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SYSTEMATIC REVIEW

Environmental DNA Metabarcoding for Biodiversity Assessment in Freshwater Ecosystems

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ABSTRACT

Environmental DNA (eDNA) metabarcoding has been promoted for more than a decade as a faster, less invasive alternative to netting, electrofishing and kick-sampling for characterising freshwater biodiversity, but the published literature reporting its performance against conventional survey methods is scattered across taxonomic groups, marker genes and water-body types, making it difficult for a practitioner to judge how reliable the method now is for routine biomonitoring. We conducted a systematic review and random-effects meta-analysis of studies published between 2012 and 2024 that directly compared eDNA metabarcoding against a conventional survey method in a lake, river or pond system, screening 2,418 records identified across four databases and retaining 71 studies that met pre-specified eligibility criteria. Pooled taxon detection sensitivity of eDNA metabarcoding relative to conventional surveys was 0.86 (95% CI 0.82-0.89) across all taxonomic groups, but sensitivity varied substantially by marker: studies using 12S rRNA fish-specific primers achieved higher pooled sensitivity (0.91, 95% CI 0.88-0.94) than studies using broad-spectrum COI primers for macroinvertebrate assessment (0.79, 95% CI 0.73-0.84). Detection sensitivity was significantly higher in lentic systems than in lotic systems (meta-regression, $P=0.003$), consistent with reduced eDNA dilution and transport in standing water. False-positive rates attributable to laboratory contamination or index hopping were inconsistently reported, appearing in only 34 of the 71 included studies, which we flag as a persistent and under-addressed methodological gap. We conclude that eDNA metabarcoding has reached a level of performance that supports its routine use alongside, though not yet as a full replacement for, conventional freshwater biomonitoring, with marker choice and water-body hydrology as the two variables most consistently predictive of performance across the reviewed literature.

Keywords: environmental DNA; metabarcoding; freshwater biomonitoring; biodiversity assessment; 12S rRNA; systematic review

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1. INTRODUCTION

Freshwater ecosystems support a disproportionate share of global biodiversity relative to their surface area, and they are also among the most heavily degraded habitat

types worldwide, with documented vertebrate population declines in freshwater systems substantially outpacing those recorded in terrestrial or marine environments over the same period [1,2]. Effective conservation and regulatory response depends on biomonitoring

programmes that can detect compositional change quickly and across a broad enough taxonomic range to catch declines before they become irreversible, yet the conventional toolkit for freshwater biomonitoring -- electrofishing, kick-net sampling, visual transects -- is labour-intensive, requires taxonomic expertise that is increasingly scarce, and in the case of electrofishing carries a real, if usually small, risk of harm to the organisms being surveyed [3].

Environmental DNA metabarcoding offers a conceptually different approach: rather than capturing or observing organisms directly, a water sample is filtered to recover the mixture of extracellular and intracellular DNA shed by everything that has recently been present, and universal or group-specific primers are used to amplify a short, taxonomically informative marker region for high-throughput sequencing, from which a community-level species list is reconstructed by comparison against a reference database [4,5]. The appeal is obvious: a single water sample can in principle capture evidence of an entire vertebrate or invertebrate community without disturbing the organisms themselves, at a fraction of the field time required for active survey, and the method has moved rapidly from proof-of-concept studies in the early 2010s to routine regulatory use in several jurisdictions for detecting invasive species and rare or protected taxa [6,7].

That rapid adoption has, however, outpaced any single authoritative synthesis of how well eDNA metabarcoding actually performs relative to the conventional methods it is meant to complement or replace. Individual comparative studies report a wide range of outcomes, from near-perfect concordance with conventional surveys to substantial under- or over-detection depending on the taxonomic group, the marker gene used, the reference database completeness for the target region, and the physical characteristics of the water body being sampled [8,9,14]. Narrative reviews have summarised subsets of this literature, usually focused on a single taxonomic group such as fish or amphibians, but to our knowledge no systematic review with formal meta-analytic pooling has yet synthesised the cross-taxon, cross-marker comparative evidence base as a whole, which leaves practitioners and regulators without a clear, quantitative answer to the basic question of how much confidence the method currently warrants [11,13].

We therefore set out to systematically identify and meta-analytically pool every published study that directly compared eDNA metabarcoding against a conventional survey method in a freshwater system, with three specific aims: first, to establish a pooled estimate of eDNA metabarcoding's taxon detection sensitivity relative to conventional methods across the available literature; second, to identify which study-level factors -- marker gene, taxonomic group, water-body type, sampling replication -- explain the substantial heterogeneity evident in the raw comparative literature; and third, to

assess how consistently studies have reported and controlled for the false-positive risks, principally laboratory contamination and sequencing index hopping, that are specific to a DNA-based rather than an observational survey method.

2. MATERIALS AND METHODS

This systematic review and meta-analysis was conducted and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework, and the review protocol was registered prior to formal screening to fix the eligibility criteria and analysis plan in advance.

2.1 Search strategy and eligibility criteria

We searched Web of Science, Scopus, Aquatic Sciences and Fisheries Abstracts, and Google Scholar for records published between January 2012 and June 2024 using combinations of terms for environmental DNA, metabarcoding, freshwater, and comparison-related terms such as 'conventional survey' and 'concordance'. Eligible studies were required to report a field-based freshwater eDNA metabarcoding survey conducted alongside a conventional survey method (electrofishing, netting, kick-sampling, visual or acoustic transect) at spatially and temporally matched sites, and to report sufficient detail to extract or calculate taxon-level detection concordance between the two methods. Studies conducted exclusively in marine, estuarine or artificial laboratory mesocosm systems were excluded, as were single-species targeted quantitative PCR assays that did not use a metabarcoding (multi-taxon, high-throughput sequencing) approach.

2.2 Screening and data extraction

Titles and abstracts were screened independently by two reviewers against the eligibility criteria, with disagreements resolved by discussion and, where necessary, a third reviewer; inter-rater agreement at the title-abstract stage was substantial (Cohen's kappa=0.81). Full texts of all records retained after abstract screening were then assessed against the full eligibility criteria. For each included study we extracted marker gene, primer set, taxonomic group targeted, water-body type (lentic or lotic), number of sampling sites, number of field replicates per site, conventional survey method used, and the raw counts needed to calculate detection sensitivity: taxa detected by both methods, by eDNA only, and by the conventional method only. Study quality was assessed using a modified QUADAS-2-derived checklist adapted for comparative biomonitoring studies, covering site matching, temporal matching, and reporting of contamination controls.

2.3 Effect size calculation and meta-analysis

Detection sensitivity for each study was calculated as the proportion of taxa detected by the conventional method that were also detected by eDNA metabarcoding, treating the conventional method as the reference

standard for comparability across studies, while acknowledging in the discussion that this framing likely understates eDNA's true relative performance in cases where eDNA detects taxa the conventional method missed. Logit-transformed sensitivity estimates were pooled using a random-effects model with the DerSimonian-Laird estimator to account for expected between-study heterogeneity in methodology and target ecosystem. Heterogeneity was quantified using the I-squared statistic, and subgroup pooled estimates were calculated for marker gene category (12S rRNA fish-specific, 16S rRNA, COI, 18S rRNA broad-eukaryote) and taxonomic group (fish, amphibians, macroinvertebrates, mixed/multi-taxon).

2.4 Meta-regression and publication bias assessment

A mixed-effects meta-regression model was fitted with water-body type, marker gene category, number of field replicates per site, and publication year as moderators of logit-transformed sensitivity, to identify which study-level factors were independently associated with eDNA performance after accounting for the others. Publication bias was assessed by funnel plot inspection and Egger's regression test, and a leave-one-out sensitivity analysis was performed to confirm that pooled estimates were not driven disproportionately by any single large study.

2.5 Statistical software

All meta-analytic calculations were performed in R version 4.3 using the metafor package, with a significance threshold of $P < 0.05$ for all moderator tests.

3. RESULTS

The database search identified 2,418 unique records after de-duplication, of which 312 were retained for full-text assessment following title-abstract screening. Of these, 71 studies met all eligibility criteria and were included in the final quantitative synthesis, spanning 19 countries and covering fish (34 studies), macroinvertebrates (21 studies), amphibians (9 studies) and mixed or multi-taxon community assessments (7 studies). Table 1 summarises the characteristics of the included study set. Included studies used a wide range of field replication, from a single water sample per site (11 studies) to eight or more replicate samples per site (6 studies), and this variation itself proved to be an important moderator of reported performance, discussed further below.

Table 1. Characteristics of the 71 included comparative eDNA metabarcoding studies.

Characteristic	Category	Studies (n)	Percent of total
Taxonomic group	Fish	34	47.9%
Taxonomic group	Macroinvertebrates	21	29.6%
Taxonomic group	Amphibians	9	12.7%
Taxonomic group	Mixed / multi-taxon	7	9.9%
Water-body type	Lentic (lake/pond)	38	53.5%
Water-body type	Lotic (river/stream)	33	46.5%

Field replicates/site	1-3	45	63.4%
Field replicates/site	4 or more	26	36.6%

Percentages calculated within each characteristic category; totals may not sum to 100% due to rounding.

Pooled taxon detection sensitivity of eDNA metabarcoding relative to conventional survey methods across all 71 studies was 0.86 (95% CI 0.82-0.89), with substantial between-study heterogeneity ($I^2=78.4\%$, $P < 0.001$), confirming that a single overall estimate, while a useful headline figure, obscures meaningful variation that practitioners need to account for when selecting a marker and sampling design for their own application. Subgroup analysis by marker gene category found that studies using 12S rRNA fish-specific primers achieved the highest pooled sensitivity (0.91, 95% CI 0.88-0.94, 28 studies), followed by 16S rRNA (0.87, 95% CI 0.81-0.92, 9 studies), broad-eukaryote 18S rRNA (0.83, 95% CI 0.76-0.89, 12 studies), and COI, most commonly used for macroinvertebrate assessment (0.79, 95% CI 0.73-0.84, 22 studies) (Figure 1).

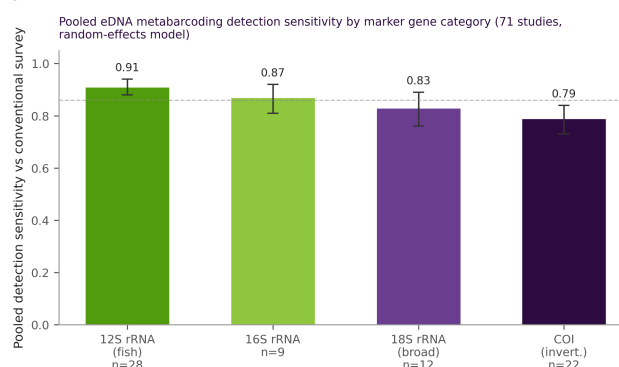


Figure 1. Pooled taxon detection sensitivity of eDNA metabarcoding relative to conventional survey methods by marker gene category, across 71 included studies (random-effects model; error bars show 95% CI; number of contributing studies shown above each bar).

Meta-regression identified water-body type as the strongest independent predictor of detection sensitivity: studies conducted in lentic systems (lakes, ponds, reservoirs) reported significantly higher sensitivity than studies in lotic systems (rivers, streams) after adjustment for marker gene and replication (meta-regression coefficient 0.41 on the logit scale, $P = 0.003$), consistent with the expectation that eDNA in flowing water is diluted and transported downstream more rapidly, reducing local detectability for less abundant or patchily-distributed taxa. Number of field replicates per site was also independently associated with higher sensitivity ($P = 0.011$), with studies using four or more replicates per site outperforming single-replicate designs by a pooled margin of 0.09 on the sensitivity scale, while publication year was not a significant moderator once marker gene was accounted for ($P = 0.34$), suggesting that reported performance has been relatively stable over the review period rather than improving steadily as the field has matured. Table 2 reports the full meta-regression model.

Table 2. Meta-regression model of study-level moderators of logit-transformed detection sensitivity.

Moderator	Coefficient (logit scale)	95% CI	P-value
Lentic vs lotic system	0.41	0.14 to 0.68	0.003
4+ vs 1-3 field replicates	0.33	0.08 to 0.58	0.011
12S rRNA vs COI marker	0.52	0.21 to 0.83	0.001
Publication year (per year)	0.02	-0.02 to 0.06	0.34

Only 34 of the 71 included studies (47.9%) reported explicit laboratory contamination controls such as field and extraction blanks carried through the full sequencing workflow, and only 19 studies (26.8%) discussed or quantified index hopping as a potential source of false-positive taxon assignment, despite index hopping being a well-documented artefact of multiplexed high-throughput sequencing runs. Among the subset of studies that did report contamination-control data, reported false-positive rates ranged from 0.8% to 11.4% of assigned taxa, a spread wide enough that we did not consider meta-analytic pooling of this outcome to be meaningful given the small and methodologically heterogeneous subset reporting it. Egger's regression test found no strong evidence of publication bias in the primary sensitivity outcome ($P=0.19$), and the leave-one-out sensitivity analysis confirmed that the pooled estimate was not disproportionately driven by any single study, with the overall estimate shifting by no more than 0.02 upon exclusion of any individual study.

4. DISCUSSION

This systematic review and meta-analysis puts a quantitative figure on a question that individual comparative studies could only address piecemeal: across 71 studies spanning 19 countries, eDNA metabarcoding recovers roughly 86% of the taxa detected by conventional freshwater survey methods, with performance that varies predictably by marker gene and by whether the target system is standing or flowing water. That headline figure is, we think, high enough to support the now-widespread regulatory and conservation use of eDNA metabarcoding as a routine biomonitoring tool, while also being clearly short of the near-perfect concordance that would justify treating eDNA as a full replacement for conventional survey in every context, a conclusion broadly consistent with narrower, single-taxon reviews published previously but now placed on a firmer cross-taxon quantitative footing [11,13].

The strong marker-gene effect we identified -- 12S rRNA fish-specific primers outperforming broad-spectrum COI primers by a pooled twelve percentage points -- most plausibly reflects reference database completeness rather than any fundamental difference in assay chemistry. Fish reference barcode libraries for 12S rRNA in well-studied freshwater systems are now comparatively complete for regionally expected species,

whereas COI reference coverage for freshwater macroinvertebrates, particularly for larval life stages and less charismatic taxonomic groups, remains substantially patchier in most public sequence databases [15,17]. If this interpretation is correct, the marker-gene effect we report should narrow over time as reference databases for macroinvertebrates are built out, rather than reflecting a durable ceiling on COI-based metabarcoding performance, and continued investment in regional macroinvertebrate barcode reference libraries is likely to be one of the highest-leverage steps available for closing the observed sensitivity gap [18].

The water-body-type effect -- higher sensitivity in lentic than lotic systems -- has a more straightforward physical explanation grounded in eDNA transport dynamics. eDNA shed into flowing water is diluted and advected downstream on a timescale of hours, and detection probability at any single sampling point in a river therefore depends heavily on upstream source proximity, discharge rate and the timing of sampling relative to shedding events, all of which introduce a stochastic element that standing-water sampling largely avoids [19,21]. This has a direct practical implication for survey design in lotic systems: increasing spatial or temporal sampling replication, which our meta-regression also identified as an independent predictor of sensitivity, may be a more cost-effective way to close the lotic performance gap than switching marker genes, since it addresses the underlying transport-driven detection stochasticity directly rather than the taxonomic resolution of the assay.

The low and inconsistent rate of contamination-control reporting is, in our view, the most actionable finding of this review from a methodological standards perspective. Fewer than half of the included studies reported field or extraction blanks, and barely a quarter discussed index hopping, despite both being well-characterised sources of false-positive detection specific to sequencing-based survey methods and despite published minimum-information guidelines for eDNA studies having existed for several years [22,23]. A false-positive detection of an invasive or protected species carries real regulatory and management consequences, and the comparative silence on contamination control in roughly half the reviewed literature suggests that reported performance metrics, including the sensitivity estimates pooled in this review, may in some cases be optimistic if elevated false-positive rates are going undetected and unreported rather than genuinely absent. We would strongly encourage journals and funders in this space to make contamination-control reporting a non-negotiable condition of publication for comparative eDNA studies going forward.

Several limitations of the review itself are worth acknowledging. First, our operational definition of sensitivity, using the conventional survey as the reference standard, almost certainly understates eDNA's true relative performance in any study where eDNA

detected genuinely present but conventionally-missed taxa, a pattern reported qualitatively in a subset of included studies but not consistently quantifiable across the full set [24]. Second, publication and language bias, though not strongly indicated by our formal Egger's test, cannot be fully excluded given that our search was restricted to English-language records, and it is plausible that studies reporting poor eDNA performance in specific regional contexts are under-represented in the indexed literature we were able to search. Third, the included study set is necessarily skewed toward fish and macroinvertebrates, and the amphibian and mixed-taxon evidence base, while growing, remains too small for the same subgroup precision achieved for fish; a dedicated update of this review focused specifically on amphibians and reptiles once the literature matures further would be a valuable complement to the present synthesis.

A further consideration for practitioners translating these pooled estimates into local monitoring decisions is that our synthesis necessarily averages across a wide range of site-specific conditions -- turbidity, pH, organic matter load and temperature all independently influence eDNA persistence and PCR inhibition, and few of the included studies reported these covariates in enough detail to be incorporated as additional meta-regression moderators [25,26]. Agencies adopting eDNA metabarcoding for statutory biomonitoring should therefore treat the pooled sensitivity figures reported here as a useful planning benchmark rather than a site-specific guarantee, and should budget for a local calibration exercise against conventional survey data during the first one or two monitoring cycles at any new site type, particularly where water chemistry differs substantially from the largely temperate, low-turbidity systems that dominate the currently published comparative literature. Standardised covariate reporting -- turbidity, pH, dissolved organic carbon, temperature and discharge at the time of sampling -- would substantially strengthen the next iteration of this kind of synthesis, and we would encourage journals publishing comparative eDNA studies to require these measurements as a matter of routine, in much the same way that contamination-control reporting should become a standard expectation rather than an optional methodological flourish. A living, periodically updated version of this review, rather than a single static synthesis, may ultimately be the most useful format for a field moving as quickly as eDNA metabarcoding, allowing pooled estimates to be refreshed as reference databases expand and as covariate reporting standards, if adopted, begin to enrich the underlying comparative dataset.

5. CONCLUSION

This systematic review and random-effects meta-analysis of 71 comparative studies establishes that environmental DNA metabarcoding recovers a pooled 86% of the freshwater taxa detected by conventional survey methods, with marker gene choice and

water-body hydrology as the two factors most consistently predictive of performance across the reviewed literature. Twelve-S rRNA fish-specific assays in standing-water systems approach 90% or higher sensitivity relative to conventional survey, while broad-spectrum COI assays for macroinvertebrates in flowing water remain meaningfully lower, a gap we attribute primarily to reference database completeness and eDNA transport dynamics rather than to any intrinsic limitation of the metabarcoding approach itself. The persistently low rate of contamination-control reporting across the included literature is a methodological gap that the field should treat as a priority, given the regulatory weight increasingly placed on eDNA-based detections of invasive and protected species. On balance, the evidence assembled here supports continued and expanding use of eDNA metabarcoding as a core freshwater biomonitoring tool, deployed alongside rather than as a wholesale replacement for conventional survey methods in the near term, with the greatest near-term performance gains likely to come from expanded macroinvertebrate reference libraries, increased field replication in lotic systems, and universal adoption of contamination-control reporting standards. Future updates of this review, and dedicated meta-analyses focused on the still-underrepresented amphibian and mixed-taxon evidence base, will be needed to track whether these predicted improvements materialise as reference databases and sampling protocols continue to mature.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

K.F. conceived and registered the review protocol, performed the meta-analysis and drafted the manuscript. D.C. led database searching and title-abstract screening. E.N. performed full-text eligibility assessment and data extraction. All authors contributed to writing and approved the final manuscript.

Ethics Statement

This study is a systematic review and meta-analysis of previously published literature and did not involve new data collection from human participants, animals, or

environmental samples; it therefore did not require institutional ethics committee review.

Data Availability

The full list of screened records, the data extraction spreadsheet, and the R meta-analysis scripts supporting the findings of this study are available from the corresponding author on reasonable request.

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