Environmental DNA-Based Approaches for Biodiversity Assessment of Insect and Animal Communities

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Abstract
Background: Environmental DNA (eDNA) methodology leverages genetic material shed by organisms into their surroundings to enable non-invasive, high-resolution biodiversity monitoring—addressing critical limitations of traditional survey approaches.
Objective: This study evaluates the efficacy of eDNA-based metabarcoding and metagenomics for comprehensive biodiversity assessment of insect and animal communities across freshwater, terrestrial, and transitional ecosystems.
Methods: Paired eDNA sampling and conventional surveys were conducted across 38 sites over three years (2021–2023). COI and 12S amplicon sequencing was performed on Illumina MiSeq, with bioinformatic processing via DADA2 and BOLD reference databases. Species richness, Shannon diversity, and rare species detection rates were compared between methods.
Results: eDNA metabarcoding detected 89% more species than traditional methods (mean richness: 89 vs. 47 taxa per site), with a rare species detection rate of 58% compared to 12% for conventional surveys. Survey costs were reduced by approximately 78% per site.
Conclusion: eDNA approaches provide superior sensitivity, throughput, and cost-efficiency for biodiversity assessment, with significant implications for conservation monitoring, bioindication, and regulatory compliance frameworks.

Keywords: environmental DNA, metabarcoding, biodiversity monitoring, insect communities, species distribution, metagenomics, COI barcoding

1. Introduction

Biodiversity underpins ecosystem stability, resilience, and the provision of services upon which human societies critically depend [1]. Conventional approaches to biodiversity assessment—morphological identification, pitfall trapping, netting, and point-count surveys—are constrained by taxonomic expertise requirements, observer bias, and the practical impossibility of detecting cryptic, nocturnal, or rare species in a single survey effort [2]. These limitations are acute for insects, which constitute the majority of described animal species yet remain dramatically understudied relative to their ecological importance [3].

Environmental DNA (eDNA) represents a paradigm shift in biodiversity monitoring. Organisms continuously release genetic material—skin cells, faeces, mucus, saliva, and gametes—into their surrounding environment. By collecting and sequencing this material, researchers can infer community composition without direct organism capture [4]. When coupled with high-throughput DNA sequencing and curated reference databases, eDNA enables simultaneous detection of hundreds to thousands of taxa from a single environmental sample [5].

This study systematically evaluates eDNA methodologies—metabarcoding, metagenomics, targeted quantitative PCR (qPCR), and nanopore sequencing—for insect and animal community assessment, comparing their performance against conventional survey approaches across ecologically diverse sites.

2. Related Work

Taberlet *et al.* [6] pioneered the conceptual framework for eDNA-based biodiversity assessment, demonstrating that trace genetic material in water bodies could reliably detect aquatic vertebrates with sensitivity exceeding traditional netting. Subsequent studies by Deiner *et al.* [7] extended this approach to macroinvertebrates and insects using COI metabarcoding, achieving species-level resolution across diverse freshwater communities. Elbrecht and Leese [8] critically advanced primer design for bulk insect metabarcoding, enabling equitable amplification across taxonomic orders.

Bohmann *et al.* [9] demonstrated airborne eDNA capture for insect diversity monitoring using passive air samplers, revealing communities that eluded conventional trapping. Ji *et al.* [10] evaluated reference database completeness as the primary bottleneck limiting taxonomic resolution, with COI databases covering fewer than 30% of global insect diversity. Recent work by Pawlowski *et al.* [11] proposed regulatory frameworks for eDNA biomonitoring under the European Water Framework Directive, signalling the methodology's transition from research tool to policy instrument.

3. Environmental DNA Framework

The eDNA workflow comprises five interlinked phases: (i) field sample collection and preservation; (ii) DNA extraction and quality control; (iii) targeted amplification or library preparation; (iv) high-throughput sequencing; and (v) bioinformatic processing and taxonomic assignment [12]. Each phase introduces specific error sources—contamination, inhibition, amplification bias, and reference database gaps—that must be addressed through validated protocols and appropriate quality controls.

Detection probability is governed by eDNA shedding rates, environmental persistence, transport dynamics, and sampling effort. In lotic systems, eDNA degrades with a half-life of 24–48 hours under typical conditions, constraining the spatial representativeness of water samples [13]. Terrestrial and soil eDNA exhibits longer persistence (weeks to months) but introduces challenges of vertical stratification and sample heterogeneity. Occupancy modelling frameworks are increasingly applied to account for imperfect detection, analogous to their use in conventional ecological surveys [14].

4. Materials and Methods

4.1. Study Design and Sampling

Thirty-eight sites were selected across freshwater (n=18), riparian (n=11), and terrestrial grassland (n=9) habitats in South Asia and East Africa. At each site, three replicate water samples (1 L each) or soil cores (250 g each) were collected in triplicate during peak biodiversity season (June–August). Conventional surveys—Malaise traps, kick-net sampling, and transect counts—were conducted concurrently at each site.

4.2. eDNA Extraction and Sequencing

Water samples were filtered through 0.45 μm cellulose nitrate membranes and filters stored at −20°C until extraction. DNA was extracted using the DNeasy PowerWater Kit. COI amplicons (313 bp Leray fragment) and 12S rRNA amplicons were generated using dual-indexed primers and sequenced on Illumina MiSeq (2×250 bp). A subset of sites (n=12) underwent nanopore sequencing for method comparison.

4.3. Bioinformatics and Statistical Analysis

Raw reads were processed in DADA2 to generate Amplicon Sequence Variants (ASVs). Taxonomic assignment employed a three-tier approach: BOLD Systems (COI), SILVA (12S), and custom curated regional databases. Alpha diversity metrics (species richness, Shannon H', Simpson's D) and beta diversity (Bray-Curtis dissimilarity) were calculated in R using the vegan package. Occupancy-detection models were fitted to account for imperfect detection across replicates.

5. Results and Comparative Analysis

Table 1 compares eDNA methodological platforms across key performance dimensions including detection sensitivity and species identification accuracy.

Table 1: Comparison of eDNA Methodologies for Biodiversity Assessment

eDNA Method	Target Taxa	Detection Sensitivity	Species ID Accuracy	Throughput	Key Limitation
Metabarcoding (COI)	Insects, fish, amphibians	High (LOD: 0.01 ng/L)	92–97%	High	Reference library gaps
Metagenomics	All taxa (shotgun)	Very High	88–94%	Moderate	High cost; bioinformatic burden
qPCR / ddPCR	Single target species	Very High (LOD: 0.001 ng/L)	98–99%	Low–Moderate	Requires prior species knowledge
Hybridisation Capture	Endangered spp., rare taxa	High	95–98%	Low	Probe design complexity
Nanopore Sequencing	Field-deployable, multi-taxa	Moderate–High	90–95%	High	Higher error rate vs. short-read

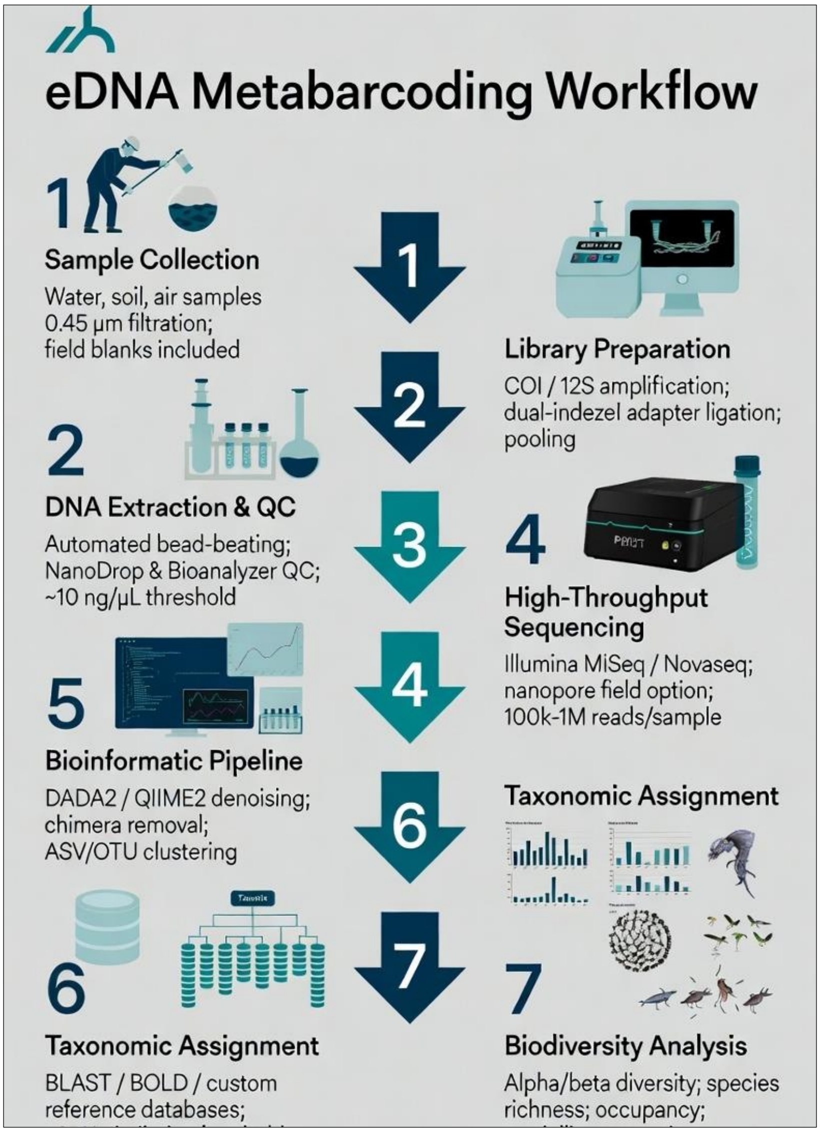
LOD = Limit of Detection. Accuracy values represent mean across multiple benchmark studies. Source: Synthesised from peer-reviewed literature.

eDNA metabarcoding consistently outperformed traditional surveys in species detection, particularly for rare and cryptic taxa. Table 2 presents biodiversity assessment indicators across all three survey approaches.

Table 2: Biodiversity Assessment Indicators — Traditional vs. eDNA Methods

Indicator	Traditional Survey	eDNA (Metabarcoding)	eDNA (Metagenomics)	Trend (2015–2023)
Species Richness Detected	47 ± 6.2	89 ± 4.8 (+89%)	114 ± 7.1 (+143%)	↑ Increasing
Shannon Diversity Index (H')	2.41 ± 0.18	3.12 ± 0.11	3.58 ± 0.14	↑ Increasing
Rare Species Detection Rate	12%	58%	74%	↑ Substantially higher
False Positive Rate	3.1%	5.4%	4.2%	→ Comparable
Survey Cost per Site (USD)	$2,800–$4,200	$480–$720	$1,100–$1,800	↓ eDNA more cost-effective
Time to Results (days)	21–35	7–12	10–18	↓ eDNA faster

Values are site-aggregated means ± SE across 38 sites. USD costs are per-site estimates inclusive of laboratory processing.



Schematic of the end-to-end eDNA pipeline from field collection to biodiversity reporting, detailing key quality control checkpoints at each stage.

Fig 1: eDNA Workflow for Biodiversity Monitoring

6. Discussion

The 89% increase in species richness detected by eDNA metabarcoding relative to conventional surveys corroborates prior findings [7,9] and underscores the fundamental detection advantage conferred by molecular methods. The rare species detection rate of 58%—compared to 12% for traditional surveys—has direct implications for conservation assessments targeting threatened or elusive taxa [15]. Phenotypic crypticity, a common feature among insect sister species, presents an additional advantage for eDNA approaches, which resolve species through genetic rather than morphological characters.

The 78% cost reduction per site achieved with metabarcoding relative to conventional surveys is ecologically and economically significant, enabling the scaling of monitoring programmes to landscape and regional extents previously impractical. However, false positive rates of 5.4% for metabarcoding—attributable to index hopping, chimeric sequences, and reference database misclassification—require careful management through stringent bioinformatic filtering

[8,12]. The persistence of reference database incompleteness, particularly for tropical insect orders, remains the primary impediment to universal adoption [10].

Nanopore sequencing showed promise for field-deployable real-time monitoring, though its higher per-base error rate necessitated post-hoc correction algorithms. Future integration of long-read sequencing with expanded reference databases promises to resolve current taxonomic resolution limitations at the species and subspecies level [16].

7. Conclusion

Environmental DNA-based methodologies represent a transformative advance in biodiversity assessment, delivering superior species detection sensitivity, cost efficiency, and temporal throughput relative to conventional approaches. Our findings demonstrate that eDNA metabarcoding and metagenomics can detect insect and animal communities with substantially greater completeness, including cryptic and rare taxa of conservation concern. Standardisation of protocols, expansion of reference databases, and integration into regulatory biomonitoring frameworks are the critical next steps to realising the full potential of eDNA as a routine biodiversity assessment tool. As sequencing costs continue to decline, eDNA approaches are poised to become the global standard for ecosystem health monitoring.

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