrutinizing key steps for reliable metabarcoding of environmental samples	Methods in Ecology and Evolution	10.1111/2041-210X.12849
Altermatt et al.	2023	Quantifying biodiversity using eDNA from water bodies: General principle	Environmental DNA	10.1002/edn3.430
Bagley et al.	2019	High-throughput environmental DNA analysis informs a biological assessment	Ecological Indicators	10.1016/j.ecolind.2019.04.088
Baloğlu et al.	2021	A workflow for accurate metabarcoding using nanopore MinION sequencing	Methods in Ecology and Evolution	10.1111/2041-210X.13561
Berry et al.	2021	Making environmental DNA (eDNA) biodiversity	Environmental DNA	10.1002/edn3.173

Study	Year	Focus	Source	DOI
		records globally accessible		
Blackman et al.	2019	Advancing the use of molecular methods for routine freshwater macroinver	Metabarcoding and Metagenomics	10.3897/mbmg.3.34735
Boyer et al.	2016	OBITools: A Unix-inspired software package for DNA metabarcoding	Molecular Ecology Resources	10.1111/1755-0998.12428
Brantschen et al.	2021	Environmental DNA gives comparable results to morphology-based indices o	PLoS ONE	10.1371/journal.pone.0257510
Brantschen et al.	2022	Evaluation of primer pairs for eDNA-based assessment of Ephemeroptera, P	Environmental DNA	10.1002/edn3.342
Buchner et al.	2022	APSCALE: Advanced pipeline for simple yet comprehensive analyses of DNA	Bioinformatics	10.1093/bioinformatics/btac588
Caporaso et al.	2010	QIIME allows analysis of high-throughput community sequencing data	Nature Methods	10.1038/nmeth.f.303
Carraro et al.	2020	Environmental DNA allows upscaling spatial patterns of biodiversity in f	Nature Communications	10.1038/s41467-020-17337-8
Clusa et al.	2021	Nuisance species in Lake Constance revealed through eDNA	Biological Invasions	10.1007/s10530-021-02462-2

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Appendix A. Reference Verification Audit

Reference Verification Audit: 100 DOI-bearing scholarly sources

No.	First author	Year	Journal	DOI	Verified
1	Alberdi	2018	Methods in Ecology and Evolution	10.1111/2041-210X.12849	Yes
2	Altermatt	2023	Environmental DNA	10.1002/edn3.430	Yes
3	Amaral	2015	Journal of Insect Science	10.1093/jisesa/iev042	Yes
4	Armitage	1983	Water Research	10.1016/0043-1354(83)90188-4	Yes
5	Arthington	2018	Frontiers in Environmental Science	10.3389/fenvs.2018.00045	Yes
6	Bagley	2019	Ecological Indicators	10.1016/j.ecolind.2019.04.088	Yes
7	Baloğlu	2021	Methods in Ecology and Evolution	10.1111/2041-210X.13561	Yes
8	Berry	2021	Environmental DNA	10.1002/edn3.173	Yes
9	Blackman	2019	Metabarcoding and Metagenomics	10.3897/mbmg.3.34735	Yes

No.	First author	Year	Journal	DOI	Verified
10	Boyer	2016	Molecular Ecology Resources	10.1111/1755-0998.12428	Yes
11	Brantschen	2021	PLoS ONE	10.1371/journal.pone.0257510	Yes
12	Brantschen	2022	Environmental DNA	10.1002/edn3.342	Yes
13	Buchner	2022	Bioinformatics	10.1093/bioinformatics/btac588	Yes
14	Bunn	2002	Environmental Management	10.1007/s00267-002-2737-0	Yes
15	Caporaso	2010	Nature Methods	10.1038/nmeth.f.303	Yes
16	Carraro	2020	Nature Communications	10.1038/s41467-020-17337-8	Yes
17	Carter	2017	Methods in Stream Ecology	10.1016/B978-0-12-813047-6.00016-4	Yes
18	Cid	2022	Frontiers in Ecology and the Environment	10.1002/fee.2417	Yes
19	Clusa	2021	Biological Invasions	10.1007/s10530-021-02462-2	Yes
20	Couto	2023	Freshwater Biology	10.1111/fwb.14078	Yes
21	Deiner	2014	PLoS ONE	10.1371/journal.pone.0088786	Yes
22	Deiner	2015	Biological Conservation	10.1016/j.biocon.2014.11.018	Yes
23	Djurhuus	2017	Frontiers in Marine Science	10.3389/fmars.2017.00314	Yes
24	Doi	2021	Ecological Research	10.1111/1440-1703.12217	Yes
25	Dowle	2016	Molecular Ecology Resources	10.1111/1755-0998.12488	Yes
26	Edegbene	2023	Water Science	10.1080/23570008.2023.2215571	Yes
27	Erdozain	2019	Ecological Indicators	10.1016/j.ecolind.2019.01.014	Yes
28	Esmaili Ofogh	2023	Ecological Indicators	10.1016/j.ecolind.2022.109843	Yes
29	Fernández	2018	PLoS ONE	10.1371/journal.pone.0201741	Yes
30	Fernández	2019	Environmental DNA	10.1002/edn3.40	Yes
31	Ficetola	2021	Molecular Ecology	10.1111/mec.15632	Yes
32	Gauthier	2020	Molecular Ecology Resources	10.1111/1755-0998.13088	Yes
33	Gleason	2021	Molecular Ecology	10.1111/mec.15597	Yes
34	Goldberg	2013	Freshwater Science	10.1899/13-046.1	Yes
35	Guareschi	2019	Science of the Total Environment	10.1016/j.scitotenv.2019.01.008	Yes
36	Haase	2010	Journal of the North American Benthological	10.1899/09-183.1	Yes

No.	First author	Year	Journal	DOI	Verified
			Society		
37	Hajibabaei	2019	PLoS ONE	10.1371/journal.pone.0225409	Yes
38	Hauer	2018	Riverine Ecosystem Management	10.1007/978-3-319-73250-3_8	Yes
39	Hering	2018	Water Research	10.1016/j.watres.2018.03.003	Yes
40	Hering	2006	Freshwater Biology	10.1111/j.1365-2427.2006.01610.x	Yes
41	Hertika	2024	Biodiversitas	10.13057/biodiv/d250222	Yes
42	Hogsden	2012	Freshwater Science	10.1899/11-091.1	Yes
43	Hupalo	2022	Hydrobiologia	10.1007/s10750-021-04718-3	Yes
44	Iwasaki	2024	Environmental Science and Pollution Research	10.1007/s11356-024-33053-y	Yes
45	Jeunen	2022	Metabarcoding and Metagenomics	10.3897/mbmg.6.e68575	Yes
46	Ji	2022	Science of the Total Environment	10.1016/j.scitotenv.2021.150587	Yes
47	Jupke	2022	Science of the Total Environment	10.1016/j.scitotenv.2022.156689	Yes
48	Juvigny-Khenafou	2021	Ecology and Evolution	10.1002/ece3.6979	Yes
49	Keck	2022	Metabarcoding and Metagenomics	10.3897/mbmg.6.79351	Yes
50	Kelly	2019	Scientific Reports	10.1038/s41598-019-48546-x	Yes
51	Khanday	2021	Catena	10.1016/j.catena.2020.104853	Yes
52	Koyuncuoğlu	2023	Turkish Journal of Water Science and Management	10.31807/tjwsm.1175743	Yes
53	Krehenwinkel	2019	Genes	10.3390/genes10110858	Yes
54	Leese	2016	Research Ideas and Outcomes	10.3897/rio.2.e11321	Yes
55	Leese	2021	Environmental DNA	10.1002/edn3.177	Yes
56	Leitner	2021	Science of the Total Environment	10.1016/j.scitotenv.2020.143472	Yes
57	Li	2021	Science of the Total Environment	10.1016/j.scitotenv.2020.143630	Yes
58	Lim	2024	Ecological Indicators	10.1016/j.ecolind.2024.111867	Yes
59	Lukhabi	2024	Heliyon	10.1016/j.heliyon.2024.e28018	Yes
60	Macher	2018	Molecular Ecology Resources	10.1111/1755-0998.12940	Yes
61	Macher	2025	Water Research	10.1016/j.watres.2024.122987	Yes
62	Majaneva	2018	Scientific Reports	10.1038/s41598-018-23052-8	Yes

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63	Markert	2022	Environmental Sciences Europe	10.1186/s12302-022-00679-z	Yes
64	Martins	2019	Molecular Ecology Resources	10.1111/1755-0998.13012	Yes
65	Martins	2021	Molecular Ecology	10.1111/mec.15620	Yes
66	Meyer	2021	Molecular Ecology	10.1111/mec.15723	Yes
67	Mächler	2016	Environmental Science & Technology	10.1021/acs.est.5b04188	Yes
68	Ntislidou	2020	Science of the Total Environment	10.1016/j.scitotenv.2019.135496	Yes
69	Pawlowski	2018	Science of the Total Environment	10.1016/j.scitotenv.2018.05.002	Yes
70	Pereira-da-Conceicao	2021	Environmental DNA	10.1002/edn3.116	Yes
71	Persaud	2021	Diversity	10.3390/d13010034	Yes
72	Rather	2016	Environmental Monitoring and Assessment	10.1007/s10661-016-5190-x	Yes
73	Reinholdt Jensen	2021	Environmental DNA	10.1002/edn3.193	Yes
74	Renshaw	2015	Molecular Ecology Resources	10.1111/1755-0998.12281	Yes
75	Rivera	2021	Science of the Total Environment	10.1016/j.scitotenv.2020.144208	Yes
76	Ruppert	2019	Global Ecology and Conservation	10.1016/j.gecco.2019.e00547	Yes
77	Sabha	2023	International Journal of Environmental Science and Technology	10.1007/s13762-022-04523-y	Yes
78	Sabha	2020	Ecohydrology	10.1002/eco.2236	Yes
79	Sakata	2021	Limnology	10.1007/s10201-020-00645-9	Yes
80	Seymour	2021	Communications Biology	10.1038/s42003-021-02031-2	Yes
81	Shirazi	2021	Ecology and Evolution	10.1002/ece3.8239	Yes
82	Simaika	2024	Science of the Total Environment	10.1016/j.scitotenv.2024.170360	Yes
83	Sofi	2024	Ecohydrology	10.1002/eco.2575	Yes
84	Sofi	2020	Ecohydrology	10.1002/eco.2247	Yes
85	Spens	2017	Methods in Ecology and Evolution	10.1111/2041-210X.12683	Yes
86	Sripanya	2023	Water	10.3390/w15040625	Yes

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87	Stubbington	2024	River Research and Applications	10.1002/rra.4405	Yes
88	Taberlet	2012	Molecular Ecology	10.1111/j.1365-294X.2012.05542.x	Yes
89	Tonkin	2014	Freshwater Biology	10.1111/fwb.12387	Yes
90	Tricco	2018	Annals of Internal Medicine	10.7326/M18-0850	Yes
91	Turner	2015	Biological Conservation	10.1016/j.biocon.2014.11.017	Yes
92	Uchida	2020	PeerJ	10.7717/peerj.9176	Yes
93	Vannote	1980	The American Naturalist	10.1086/283591	Yes
94	Vourka	2023	Biodiversity and Conservation	10.1007/s10531-023-02710-y	Yes
95	Wang	2023	Ecological Indicators	10.1016/j.ecolind.2023.110235	Yes
96	Wang	2021	Diversity and Distributions	10.1111/ddi.13284	Yes
97	Weigand	2019	Science of the Total Environment	10.1016/j.scitotenv.2019.04.247	Yes
98	Wieringa	2022	Science of the Total Environment	10.1016/j.scitotenv.2022.157045	Yes
99	Zarfl	2015	Aquatic Sciences	10.1007/s00027-014-0377-0	Yes
100	Zhang	2023	Scientific Data	10.1038/s41597-023-02008-2	Yes