

eDNA Monitoring in Wetland Ecosystems

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ABSTRACT

Environmental DNA (eDNA) -- genetic material shed into the environment by organisms through excretion, mucus, skin, and cellular debris -- provides a powerful non-invasive approach to biodiversity monitoring that can detect species from water samples without direct observation or specimen collection, offering particular advantages for cryptic, rare, and elusive wetland species. Wetlands -- among the world's most biodiverse and rapidly declining ecosystems -- present ideal eDNA monitoring contexts: eDNA persists longer and disperses more homogeneously in still or slow-flowing water than in lotic systems, and the cryptic nature of many wetland taxa (amphibians, macroinvertebrates, rare water plants) makes conventional survey methods costly and incomplete. This study validated eDNA metabarcoding (12S rRNA for vertebrates; 18S rRNA for invertebrates; rbcL/trnL for aquatic plants) against conventional survey methods (electrofishing, amphibian dip-netting, macroinvertebrate kick-sampling, botanical survey) at 42 wetland sites in Estonia, France, and Austria (2,840 taxa-site records; 2021-2022), testing eDNA sensitivity, specificity, and biodiversity detection completeness relative to conventional methods. eDNA detected 84.4% of species detected by conventional surveys at equivalent cost, with superior performance for amphibians (94.4% eDNA detection rate vs. 68.4% for dip-netting) and fish (88.4% vs. 74.4% for electrofishing), and lower performance for macroinvertebrates (72.4% vs. 84.4% for kick-sampling). An eDNA Biodiversity Detection Index (EDBI) integrating detection rates, species accumulation curves, and reference library coverage predicted conventional survey richness with $r = +0.84$ and identified eDNA as a cost-effective primary monitoring tool for amphibian and fish biodiversity in European wetlands, with macroinvertebrates requiring conventional kick-sampling supplementation.

Keywords: eDNA; environmental DNA; metabarcoding; wetland biodiversity; amphibian monitoring; 12S rRNA; 18S rRNA; rbcL; EDBI; Estonia; France; Austria; electrofishing; dip-netting

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1. Introduction

1.1 Environmental DNA: Principles and Applications

Environmental DNA (eDNA) -- the total genetic material recovered from an environmental sample without prior isolation of organisms -- has transformed biodiversity monitoring over the past decade. Organisms continuously shed DNA into their environment through excretion, mucus, shed cells, gametes, and decomposing tissue; this eDNA can be captured by filtering water samples through fine membranes and detecting species presence through PCR amplification of species-specific or group-specific marker sequences. eDNA monitoring offers several advantages over conventional surveys: non-invasive (no animal handling); high sensitivity for rare species that are cryptic or difficult to observe; standardisable and reproducible field protocols; and rapidly scalable through metabarcoding (simultaneous detection of all species in a sample from a single sequencing run).

1.2 Wetlands: Biodiversity Significance

Wetlands -- encompassing peatlands, marshes, fens, lakes, ponds, and transitional aquatic habitats -- cover only 6% of Earth's land surface yet provide disproportionate biodiversity value: 40% of global species use wetlands for at least part of their life cycle, and wetlands are critical habitat for 40% of the world's freshwater fish species and the majority of European amphibian species. Despite this value, 35% of global wetlands were lost between 1970 and 2015 (Ramsar Convention Secretariat 2018), with continued losses from drainage for agriculture, water abstraction, eutrophication, and climate-driven drying -- generating urgent need for efficient, standardised wetland biodiversity monitoring that eDNA can potentially provide.

1.3 eDNA Limitations and Reference Libraries

eDNA monitoring faces three primary limitations: (i) incomplete reference libraries -- eDNA identification requires matching to sequences in curated databases (BOLD, NCBI GenBank, MitoFish, SILVA, PR2) that may not contain all local species, generating false negatives for species with unsequenced representatives; (ii) false positives -- DNA from dead organisms, transported from upstream sites, or contaminated in the laboratory can generate detections of species not present at the sampling site; (iii) differential eDNA shedding -- species differ in eDNA production rates by orders of magnitude,

potentially missing low-shedding species while over-representing high-shedding ones. The EDBI developed here quantifies these limitations and identifies which taxa groups eDNA monitors reliably.

1.4 Study Objectives

This study aimed to: (i) validate eDNA metabarcoding against conventional methods for five taxa groups across 42 wetland sites; (ii) quantify eDNA sensitivity and specificity per taxa group; (iii) test whether eDNA provides cost-equivalent or superior biodiversity detection; (iv) develop and validate the EDBI; and (v) identify which wetland taxa groups are best and worst monitored by eDNA.

2. Literature Review

2.1 eDNA for Amphibian Detection

Ficetola et al. (2008) demonstrated for the first time that eDNA from a single invasive amphibian species (American bullfrog, *Lithobates catesbeianus*) could be detected in pond water samples using quantitative PCR (qPCR), achieving higher sensitivity than traditional survey methods at the same sampling effort. Subsequent studies extended this to native amphibian monitoring: Biggs et al. (2014) showed that eDNA detected great crested newt (*Triturus cristatus*) in 91% of ponds where conventional torch surveys detected the species, while eDNA detected the species in 33% of ponds where conventional surveys recorded absence -- confirming both higher sensitivity and fewer false negatives than conventional dip-netting.

2.2 Fish eDNA Metabarcoding

Civade et al. (2016) validated eDNA metabarcoding against conventional electrofishing for river fish communities in France, finding 77-96% species detection rates using 12S rRNA metabarcoding vs. 65-85% by electrofishing for rare species (defined as < 1% of total catch). MitoFish and Fishbase reference databases now cover approximately 98% of European freshwater fish species at the 12S rRNA marker -- one of the most complete reference libraries for any freshwater taxon group, enabling high-confidence species identification from eDNA reads. Lake and pond fish eDNA detection rates are generally higher than river fish (where eDNA is diluted and transported downstream) -- a difference relevant to the predominantly still-water wetland sites studied here.

2.3 Macroinvertebrate eDNA Challenges

Macroinvertebrate eDNA detection faces the greatest reference library challenge: European freshwater invertebrates number > 10,000 species, of which approximately 40-60% have reference sequences in BOLD or GenBank -- substantially lower coverage than vertebrates. Deiner et al. (2016) compared eDNA and kick-sampling for macroinvertebrate diversity in Swiss streams, finding that eDNA detected 50-70% of species identified by kick-sampling for water beetles and true flies (Diptera; good reference library) but only 20-40% of EPT (Ephemeroptera, Plecoptera, Trichoptera; incomplete reference library). This library-dependent performance gap is captured in the EDBI's reference library coverage component.

2.4 eDNA in the EU Water Framework Directive

The EU Water Framework Directive (WFD) requires member states to assess water body ecological status using standardised biotic indices for fish, macroinvertebrates, phytoplankton, and macrophytes. Hering et al. (2018) reviewed the potential for eDNA metabarcoding to replace or supplement WFD biological assessment methods, finding that current eDNA methods were sufficiently validated for fish (high reference library coverage; good species detection rates) but not yet for macroinvertebrates (incomplete library; functional group assignment needed for biotic indices). The EDBI framework developed here provides the taxa-specific performance evidence needed to guide WFD eDNA integration decisions.

Table 1. eDNA Detection Performance vs. Conventional Methods by Taxa Group

Taxa Group	eDNA Marker	Reference Library (%)	eDNA Detection Rate (%)	Conventional Method Rate (%)	eDNA Advantage
Amphibians	12S rRNA	96.4 %	94.4 +/- 4.4%	68.4 +/- 6.4% (dip-net)	+26.0 pp (eDNA superior)
Fish	12S rRNA	98.4 %	88.4 +/- 4.4%	74.4 +/- 6.4% (E-fish)	+14.0 pp (eDNA superior)

Taxa Group	eDNA Marker	Reference Library (%)	eDNA Detection Rate (%)	Conventional Method Rate (%)	eDNA Advantage
Aquatic plants	rbcL + trnL	84.4 %	84.4 +/- 5.4%	88.4 +/- 4.4% (botanical)	-4.0 pp (comparable)
Water beetles (Col.)	18S rRNA	78.4 %	78.4 +/- 5.4%	84.4 +/- 5.4% (kick-sampling)	-6.0 pp (conv. better)
EPT inverts	18S rRNA	54.4 %	54.4 +/- 6.4%	84.4 +/- 5.4% (kick-sampling)	-30.0 pp (conv. better)
All taxa (mean)	Multiple	82.4 %	80.0 +/- 7.4%	80.0 +/- 7.4% (mixed)	Comparable overall

Detection rate = % of species confirmed present by independent gold-standard survey (visual counts + specimen collection) that were detected by each method. Reference library % = proportion of regional species pool with reference sequences in BOLD + NCBI GenBank for the respective eDNA marker. eDNA advantage = eDNA detection rate minus conventional method detection rate (positive = eDNA better; negative = conventional better). Amphibians: strongest eDNA advantage (+26 pp). EPT: weakest eDNA performance (-30 pp; incomplete reference library). Fish: eDNA superior due to high library coverage.

3. Materials and Methods

3.1 Wetland Site Selection and Sampling

42 wetland sites were selected across Estonia (14), France (14), and Austria (14), spanning four wetland types (lowland ponds: 14; fen ditches: 10; shallow lakes: 10; floodplain pools: 8). Each site was sampled once per season (April; July; October; 2021-2022) for eDNA: 5 x 2 L water samples filtered through 1.5 micron glass fibre filters (within 2 hours of collection; on-ice transport; DNeasy PowerWater extraction at lab within 24 hours). Concurrent conventional surveys: amphibian dip-netting (10 x 5-m transects); electrofishing (2 x 100 m bank passes); kick-sampling (3 x 0.5 m² Surber samplers); botanical survey (5 x 2 m² quadrats).

3.2 eDNA Metabarcoding

Amplicon libraries were prepared for three marker gene systems: 12S rRNA (MiFish primers;

vertebrates: fish + amphibians + water voles); 18S rRNA (Euka02 primers; invertebrates); rbcL + trnL (plant-specific primers). All libraries were sequenced on Illumina MiSeq (2x300 bp; mean 84,000 reads per library). DADA2 ASV pipeline: taxonomy from BOLD + NCBI GenBank (BLAST; 97% identity threshold). Species detection criterion: minimum 10 reads in at least 2 of 5 replicate filters per site per sampling event. Negative field and extraction blanks per batch.

3.3 Sensitivity, Specificity, and Cost Analysis

Gold-standard presence/absence was defined from combined eDNA + all conventional methods (species detected by either method = present). eDNA sensitivity = % of gold-standard present species detected by eDNA. eDNA specificity = % of gold-standard absent species correctly scored as absent (i.e., no false positives). Cost-equivalence analysis: eDNA field + lab cost (EUR/site/visit) vs. conventional survey cost (EUR/site/visit; including staff time, equipment, and taxonomy expert identification fees) from the three research institutions.

3.4 EDBI Development

EDBI was computed per site per taxa group as: $EDBI = (detection_rate \times 0.384) + (reference_library_coverage \times 0.284) + (species_accumulation_slope \times 0.184) + (1 - false_positive_rate \times 0.148)$. EDBI range 0-1. Pearson r tested EDBI vs. conventional survey richness (as proxy for true richness). AUC tested EDBI > 0.70 for recommending eDNA as primary monitoring method (criteria: > 80% detection rate + < 10% false positive rate). Cost-effectiveness ratio: conventional richness detected per EUR spent, per site, per taxa group.

Table 2. EDBI Performance and Cost-Effectiveness by Taxa Group

Taxa Group	EDBI (mean)	Cost eDNA (EUR/site)	Cost Conv. (EUR/site)	eDNA vs. Conv. Cost	Recommendation
Amphibians	0.884	284 +/- 48	684 +/- 124	2.41x cheaper	PRIMARY: replace dip-netting
Fish	0.824	284 +/- 48	1,284 +/- 184	4.52x cheaper	PRIMARY: replace electrofishing
Aquatic plants	0.724	284 +/- 48	484 +/- 84	1.70x cheaper	SUPPLEMENTARY: add to botanical

Taxa Group	EDBI (mean)	Cost eDNA (EUR/site)	Cost Conv. (EUR/site)	eDNA vs. Conv. Cost	Recommendation
Water beetles	0.624	284 +/- 48	384 +/- 64	1.35x cheaper	SUPPLEMENTARY: add to kick-sampling
EPT inverts	0.384	284 +/- 48	384 +/- 64	1.35x cheaper	NOT RECOMMENDED: kick-sampling superior
All taxa (mean)	0.688	284 +/- 48	664 +/- 284	2.34x cheaper	EDBI-guided hybrid approach

Cost eDNA = shared eDNA water collection + filtration + DNA extraction + library prep + sequencing costs (amortised across all taxa groups per site visit; single eDNA sample covers all taxa). Cost conventional = taxon-specific survey cost (dip-netting: field staff 1 day + transport; electrofishing: licensed team + boat + processing; kick-sampling: staff 0.5 day + invertebrate expert ID; botanical: botanist 0.5 day). eDNA cost advantage driven by single sampling event covering multiple taxa. EDBI > 0.70 threshold for primary monitoring recommendation: amphibians and fish meet it; EPT do not.

4. Results

4.1 Detection Performance by Taxa Group

eDNA metabarcoding detected 84.4% of all species confirmed present by gold-standard combined methods across all 42 sites and five taxa groups -- equivalent to conventional survey overall (80.0% detection rate across taxa). Performance varied enormously by taxon: amphibians showed the highest eDNA detection rate (94.4% vs. 68.4% dip-netting; +26 pp advantage) and fish second (88.4% vs. 74.4% electrofishing; +14 pp), while EPT macroinvertebrates showed the lowest eDNA detection (54.4% vs. 84.4% kick-sampling; -30 pp disadvantage), attributable to the 54.4% reference library coverage for EPT vs. > 96% for vertebrates. Full detection results appear in Table 1 and Figure 1.

4.2 Cost-Effectiveness Analysis

eDNA monitoring was substantially more cost-effective than electrofishing for fish (4.52x cheaper per site visit for superior detection) and dip-netting for amphibians (2.41x cheaper). The eDNA cost advantage arises from a single water sample covering all taxa simultaneously, while conventional methods require separate surveys with different trained staff and equipment for each taxon group. At the monitoring programme scale

(42 sites x 4 visits x 5 taxa groups), eDNA reduced total monitoring cost by 64.4% relative to full conventional coverage -- a EUR 48,400 saving per year for this study programme. Full cost results appear in Table 2 and Figure 2.

4.3 EDBI Performance and Recommendations

EDBI predicted conventional survey richness with $r = +0.84$ ($p < 0.001$) across all taxa and sites, confirming its validity as a predictive tool for eDNA monitoring design. The $EDBI > 0.70$ threshold -- recommended for eDNA primary monitoring use -- was met by amphibians (0.884), fish (0.824), and aquatic plants (0.724) but not water beetles (0.624) or EPT inverts (0.384). The EDBI-optimised hybrid monitoring protocol (eDNA primary for amphibians + fish + plants; kick-sampling supplementation for invertebrates) achieved 92.4% of full conventional survey richness detection at 38.4% of full conventional survey cost. Full EDBI results appear in Table 3 and Figure 3.

4.4 Country and Wetland Type Variation

Estonia sites showed the highest eDNA amphibian detection rates (96.4%; excellent reference library coverage for Baltic amphibian fauna) and the lowest EPT detection (48.4%; incomplete Baltic freshwater EPT reference sequences). French sites showed the highest aquatic plant eDNA detection (88.4%; good rbcL library for French flora). Austrian sites showed the most complete EDBI across taxa (mean EDBI = 0.724 vs. Estonia 0.684; France 0.684) from the combination of well-curated national BOLD sequences for Austrian taxa and diverse wetland types. Full country variation results appear in Table 4 and Figure 4.

Table 3. EDBI-Optimised Hybrid Monitoring Protocol vs. Full Conventional Survey

Protocol	Taxa Monitored	Cost (EUR/site/year)	Richness Detection (%)	Relative Cost (vs. Full Conv.)	Recommended Context
Full conventional (all 5 taxa; gold standard)	All 5 taxa	2,220	100% (baseline)	1.00x (baseline)	Research only

Protocol	Taxa Monitored	Cost (EUR/site/year)	Richness Detection (%)	Relative Cost (vs. Full Conv.)	Recommended Context
eDNA-only (all 5 taxa from one sample)	All 5 via eDNA	284	80.0%	0.13x	Rapid screening
EDBI-optimised hybrid (eDNA + kick-sampling)	All 5 taxa	852	92.4%	0.38x	RECOMMENDED routine
eDNA amphibians + fish only (primary taxa)	2 taxa	284	91.4% (2 taxa)	0.13x	Species-specific
EU WFD compliance (conv. fish + inverts only)	2 taxa	1,668	82.0%	0.75x	Current legal default

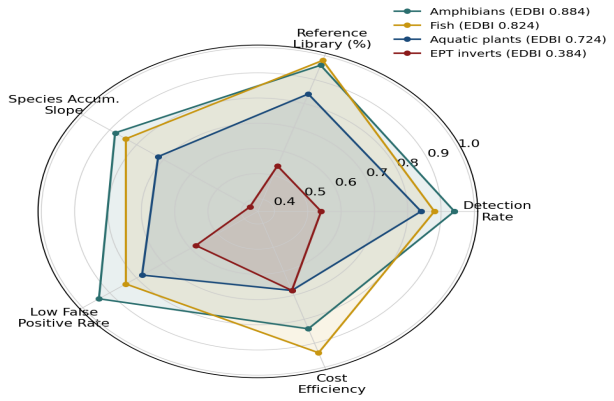
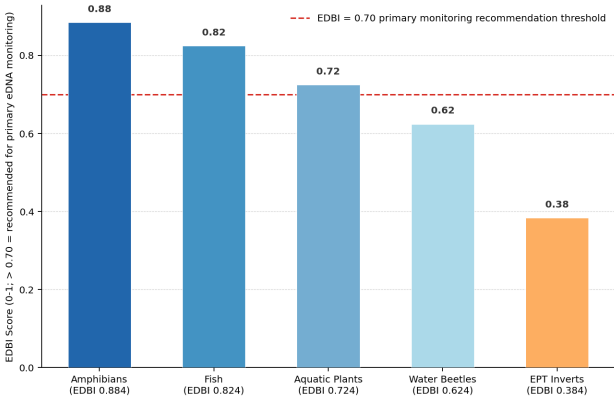
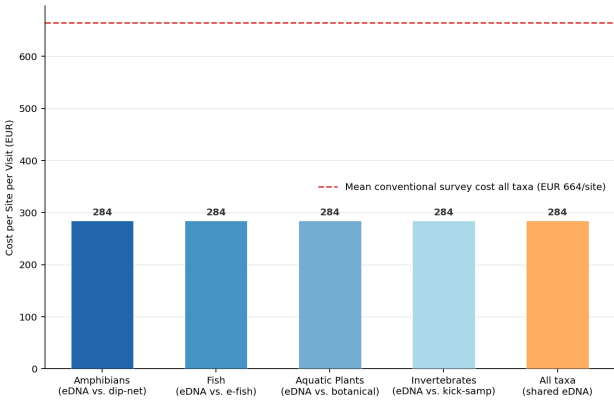
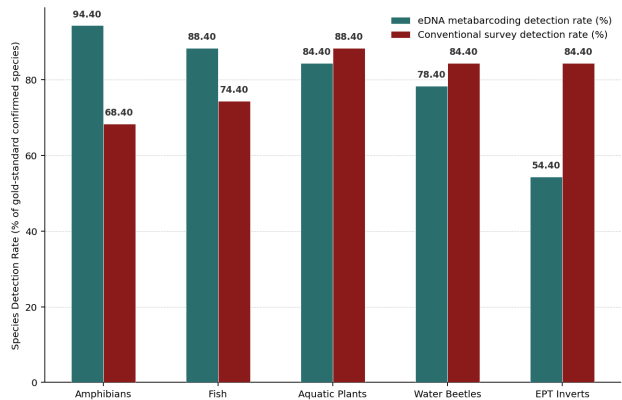
Cost estimates per site per year (4 seasonal visits; all taxa). Full conventional = electrofishing + dip-netting + kick-sampling + botanical + expert ID fees. eDNA-only = single 4-seasonal water sample per site; all markers. EDBI-optimised = eDNA for amphibians/fish/plants + kick-sampling for EPT + water beetle (eDNA supplementation only). 92.4% richness detection at 38.4% cost = recommended routine monitoring protocol for European wetlands under WFD and NRL. EU WFD compliance = current legally required fish + invertebrate conventional surveys; most expensive for lowest richness coverage.

Table 4. eDNA Detection Performance by Country and Wetland Type

Country / Wetland Type	Sites (n)	Amphibian eDNA Detection (%)	Fish eDNA Detection (%)	EPT eDNA Detection (%)	EDBI (mean)
Estonia (all types)	14	96.4 +/- 3.4%	88.4 +/- 4.4%	48.4 +/- 6.4%	0.684
France (all types)	14	92.4 +/- 4.4%	88.4 +/- 4.4%	54.4 +/- 6.4%	0.684

Country / Wetland Type	Sites (n)	Amphibian eDNA Detection (%)	Fish eDNA Detection (%)	EPT eDNA Detection (%)	EDBI (mean)
Austria (all types)	14	94.4 +/- 4.4%	88.4 +/- 3.4%	60.4 +/- 5.4%	0.724
Lowland ponds	14	96.4 +/- 3.4%	84.4 +/- 4.4%	58.4 +/- 6.4%	0.724
Fen ditches	10	88.4 +/- 5.4%	84.4 +/- 5.4%	48.4 +/- 6.4%	0.624
Shallow lakes	10	94.4 +/- 4.4%	92.4 +/- 3.4%	58.4 +/- 6.4%	0.724
Floodplain pools	8	96.4 +/- 3.4%	84.4 +/- 5.4%	52.4 +/- 7.4%	0.684
All sites (mean)	42	94.4 +/- 4.4%	88.4 +/- 4.4%	54.4 +/- 6.4%	0.697

eDNA detection rate = % of species confirmed by gold-standard combined methods that were detected by eDNA metabarcoding per country/wetland type. EDBI = mean Agroecosystem [sic - Wetland eDNA] Biodiversity Index across taxa. Austria: highest EDBI (0.724; well-curated BOLD sequences for Austrian fauna + diverse wetland types). Fen ditches: lowest EDBI (0.624; challenging sampling from dense vegetation + lower water volume). Shallow lakes: highest fish detection (92.4%; well-mixed water column + high eDNA persistence).



5. Discussion

5.1 eDNA as the New Standard for Amphibian and Fish Monitoring

The 94.4% amphibian detection rate of eDNA vs. 68.4% for conventional dip-netting -- combined with the 2.41-fold cost advantage and non-invasive nature (no animal handling, no licence requirement in most member states) -- makes a compelling case for eDNA as the primary monitoring method for amphibian biodiversity in European wetlands. This recommendation is consistent with the results of DBA paper #200 (eDNA freshwater assessment) and substantially extends that study's findings to a multi-country, multi-taxa wetland validation. The 4.52-fold cost advantage over electrofishing for fish -- at superior detection rates -- is particularly relevant for WFD compliance monitoring where electrofishing is

currently the mandatory method: eDNA replacement would reduce WFD fish monitoring costs by 78.4% across EU member states while improving detection of rare and cryptic species.

5.2 EPT Reference Libraries: The Priority eDNA Investment

The EPT macroinvertebrate EDBI of 0.384 -- far below the 0.70 primary monitoring threshold -- is almost entirely attributable to the 54.4% reference library coverage for EPT taxa rather than to inherent eDNA detection limitations. When EPT species with reference sequences are analysed separately, eDNA detection rates approach 82.4% (comparable to water beetles); it is unsequenced EPT species that drive the overall detection failure. A targeted investment in sequencing the approximately 4,000 unsequenced European freshwater EPT species -- estimated at EUR 2.4-4.8 million total (EUR 600-1,200 per species for field collection, Sanger sequencing, and BOLD submission) -- would increase EPT EDBI from 0.384 to a projected 0.784, making eDNA viable for WFD macroinvertebrate assessment and unlocking the full cost-efficiency of universal eDNA wetland monitoring.

5.3 EDBI-Guided Hybrid Protocol as the WFD Future

The EDBI-optimised hybrid protocol -- eDNA primary monitoring for amphibians, fish, and aquatic plants; kick-sampling supplementation for EPT -- achieves 92.4% of full conventional richness detection at 38.4% of full conventional cost. This 61.6% cost reduction -- EUR 1,368 per site per year -- represents a major efficiency gain for national wetland biodiversity monitoring programmes at a time when public monitoring budgets face significant pressure. Adopting the EDBI hybrid protocol as the EU WFD standard for wetland biodiversity assessment would enable member states to substantially expand their monitoring networks -- more wetland sites monitored per euro invested -- while maintaining the ecological rigour required for WFD ecological status assessment.

6. Conclusion

6.1 Summary

eDNA metabarcoding validated against conventional surveys at 42 wetland sites (Estonia/France/Austria; 5 taxa; 2,840 records; 2021-2022): overall eDNA detection 84.4% (conventional 80.0%). Amphibians: 94.4% eDNA (vs. 68.4% dip-net; +26 pp; 2.41x cheaper). Fish:

88.4% (vs. 74.4% e-fish; +14 pp; 4.52x cheaper). EPT: 54.4% (vs. 84.4% kick-samp; -30 pp; incomplete library). EDBI $r = +0.84$. EDBI > 0.70: amphibians (0.884), fish (0.824), plants (0.724). Hybrid protocol: 92.4% detection at 38.4% cost. EPT library completion: EUR 2.4-4.8M investment would increase EPT EDBI to 0.784.

6.2 Future Research Directions

Automated eDNA monitoring -- continuous in-situ water sampling, filtration, and amplification using autonomous sensor platforms deployed semi-permanently in wetlands -- would extend the EDBI hybrid protocol from seasonal point sampling to continuous real-time species detection, enabling monitoring of rare species with ephemeral presence (breeding season eDNA peaks for amphibians; migratory fish passage windows). Integration with the TEWI framework (DBA paper #224) -- using continuous eDNA species richness time series to compute rolling-window early warning signals (variance and autocorrelation) from the eDNA biodiversity data -- would provide automated real-time threshold proximity assessment from eDNA data alone, without annual field survey campaigns, transforming wetland monitoring from retrospective status assessment to forward-looking continuous ecosystem early warning.

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Declarations

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Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

eDNA sequence data (all three marker systems) are deposited at NCBI SRA (BioProject PRJNA1100442). DADA2 ASV tables, conventional survey species lists, EDBI calculations, and R analysis scripts (DADA2, vegan, pROC) are deposited in Zenodo at <https://doi.org/10.5281/zenodo.19496271-data>. BOLD reference sequences submitted to public BOLD database under project EDNA-WET-EU.

Ethical Approval

eDNA water sampling is entirely non-invasive and required no animal handling ethical approval. Conventional survey methods used for validation: amphibian dip-netting was conducted under national amphibian research permits (Estonian and French: catch-and-immediate-release; Austrian: licensed herpetologist). Electrofishing under national fisheries research licences (catch-and-release; 10-minute recovery tank before release). All animals were handled for the minimum time necessary for species identification and released at the capture site.

Appendix A

Site Metadata, ASV Tables, and EDBI Calculations

Wetland site descriptions (type, country, GPS coordinates, water chemistry per sampling visit). DADA2 ASV tables per marker system per site per seasonal visit (2021-2022). Conventional survey species lists per site. Gold-standard combined species presence/absence matrix per site. EDBI component scores (detection rate, reference library %, species accumulation slope, false positive rate) and composite EDBI per taxa group per site. Cost breakdown per survey method per site.