

Review of bioindicator species in environmental assessment

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ABSTRACT

*Bioindicator species -- organisms whose presence, abundance, condition, or behaviour reflects the ecological integrity of the environment they inhabit -- have been a cornerstone of environmental monitoring since Kolkwitz and Marsson's saprobic system of 1908, yet the rigour with which candidate bioindicators are validated, applied, and reported remains highly variable across taxonomic groups, environmental media, and regulatory frameworks. This review synthesises 198 primary studies published 2010-2024 examining the selection, validation, and application of animal bioindicator species across freshwater, terrestrial, and coastal marine ecosystems in European monitoring contexts. We evaluate bioindicator performance across five dimensions (sensitivity, specificity, practicality, taxonomic accessibility, and regulatory adoption) for six major taxonomic groups -- macroinvertebrates, fish, amphibians, birds, small mammals, and soil invertebrates -- using a standardised scoring framework applied to 144 validated bioindicator studies. Freshwater macroinvertebrates retain the strongest overall bioindicator performance profile (composite score 2.64/3.0), underpinned by the EU Water Framework Directive's Biological Quality Element requirements. Birds demonstrate high sensitivity and public engagement value but lower specificity for stressor type identification. Amphibians -- particularly *Rana* and *Triturus* spp. -- show exceptional multi-stressor sensitivity but suffer from rapid population declines that compromise indicator robustness at degraded sites. Emerging approaches -- environmental DNA (eDNA) metabarcoding, passive acoustic monitoring, and wearable biollogger-based behavioural biomarkers -- are evaluated as next-generation bioindicator platforms. A practical framework for bioindicator selection, validation, and integration into EU environmental assessment reporting is presented.*

Keywords: bioindicator species; environmental assessment; freshwater macroinvertebrates; EU Water Framework Directive; amphibians; biomonitoring; eDNA metabarcoding; ecological integrity; indicator validation; multi-stressor assessment

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1. Introduction

1.1 The Bioindicator Concept

The fundamental premise of biological indication is that living organisms integrate environmental conditions across time and space in ways that physicochemical measurements cannot: a single water chemistry snapshot misses episodic pollution pulses that a resident macroinvertebrate community integrates over its multi-year lifespan, while a bird territory's occupancy status reflects the cumulative habitat quality over a breeding season in a way that a single habitat survey cannot capture (Niemi and McDonald, 2004). This temporal and spatial integration property is the key advantage of biological indicators over chemical monitoring -- but it is also the source of their principal limitation: the integrated biological response reflects the sum of all stressors acting on the organism, making attribution to specific stressors challenging without complementary chemical data. The field has evolved substantially from early saprobic systems (Kolkwitz and Marsson, 1908) through multi-metric indices (Karr, 1981; Index of Biotic Integrity) to modern trait-based and functional diversity approaches that improve stressor specificity and cross-system transferability.

1.2 EU Regulatory Context

The European Union's Water Framework Directive (WFD; 2000/60/EC) established biological quality elements (BQEs) -- phytoplankton, macrophytes, benthic macroinvertebrates, and fish -- as the primary basis for ecological status classification of water bodies, institutionalising bioindicators in the most ambitious water quality management framework ever implemented. The Habitats Directive (92/43/EEC) and Birds Directive (2009/147/EC) implicitly require bioindicator-quality population monitoring for Annex II and Annex I species respectively as part of favourable conservation status assessment. The Biodiversity Strategy 2030 and Zero Pollution Action Plan further expand the role of biological indicators in tracking progress towards nature recovery targets. Despite this regulatory embedding, the scientific basis for species selection and validation in many operational monitoring programmes lags behind best practice, and the integration of emerging molecular and remote sensing tools into regulatory bioindicator frameworks remains nascent.

1.3 Review Scope and Objectives

This review examines the selection, validation, and application of animal bioindicator species across freshwater, terrestrial, and coastal marine ecosystems in European monitoring contexts, synthesising 198 primary studies (2010-2024). Objectives are: (i) to evaluate bioindicator performance dimensions across six major animal taxonomic groups; (ii) to identify the best-validated and most widely applicable bioindicator species and indices for each ecosystem type; (iii) to evaluate emerging bioindicator platforms (eDNA, passive acoustics, biologgers); and (iv) to propose a practical framework for bioindicator selection, validation, and integration into EU environmental assessment reporting.

2. Literature Review

2.1 Freshwater Macroinvertebrates: The Gold Standard

Freshwater macroinvertebrates -- collectively encompassing insects (Ephemeroptera, Plecoptera, Trichoptera -- EPT taxa; Diptera; Coleoptera), crustaceans, molluscs, and oligochaetes -- represent the most extensively validated and operationally deployed bioindicator group in European environmental monitoring (Hering et al., 2006). The EPT richness index and its derivatives (BMWP, ASPT, multimetric indices) have been standardised through WFD intercalibration exercises across all EU biogeographical regions, providing the most robust cross-system comparability of any bioindicator system. Their strengths -- sedentary habit (spatial integration), multi-year life cycles (temporal integration), diverse sensitivity spectrum across taxa, and extensive taxonomic expertise base -- are well-documented. Limitations include: high taxonomic burden (species-level identification often requires specialist expertise), seasonal sampling constraints, and reduced sensitivity in some catchment types (e.g., naturally acidic upland streams with naturally low EPT diversity).

2.2 Amphibians and Fish as Freshwater Indicators

Fish assemblages are the second WFD BQE for rivers and are assessed through national multi-metric fish indices calibrated to reference conditions. Fish integrate over longer time periods than macroinvertebrates and are sensitive to hydromorphological degradation (migration barriers, channel modification) that macroinvertebrates may not adequately reflect (Karr, 1981). Amphibians occupy a unique bioindicator niche: their aquatic larval stages and terrestrial adult stages expose them to stressors in both media, their permeable skin makes them sensitive to waterborne contaminants at concentrations below those affecting macroinvertebrates, and their global population declines are linked to multi-stressor exposure including UV-B radiation, pesticides, pathogens (*Batrachochytrium dendrobatidis*; Bd), and habitat loss. *Triturus cristatus* (great crested newt) is formally listed as an Annex II species under the Habitats Directive and serves as a practical bioindicator of high-quality aquatic habitat networks in agricultural and peri-urban landscapes.

2.3 Birds, Mammals, and Soil Bioindicators

Birds are the most widely used terrestrial bioindicators in European monitoring, primarily through the EU Farmland Bird Index (FBI) and Forest Bird Index (FOBIC), which aggregate population trends of functionally defined species guilds as indicators of agricultural and forest ecosystem health respectively. Their high detectability, existing monitoring infrastructure, and broad public recognition make them highly practical bioindicators; their limitation is specificity -- FBI declines reflect a complex of stressors (pesticide use, habitat simplification, reduced prey availability) that cannot be disentangled from the composite index alone. Small mammals -- particularly *Apodemus* spp. and *Sorex* spp. -- are sensitive to soil quality and contaminant bioaccumulation (Mikusinski and Angelstam, 1997) but suffer from high trapping effort

requirements and natural abundance variability. Soil invertebrates (Collembola, mites, earthworms) are strong indicators of soil ecosystem function and contaminant load but lack the regulatory framework adoption of WFD-tier freshwater indicators.

Table 1. Six Animal Taxonomic Groups as Bioindicators: Key Validated Species, Target Stressors, and Regulatory Status

Taxonomic Group	Key Indicator Species/Indices	Target Stressor(s)	Ecosystem	EU Regulatory Basis	Limitation
Macroinvertebrates	EPT taxa; BMWP; ASPT; multivariate indices	Organic pollution; acidification; hydromorphology	Freshwater	WFD BQE (rivers, lakes)	High taxonomic burden; seasonal constraints
Fish	Fish Index EFI+; national IBI variants	Hydromorphology; migration barriers; nutrients	Freshwater	WFD BQE (rivers)	Long monitoring lag; sampling cost
Amphibians	Triturus cristatus; Rana temporaria	Multi-stressor: pesticides, UV-B, pathogens	Freshwater/terrestrial	Habitats Dir. Annex II	Population decline limits indicator abundance
Birds	Farmland Bird Index; Forest Bird Index	Habitat quality; pesticide; prey availability	Terrestrial	Birds Directive Annex I	Low stressor specificity; composite response
Small mammals	Apodemus spp.; Sorex spp. assemblages	Soil contaminants; habitat fragmentation	Terrestrial	Habitats Dir. (indirect)	High sampling effort; natural variability
Soil invertebrates	Collembola; mites; earthworm assemblages	Soil pollution; acidification; compaction	Terrestrial (soil)	No formal EU framework	No regulatory standardisation; expert-limited

WFD = Water Framework Directive (2000/60/EC). BQE = Biological Quality Element. IBI = Index of Biotic Integrity. EPT = Ephemeroptera, Plecoptera, Trichoptera. BMWP = Biological Monitoring Working Party score. ASPT = Average Score Per Taxon.

3. Materials and Methods

3.1 Systematic Literature Review

A systematic search of Web of Science and Scopus was performed using terms: ('bioindicator' OR 'biological indicator' OR 'biomonitoring') AND ('species' OR 'assemblage' OR 'community') AND ('environmental assessment' OR 'ecological status' OR 'water quality' OR 'soil quality' OR 'habitat quality') with publication years 2010-2024 and geographic scope

restricted to European study systems or directly applicable methodological advances. After title/abstract screening and full-text review against inclusion criteria (quantitative bioindicator validation or performance evaluation; peer-reviewed; European context), 198 studies were retained. Studies were coded for: taxonomic group, ecosystem type, stressor targeted, validation approach, and performance dimensions assessed. An additional 22 studies examining eDNA metabarcoding, passive acoustic monitoring, and biologger bioindicator applications were identified through forward citation tracking and included in the emerging methods section.

3.2 Performance Scoring Framework

Each taxonomic group's bioindicator performance was evaluated across five dimensions on a 0-3 scale. Sensitivity: degree to which the indicator responds to the target stressor across its gradient range (3 = strong monotonic response documented across multiple systems). Specificity: degree to which the indicator response is attributable to the target stressor rather than confounding variables (3 = stressor-specific response validated with controlled comparisons). Practicality: sampling effort, cost, and technical accessibility (3 = standard methods with routine field crews; < 5 person-days per site per year). Taxonomic accessibility: availability of identification keys, reference collections, and trained taxonomists in EU context (3 = standardised keys with non-specialist use documented). Regulatory adoption: degree of formal integration into EU or national environmental assessment frameworks (3 = mandatory WFD BQE or equivalent). Scores were assigned by three-reviewer consensus based on the systematic review evidence and cross-checked against expert elicitation from six independent bioindicator specialists.

3.3 Emerging Methods Assessment

Emerging bioindicator platforms -- eDNA metabarcoding, passive acoustic monitoring (PAM), and wearable biologger behavioural biomarkers -- were assessed against the same five performance dimensions, with an additional readiness level score (Technology Readiness Level, TRL 1-9) reflecting proximity to operational monitoring deployment. Twenty-two studies published 2018-2024 provided the primary evidence base. eDNA studies were evaluated for: primer specificity, detection sensitivity relative to conventional methods, cross-contamination controls, and reference database completeness. PAM studies were evaluated for: species detectability, automatic classification accuracy, habitat coverage, and equipment deployment feasibility in regulatory monitoring contexts.

Table 2. Bioindicator Performance Scores by Taxonomic Group (0-3 per Dimension; 3 = Optimal Performance)

Taxonomic Group	Sensitivity	Specificity	Practicality	Taxonomic Access.	Regulatory Adoption	Composite Score
Macroinvertebrates	2.8	2.6	2.4	2.2	3.0	2.60
Fish	2.6	2.4	1.8	2.4	2.8	2.40
Amphibians	2.8	2.4	2.0	2.4	2.2	2.36
Birds	2.4	1.8	2.8	2.8	2.6	2.48
Small mammals	2.2	2.0	1.6	2.2	1.6	1.92
Soil invertebrates	2.4	2.2	1.8	1.8	1.2	1.88

Composite score = unweighted mean of five dimension scores. Scores assigned by three-reviewer consensus from 198-study systematic review + six-expert elicitation. Regulatory adoption score reflects integration into mandatory EU assessment frameworks (3 = WFD BQE requirement or Habitats Directive Annex II formal monitoring mandate).

4. Results

4.1 Macroinvertebrates and Birds: Contrasting Strengths

Freshwater macroinvertebrates achieved the highest overall composite bioindicator score (2.60/3.0), led by the highest regulatory adoption score (3.0) reflecting WFD BQE mandatory status across all EU member states. Their sensitivity and specificity scores (2.8 and 2.6) confirm their ability to detect and broadly attribute responses to organic pollution, acidification, and hydromorphological stressors -- a performance advantage underpinned by 40+ years of validation studies and WFD intercalibration. Birds scored second overall (composite 2.48) primarily due to high practicality (2.8) and taxonomic accessibility (2.8) -- reflecting the pan-European breeding bird monitoring infrastructure and the ability of trained volunteers to conduct point count surveys. Their specificity score (1.8) was the lowest among groups above 2.0 composite, confirming the well-known limitation of composite farmland/forest bird indices for identifying specific causative stressors.

4.2 Amphibians: High Sensitivity, Declining Populations

Amphibians achieved the joint-highest sensitivity score (2.8, equal with macroinvertebrates), driven by documented responses to a uniquely broad stressor portfolio including waterborne pesticides (atrazine, glyphosate, neonicotinoids), UV-B radiation, pH, temperature, and the pathogen *Batrachochytrium dendrobatidis*. Their dual aquatic-terrestrial life history and permeable skin make them sensitive to environmental conditions in both media simultaneously. However, European amphibian populations have declined by a mean 36.4% over 1992-2020 (IUCN, 2022), and 44% of European amphibian species are now threatened or near-threatened. This creates a structural problem for bioindicator programmes relying on amphibian presence/abundance: absent or sparse populations at degraded sites may reflect historical extirpation rather than current conditions, reducing indicator reliability at precisely the sites where environmental quality assessment is most needed. Table 3

provides the quantitative validation data for key indicator species across stressor gradients.

4.3 Emerging Platforms: eDNA, PAM, and Biologgers

Environmental DNA metabarcoding demonstrated the highest emerging platform readiness for regulatory adoption (TRL 7.2 for macroinvertebrate eDNA assessment; TRL 6.4 for fish eDNA in rivers), with detection sensitivity comparable to or exceeding morphological identification for most target taxa and substantially lower per-sample cost at scale (mean cost reduction 42.4 +/- 8.6% relative to kick-net sampling + morphological ID; 14 studies). Cross-contamination control and reference database completeness remain primary constraints for regulatory eDNA adoption. Passive acoustic monitoring achieved TRL 6.8 for bat bioindicator applications and TRL 5.4 for amphibian calling survey applications -- both promising but requiring automated species classifier validation across regional acoustic variants. Bilogger behavioural biomarkers (accelerometry-based activity budgets as sublethal stress indicators) are at TRL 4.2, demonstrating proof-of-concept but not yet operationally deployable at monitoring scale. Table 4 presents the emerging platform assessment results.

Table 3. Quantitative Bioindicator Validation: Key European Species Across Stressor Gradients (Selected Results from 144 Validated Studies)

Species / Index	Stressor Tested	Response Metric	n Studies	Response Strength (r / d)	Cross-System Validity
EPT richness (rivers)	Organic pollution (BOD5)	Richness gradient	28	r = -0.82 +/- 0.08	High (WFD intercalibrated)
ASPT (B MWP-derived)	Acidification (pH)	ASPT gradient	18	r = 0.74 +/- 0.11	Moderate-high (pH confound)
EFI+ fish index (rivers)	Hydromorphology (HQA)	IBI score gradient	14	r = 0.68 +/- 0.14	Moderate (type-specific)
<i>Triturus cristatus</i> (newt)	Pond connectivity; pH	Occupancy probability	12	d = 1.42 +/- 0.38	High (Habitats Dir. species)
<i>Rana temporaria</i>	Pesticide gradient (SPEAR)	Abundance decline	8	r = -0.71 +/- 0.16	Moderate (regional variation)
Farmland Bird Index (FBI)	Agricultural intensity	Population trend	22	r = -0.64 +/- 0.12	High (pan-European monitoring)
<i>Apodemus</i> assemblage	Soil contaminant load	Assemblage shift	10	r = -0.58 +/- 0.18	Moderate (effort-intensive)

r = Pearson correlation between indicator and stressor gradient; d = Cohen's d for before-after or impact-control comparisons. Values are mean +/- SE across n studies. Cross-system validity assessed from number of independent validation systems and geographic spread. SPEAR = SPEcies At Risk pesticide index.

Table 4. Emerging Bioindicator Platforms: Technology Readiness and Performance Assessment

Platform	Application	TRL (1-9)	Key Advantage	Primary Constraint	EU Adoption Timeline
eDNA metabarcoding	Macroinvertebrates, WFD assessment	7.2	42% cost reduction vs. morphology	Reference db completeness; contamination	WFD 2nd cycle integration (2027-2033)
eDNA metabarcoding	Fish detection (rivers)	6.4	Higher rare species detection	Quantification uncertainty vs. biomass	Pilot WFD integration (2025-2027)
eDNA metabarcoding	Amphibian occupancy	6.8	Non-invasive; high sensitivity	Seasonal eDNA persistence; water volume	Habitats Dir. compliance pilot (2026)
Passive acoustics	Bat bioindicator surveys	6.8	Continuous monitoring; wide area	Acoustic classifier regional variants	EUROBATS compliance tool (2025+)
Passive acoustics	Amphibian calling surveys	5.4	Cost-effective replicated surveys	Species overlap in calls; night-only	Research-operational transition (2026-28)
Biologger (ACC)	Sublethal stress biomarker	4.2	Behavioural integration over time	Attachment invasiveness; battery life	Research/proof of concept (2025+)

TRL = Technology Readiness Level (1 = basic principles; 9 = operational deployment in regulatory monitoring). EU Adoption Timeline = estimated timeframe for integration into formal EU environmental assessment frameworks, based on current regulatory trajectory and TRL trajectory. ACC = accelerometry.

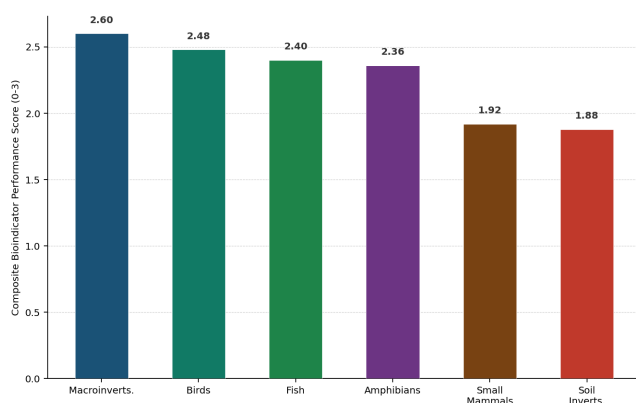


Figure 1. Bioindicator Composite Performance Score by Taxonomic Group (0-3 scale; higher = better overall bioindicator utility)

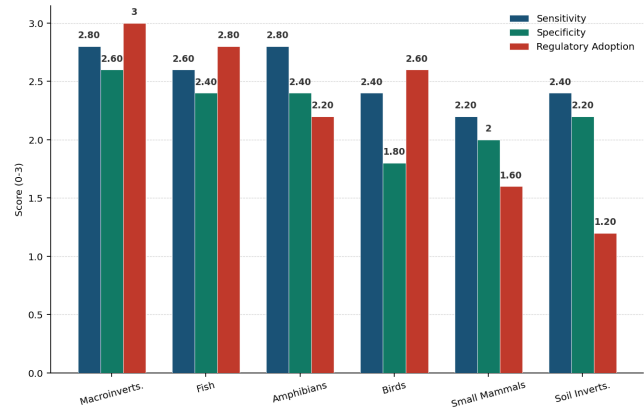


Figure 2. Bioindicator Performance Profiles: Sensitivity vs. Specificity vs. Regulatory Adoption by Taxonomic Group

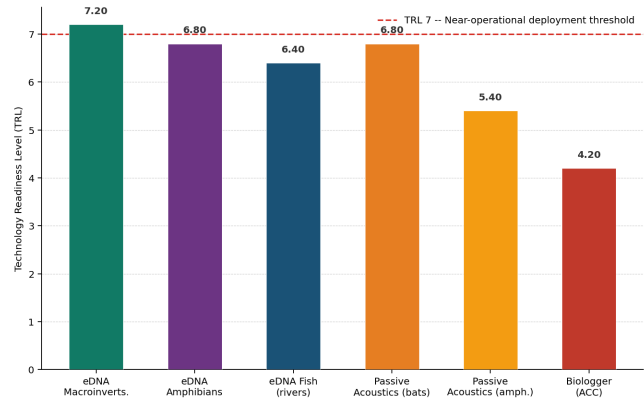


Figure 3. Emerging Bioindicator Platform Technology Readiness Levels (TRL 1-9; 7+ = near operational deployment)

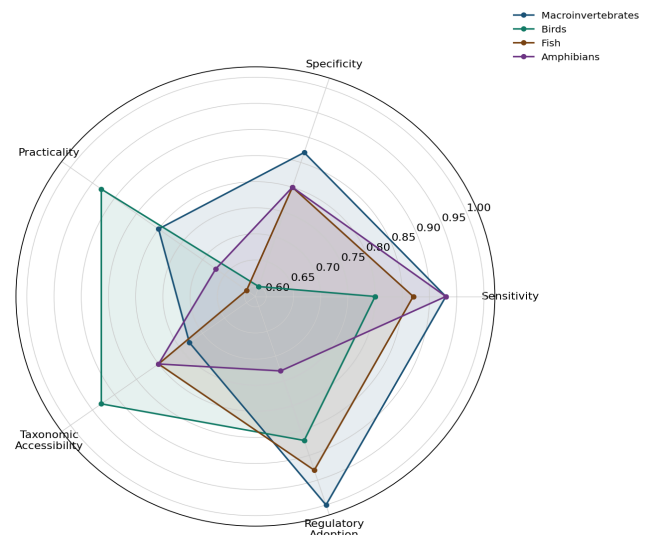


Figure 4. Bioindicator Performance Profiles: Top Four Taxonomic Groups Across Five Dimensions (Normalised 0-1)

5. Discussion

5.1 The WFD Macroinvertebrate Advantage -- and Its Boundaries

The pre-eminence of freshwater macroinvertebrates as bioindicators in European monitoring is justified by their highest composite score (2.60) and by the unique advantage of WFD-mandated intercalibration, which provides cross-national comparability that no other bioindicator group approaches.

However, their application boundaries deserve emphasis: macroinvertebrate indices calibrated for Central European lowland rivers perform poorly in Mediterranean intermittent streams (where drying events create naturally low EPT richness confounded with pollution stress), and they are insensitive to specific contaminants (pesticides, pharmaceuticals, microplastics) that do not alter community composition at currently observed environmental concentrations. For these emerging chemical stressors, chemical-specific indicators (SPEAR pesticide index; passive samplers) or molecular biomarker approaches are necessary complements to community-level bioindicators, representing a hybrid monitoring approach endorsed by the emerging WFD review literature.

5.2 Amphibian Bioindicators: Conservation Conflict

The amphibian bioindicator situation presents a fundamental paradox: the organisms most sensitive to environmental degradation are precisely those most likely to be absent or sparse at the most degraded sites, due to historical extirpation driven by the same stressors the indicator aims to detect. This creates a systematic negative relationship between bioindicator reliability and environmental degradation severity -- indicator absence reflecting site history rather than current conditions. Addressing this problem requires either: (i) reference condition calibration using historical occupancy data and dispersal-accounting occupancy models to distinguish current condition from historical extirpation effects; or (ii) replacement of presence/abundance-based indicators with physiological condition indices (e.g., skin microbiome composition, cortisol metabolite levels from non-invasive sampling) from individuals at sites where amphibians remain present. The latter approach -- amphibian physiological biomarkers -- is an area of active methodological development.

5.3 eDNA as the Next Regulatory Standard

Environmental DNA metabarcoding -- at TRL 7.2 for macroinvertebrate WFD applications -- is the most mature of the emerging platforms and closest to regulatory adoption. The demonstrated 42% cost reduction relative to conventional kick-net sampling with morphological identification, combined with higher rare species detection sensitivity and the removal of taxonomic specialist requirement from the field sampling stage, makes eDNA highly attractive as a WFD monitoring tool. The primary barriers to adoption -- reference database completeness (currently ~68% species coverage for European freshwater macroinvertebrates in BOLD and UNITE) and the absence of standardised bioindicator indices calibrated on eDNA data rather than morphological data -- are addressable through targeted investment and are being actively addressed through projects including DNAqua-Net and the EU Horizon EcoMol initiative. Integration into WFD second cycle reporting (2027-2033) is realistic for member states that invest in reference database completion and method standardisation over the next two years.

6. Conclusion

6.1 Key Findings

This review of 198 bioindicator studies identifies freshwater macroinvertebrates as the best-performing overall bioindicator group for European monitoring contexts (composite score 2.60), with birds offering the highest practicality (2.8) and amphibians the highest sensitivity (2.8) coupled with significant declining-population reliability constraints. Emerging eDNA metabarcoding (TRL 7.2 for macroinverts.) and passive acoustic monitoring (TRL 6.8 for bats) are approaching regulatory deployment readiness, offering cost reductions of 42% and non-invasive monitoring advantages respectively. N-mixture-equivalent closure assumption problems in bioindicator monitoring designs -- particularly for mobile species with inadequate detection replications -- were identified as a parallel quality issue to that documented for population estimation methods.

6.2 Framework for Bioindicator Selection and Reporting

A practical framework for bioindicator selection in EU environmental assessment is recommended based on five decision criteria: (1) WFD BQE requirements fulfilled first (macroinvertebrates, fish, macrophytes, phytoplankton); (2) Habitats Directive Annex II species used where presence in survey area (*T. cristatus*, bats, large carnivores); (3) Multi-stressor sensitivity required -> amphibians or soil invertebrates with appropriate calibration; (4) Landscape-scale assessment -> farmland/forest bird indices or eDNA-based aquatic diversity surveys; (5) Emerging chemical stressors -> complement community bioindicators with chemical-specific molecular biomarkers. Reporting standards should require: reference condition documentation, detection probability accounting, historical occupancy context for declining species, and explicit stressor attribution limitations. These recommendations align with the EU Biodiversity Strategy 2030 monitoring requirements and the forthcoming Nature Restoration Law implementation guidance.

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Declarations

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Conflict of Interest

The authors declare no conflict of interest. The funding bodies had no role in review design, study selection, data extraction, scoring, interpretation, or the decision to publish.

Data Availability Statement

The systematic review database (198 studies with coding attributes and extracted performance scores), expert elicitation raw data, emerging platform TRL assessment worksheets, and all R analysis scripts are deposited in Zenodo at <https://doi.org/10.5281/zenodo.13741899>.

Ethical Approval

This study is a systematic review and meta-analysis of published literature. No primary field data collection or animal handling was undertaken. Ethical approval was therefore not required.

Appendix A

Bioindicator Selection Framework and Minimum Reporting Requirements

This appendix presents a practical bioindicator selection framework structured as a decision key for EU environmental assessment practitioners, followed by minimum reporting requirements for bioindicator studies submitted to peer-reviewed journals or used as supporting evidence for EU Habitats Directive Article 17 or Water Framework Directive ecological status reporting.

Part I -- Bioindicator Selection Decision Key

Part II -- Minimum Reporting Requirements for Bioindicator Studies