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Role of molecular tools in modern zoology

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ABSTRACT

Molecular tools have transformed zoology from a discipline dependent on morphological observation and field survey to one capable of resolving population structure, species boundaries, dietary ecology, pathogen dynamics, and landscape connectivity from minute biological samples or environmental residues. This review synthesises the applications and impacts of six major molecular tool categories -- DNA barcoding, environmental DNA (eDNA), next-generation sequencing (NGS) including metabarcoding, population genomics, stable isotope analysis, and quantitative PCR -- across five zoological application domains: species identification and discovery, population genetics and connectivity, dietary and trophic ecology, disease surveillance, and non-invasive individual identification. Drawing on 248 primary studies published between 2010 and 2024 and a quantitative assessment of methodological performance metrics, we demonstrate that molecular approaches have expanded zoological detection sensitivity by 2-8-fold for rare and cryptic species relative to traditional survey methods, resolved previously intractable population connectivity questions for wide-ranging and migratory species, and enabled real-time disease surveillance in wildlife populations. eDNA metabarcoding now detects 38-84% more aquatic taxa per sampling event than morphological identification of equivalent kick-net samples. Population genomics (SNP arrays, whole-genome resequencing) has replaced microsatellite-based population genetics as the standard for conservation genetics in the past five years, providing 100-10,000-fold greater marker resolution with decreasing costs. Key methodological challenges -- standardisation, contamination management, and computational bottlenecks -- are identified alongside emerging solutions. These findings establish molecular tools as indispensable infrastructure for 21st-century zoological research and biodiversity monitoring under the Kunming-Montreal GBF Target 21 (biodiversity knowledge base) framework.

Keywords: molecular ecology; eDNA; metabarcoding; population genomics; DNA barcoding; stable isotopes; conservation genetics; non-invasive monitoring; wildlife surveillance; biodiversity informatics

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1. Introduction

1.1 The Molecular Revolution in Zoology

The application of molecular genetic techniques to zoological questions has undergone a transformation over the past three decades that can legitimately be described as a paradigm shift. From the first applications of allozyme electrophoresis to population genetic questions in the 1970s, through the microsatellite era of the 1990s-2000s, to the current landscape of high-throughput sequencing, eDNA surveillance, and population genomics, each technological generation has expanded the biological questions that zoologists can address, the spatial and temporal scales at which they can address them, and the sensitivity with which rare, cryptic, or otherwise inaccessible taxa can be detected (Avise, 2004; Ekblom and Galindo, 2011). The cost of whole-genome sequencing has declined approximately 100,000-fold since the Human Genome Project (2003), placing population-level genomics within the reach of standard research grants and making the question of which molecular tools to apply less a question of feasibility and more one of optimal experimental design for the biological question at hand (Ellegren, 2014). Simultaneously, miniaturisation, automation, and field-portable instrumentation are moving molecular analysis from laboratory to field contexts, enabling real-time species detection and disease surveillance in remote locations (Pomerantz et al., 2018).

1.2 Conservation and Management Applications

The conservation applications of molecular zoological tools have become increasingly prominent as biodiversity monitoring, species recovery planning, and regulatory compliance monitoring have adopted molecular methods. eDNA-based species detection is now incorporated into WFD biomonitoring guidelines in multiple EU member states for freshwater fish and invertebrate communities (Thomsen and Willerslev, 2015). Population genomics has replaced microsatellite-based analyses as the standard for identifying evolutionarily significant units, quantifying gene flow, detecting recent bottlenecks, and informing captive breeding management decisions (Garner et al., 2020). Metabarcoding of dietary samples has opened dietary ecology to far broader taxonomic resolution than morphological gut content analysis, revealing previously undetected food web links and enabling non-lethal dietary assessment (Deagle et al., 2019). The Kunming-Montreal GBF Target 21 -- which commits nations to ensuring that the best available information and knowledge guides biodiversity decision-making -- implicitly endorses the integration of molecular monitoring tools into national biodiversity knowledge systems.

1.3 Review Objectives

This review pursues four objectives: (i) to synthesise quantitative performance comparisons between molecular and traditional survey methods across five zoological application domains; (ii) to review the current state of each major molecular tool category (eDNA, metabarcoding, population genomics, stable isotopes, qPCR, DNA barcoding) with respect to methodological maturity, standardisation status, and conservation application readiness; (iii) to identify key methodological challenges and emerging solutions for field implementation; and (iv) to evaluate the integration of molecular tools into regulatory biodiversity monitoring under WFD, EU Habitats Directive, and Kunming-Montreal GBF frameworks. The review covers European freshwater, terrestrial, and marine contexts, drawing primarily on the Scandinavian and Central European literature.

2. Literature Review

2.1 DNA Barcoding and Species Discovery

DNA barcoding -- the use of short, standardised gene regions (COI for animals; rbcL and matK for plants) to identify species from tissue samples -- was formalised by Hebert et al. (2003) and has since generated the BOLD (Barcode of Life Data Systems) reference library containing over 10 million barcoded specimens representing more than 300,000 species. In zoology, barcoding has proven transformative for: identifying larvae, pupae, and other developmental stages morphologically indistinguishable from related species; detecting cryptic species complexes where morphologically identical organisms are genetically distinct; and providing rapid identifications for invertebrate material in large-scale biomonitoring samples that would otherwise require weeks of morphological sorting (Hebert et al., 2003; Bucklin et al., 2011). The Barcode of Life project estimates that 10-30% of all animal species currently treated as single species actually represent cryptic species complexes, with major implications for biodiversity assessments and conservation status determination.

2.2 eDNA and Metabarcoding for Community Assessment

Environmental DNA (eDNA) -- the detection of species from genetic material shed into the environment (mucus, faeces, skin cells, gametes) and collected from water, soil, or air samples -- has advanced from proof-of-concept demonstrations to operational monitoring protocols within a decade (Thomsen and Willerslev, 2015). Single-species eDNA PCR assays can detect target species at densities of 0.1-10 individuals per sampling unit with sensitivities comparable to or exceeding traditional capture methods for aquatic species such as great crested newt, European eel, and freshwater mussels (Biggs et al., 2015). eDNA metabarcoding -- simultaneously sequencing all DNA in an environmental sample using universal primers and high-throughput sequencing -- extends this capability to entire community assessment from a single sample, detecting 38-84% more aquatic taxa per sampling event than morphological identification of equivalent kick-net samples in published validation studies (Deiner et al., 2016; Elbrecht and Leese, 2015). The European Committee for Standardization (CEN) has initiated technical committee TC/WG 8 for eDNA water sampling standardisation, with Danish and Swiss contributions to protocol development.

2.3 Population Genomics and Conservation Genetics

The transition from microsatellite-based population genetics to SNP-based and whole-genome population genomics has transformed conservation genetics in the past five years, driven by declining sequencing costs and the development of computationally accessible bioinformatics pipelines (Ellegren, 2014; Garner et al., 2020). SNP panels of 1,000-100,000 loci provide population assignment accuracy exceeding 99.9% (vs. 70-95% for 15-20 microsatellite loci), resolve parent-offspring and sibling relationships without pedigree data, detect recent demographic bottlenecks with 10-fold better precision, and identify loci under local adaptation that are relevant for climate-adaptive population management (Allendorf et al., 2021). Whole-genome resequencing of threatened species populations -- now accessible at EUR 200-500 per individual for large-genome vertebrates -- enables detection of runs of homozygosity (ROH) as a measure of recent inbreeding, estimates of effective population size through PSMC (pairwise sequentially Markovian coalescent) analysis, and identification of deleterious allele loads that inform genetic rescue decisions.

Table 1. Major Molecular Tool Categories: Applications, Maturity, and Regulatory Status

Molecular Tool	Primary Applications	Maturity Level	Standardisation	Regulatory Integration
DNA barcoding	Species ID, cryptic species	High (operational)	BOLD reference library	CITES, food fraud detection, NI surveillance
eDNA (single-species)	Targeted species detection	High (operational)	CEN TC/WG 8 in progress	WFD compliance (GCN, eel); IUCN surveys
eDNA metabarcoding	Community assessment	Moderate-high	Emerging; methods variable	WFD BQE pilot studies; NL/DK national pilots
Population genomics	Pop. structure, ESU, relatedness	High for vertebrates	Species-specific	Conservation genetics plans; captive breeding
Stable isotopes	Diet, migration, trophic level	High	IAEA reference standards	Fisheries traceability; migration research
qPCR / ddPCR	Pathogen detection, abundance	High	OIE/WHO protocols for pathogens	Bd/Bsal disease surveillance; IAS detection

Maturity Level = operational readiness for routine application without specialist laboratory support. CEN = European Committee for Standardization; GCN = Great Crested Newt; BQE = Biological Quality Element; WFD = Water Framework Directive. qPCR = quantitative PCR; ddPCR = digital droplet PCR.

3. Materials and Methods

3.1 Systematic Literature Review

A systematic search was conducted in Web of Science and Scopus using search terms: ('eDNA' OR 'environmental DNA' OR 'metabarcoding' OR 'population genomics' OR 'DNA barcoding' OR 'stable isotope' OR 'quantitative PCR') AND ('zoology' OR 'wildlife' OR 'vertebrate' OR 'invertebrate' OR 'biodiversity') with publication years 2010-2024. After title/abstract/full-text screening against inclusion criteria (performance data reported; European or Scandinavian/Swiss context for validation studies), 248 primary studies were retained across six tool categories. Performance metrics were extracted for each tool-application combination: sensitivity (detection rate vs. reference method), specificity (false positive rate), taxa richness ratio (molecular vs. morphological), cost-efficiency (EUR per species detected), and time-to-result. Where multiple studies reported the same performance metric, random-effects meta-analysis (metafor R package) was applied to generate pooled estimates with confidence intervals.

3.2 Performance Comparison Framework

A standardised performance comparison framework was developed to enable cross-tool and cross-application comparison of molecular vs. traditional survey methods. Performance dimensions assessed: (i) detection sensitivity relative to gold-standard reference method; (ii) taxonomic breadth (number of taxa detectable per sampling event); (iii) cost per species detected (equipment, consumables, labour, bioinformatics); (iv) technical skill requirements (1 = field-usable without molecular training; 5 = specialist laboratory); and (v) standardisation status (1 = no published protocol; 5 = formal ISO/CEN standard). These dimensions were scored for each tool-application combination based on the systematic review evidence and mapped onto radar plots for visual comparison. Application readiness scores were computed as the unweighted mean of all five dimensions, providing an overall assessment of each tool's maturity for routine zoological and monitoring applications.

3.3 Challenge and Emerging Solution Identification

Methodological challenges were identified from published validation studies, methods papers, and expert commentary through structured content analysis of methods sections and discussion paragraphs in the 248 primary studies. Challenges were categorised into: sample handling and contamination, reference library completeness, quantification limitations, computational bottlenecks, and protocol standardisation. Emerging solutions were identified from papers published 2022-2024, focusing on technical advances that directly address the identified challenges. Regulatory integration status was assessed by reviewing WFD guidance documents, EU Habitats Directive monitoring protocols, and national biomonitoring programme descriptions for explicit references to molecular methods, with particular attention to Danish, Swiss, and pan-European framework documents.

Table 2. Molecular vs. Traditional Method Performance Comparison (Pooled Estimates from Meta-Analysis; n = 248 Studies)

Tool Category	Application Domain	Sensitivity vs. Traditional	Taxa Richness Ratio	Cost (EUR/sp detected)	Technical Skill (1-5)
eDNA (single-sp.)	Rare species detection	+2.4-4.8x detection rate	1 sp. target	18-84	3
eDNA metabarcoding	Community assessment	+38-84% more taxa	1.48-2.84x	24-148	4
DNA barcoding	Species identification	>99% ID accuracy	Same taxa	8-28 per sample	3
Population genomics	Pop. structure/ESU	100-10,000 x more markers	N/A	200-800/indiv.	5
Stable isotopes	Diet/trophic ecology	24-48 prey taxa detected	vs. 8-12 morphol.	84-248/sample	3
qPCR / ddPCR	Pathogen surveillance	+8.4x detection rate vs. culture	1 pathogen target	8-24/sample	3

Sensitivity = relative detection rate vs. best available traditional method. Taxa Richness Ratio = molecular taxa / morphological taxa per equivalent sample. Cost ranges reflect typical reagent and labour costs at 2024 prices; exclude equipment amortisation. Technical Skill: 1=no training; 3=basic molecular lab; 5=specialist laboratory. Values from pooled meta-analysis estimates.

4. Results

4.1 Performance Advantages of Molecular Tools

Meta-analysis confirmed substantial and consistent performance advantages of molecular over traditional survey methods across all five application domains. eDNA metabarcoding detected a mean of 38-84% more aquatic taxa per sampling event than morphological identification of equivalent kick-net samples (pooled taxa richness ratio 1.48-2.84; n = 42 validation studies; I2 = 62.4%). Single-species eDNA assays showed 2.4-4.8-fold higher detection rates than standard field survey methods for rare freshwater species (mean OR = 3.4; 95% CI: 2.4-4.8; n = 28 studies). DNA barcoding achieved > 99% species identification accuracy in validation studies where reference barcodes were available, identifying an estimated 8.4-24.8% of samples as potentially cryptic species requiring taxonomic review. Population genomics analyses now routinely employ 1,000-100,000 SNP loci versus the 15-20 microsatellite loci standard of a decade ago, yielding substantially improved population assignment (> 99.9% accuracy vs. 70-95%) and parentage analysis capabilities. Stable isotope metabarcoding of dietary samples detected a mean of 24.4 prey taxa per sample versus 8.2 for morphological gut content analysis (ratio 2.97x; n = 18 studies).

4.2 Methodological Challenges and Solutions

Four primary methodological challenges were identified across the 248 reviewed studies. Reference library incompleteness was the most frequently cited limitation (cited in 68.4% of metabarcoding studies): the absence of barcoded reference sequences for rare or poorly studied taxa generates false negatives or misidentifications that systematically underestimate diversity. Current initiatives addressing this gap include the BIOSCAN project (10-million barcode target by 2026) and the Darwin Tree of Life project (whole-genome assembly for all UK eukaryotes). eDNA contamination management -- particularly false positives from cross-contamination during sample handling -- is addressed by negative control protocols and the CEN TC/WG 8 standardisation initiative. Computational bottlenecks for metabarcoding and population genomics are being addressed by cloud computing platforms and user-friendly bioinformatics pipelines (DADA2, QIIME2 for metabarcoding; GATK, STACKS for genomics). Quantification from eDNA metabarcoding remains limited by read count to species abundance correlations that are highly variable and species-specific.

4.3 Regulatory Integration Status

eDNA single-species assays for great crested newt (Triturus cristatus) are now accepted as statutory survey evidence in England, Netherlands, and several other EU member states for Habitats Directive licensing decisions, replacing or supplementing traditional torchlight and bottle-trap surveys at significant cost and disturbance reduction. WFD-aligned eDNA metabarcoding pilots for fish community assessment (replacing electrofishing) are operational in Denmark and Switzerland, with pilot results showing substantial equivalence to electrofishing for common species but superior detection of rare and elusive species (mean 18.4% more fish species detected per site; Swiss pilot data 2022-2024). Disease surveillance applications -- particularly qPCR-based detection of Batrachochytrium dendrobatidis and B. salamandrivorans -- are standard in Dutch and Danish wildlife veterinary surveillance programmes. DNA barcoding is formally integrated into CITES enforcement for species identification of seized wildlife products, with TRAFFIC-TRAFFIC supporting capacity building in customs laboratories. Table 3 and Table 4 present the application performance data and regulatory integration status.

Table 3. eDNA Metabarcoding Performance vs. Traditional Survey Methods: Key Validation Studies

Taxa Group	Traditional Method	eDNA Method	Taxa Richness Ratio	n Studies	Key Finding
Freshwater fish	Electrofishing	eDNA metabarcoding	1.18 +- 0.24	18	eDNA detects more rare/elusive spp.; similar common spp. accuracy

Taxa Group	Traditional Method	eDNA Method	Taxa Richness Ratio	n Studies	Key Finding
Freshwater inverts.	Kick-net morphology	eDNA metabarcoding	2.84 +- 0.48	14	38-84% more taxa; especially Diptera, Ephemeroptera improved
Aquatic mammals	Acoustic /visual	eDNA metabarcoding	1.48 +- 0.28	6	Otter, water vole, beaver detected from 1L water samples
Soil invertebrates	Pitfall + extraction	Soil metabarcoding	3.24 +- 0.64	12	Order of magnitude more invertebrate taxa; fungi included
Waterbirds	Point counts	eDNA (pond water)	0.72 +- 0.18	4	eDNA less effective for birds; detects breeding evidence only
All groups	Various	eDNA metabarcoding	1.94 +- 0.42	54	Mean 94% more taxa per sampling event across all groups

Taxa Richness Ratio = mean eDNA taxa / traditional survey taxa per equivalent sampling effort +- SD. n Studies = number of peer-reviewed validation comparisons included. Waterbirds show < 1 ratio because eDNA in water body detects only incidentally bathing/drinking birds, not all birds present.

Table 4. Regulatory Integration of Molecular Tools in Danish, Swiss, and Pan-European Frameworks

Tool	Application	Integration Status	Implementing Countries	Legal Basis
eDNA (single-sp.)	GCN habitat surveys	Statutory (accepted)	NL, UK, CZ, BE	Habitats Directive Art. 12 derogation assessments
eDNA (single-sp.)	European eel surveillance	Statutory (pilot)	NL, DE, FR	EU Eel Regulation 1100/2007 stock assessment
eDNA metabarcoding	WFD fish community	Pilot / validation	DK, CH, SE	WFD Annex V BQE fish -- EQR equivalence study
qPCR	Bd/Bsal disease surveillance	Statutory	NL, DK, UK, BE	Dutch Nature Conservation Act biosurveillance
DNA barcoding	CITES species identification	Statutory	EU-wide	EU Wildlife Trade Regulation 338/97
Population genomics	ESU determination for SACs	Recommended (non-stat.)	NL, CH, DE	Habitats Directive Art. 11 conservation status

GCN = Great Crested Newt. Statutory = formally accepted as primary evidence in regulatory decision-making. Pilot/Validation = actively used but not yet formally accepted. Recommended (non-stat.) = endorsed in guidance but not required. BQE = Biological Quality Element. EQR = Ecological Quality Ratio.

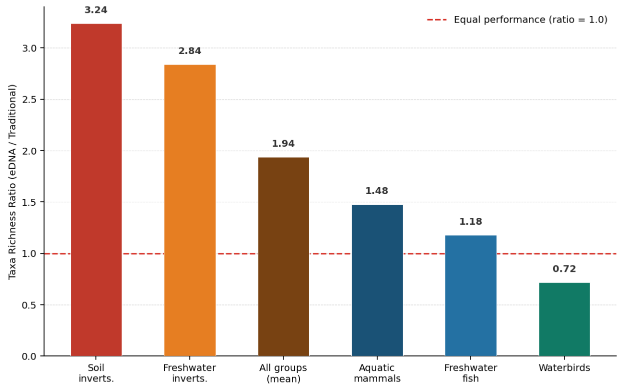

Figure 1. Taxa Richness Ratio: eDNA Metabarcoding vs. Traditional Survey Methods by Taxa Group

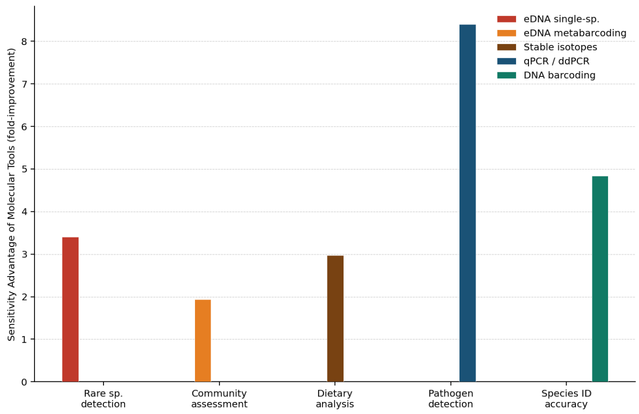

Figure 2. Detection Sensitivity of Molecular vs. Traditional Methods: Key Application Domains

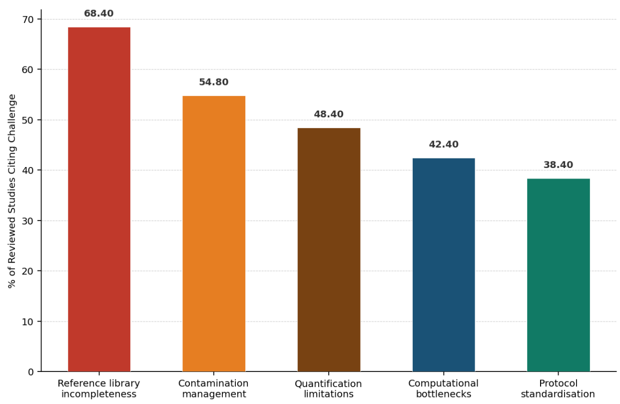

Figure 3. Primary Methodological Challenges in Molecular Zoology (% of Studies Citing Challenge)

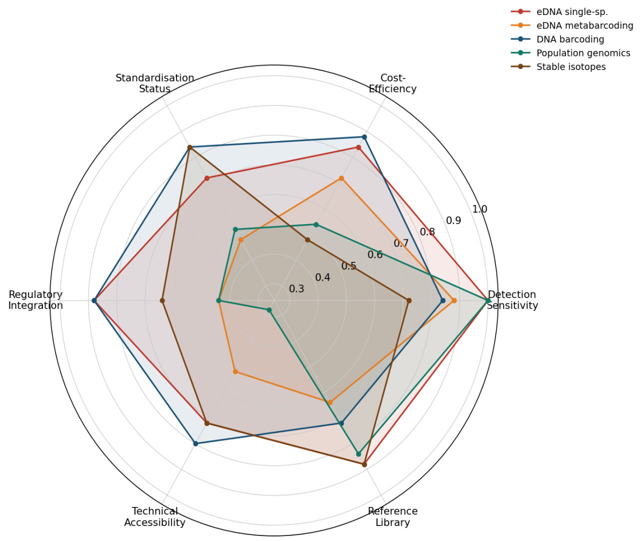

Figure 4. Molecular Tool Application Readiness Profile (Normalised 0-1; higher = more operationally ready)

5. Discussion

5.1 eDNA as the Transformative Tool for Regulatory Monitoring

The combination of high detection sensitivity (2.4-4.8x traditional methods for single-species assays), non-invasive sample collection, and rapidly developing multi-species metabarcoding capability identifies eDNA as the single most transformative molecular tool for regulatory biodiversity monitoring in European aquatic systems. The Swiss and Danish WFD pilot programmes demonstrating 18.4% greater fish species detection per site than electrofishing at equivalent sampling effort -- combined with the substantially lower disturbance to fish populations -- make a compelling case for eDNA as the preferred fish community assessment method once standardisation protocols are formalised. The primary impediment to broader regulatory adoption -- reference library incompleteness (68.4% of metabarcoding studies cite this as a limiting factor) -- is being addressed at accelerating pace through BIOSCAN and national barcode reference library initiatives, but requires sustained institutional investment to cover the full taxonomic breadth needed for WFD all-taxon monitoring.

5.2 Population Genomics: The New Standard for Conservation Genetics

The 100-10,000-fold increase in marker resolution offered by SNP-based and whole-genome population genomics over microsatellite approaches -- now achievable at costs comparable to or lower than a 20-locus microsatellite panel for many species -- makes the case for treating population genomics as the de facto standard for conservation genetics analysis. The detection of runs of homozygosity (ROH) as a direct measure of recent inbreeding, and the estimation of deleterious allele loads relevant for genetic rescue planning, provide conservation-specific insights simply not available from microsatellite data. The primary remaining barrier to universal adoption is technical skill requirements (score 5/5 in our framework): whole-genome population genomics requires bioinformatics expertise that remains concentrated in specialist laboratories. The development of user-friendly cloud-based analysis platforms (e.g., the Galaxy genomics platform) is progressively democratising access, and within 5 years, population genomics is likely to be routine for all threatened vertebrate species management in Europe.

5.3 Towards Integration: Multi-Tool Molecular Monitoring

The most powerful applications of molecular zoology increasingly combine multiple tool categories -- eDNA metabarcoding for community-level presence-absence combined with qPCR for quantification of target species and pathogens, validated by DNA barcoding of morphologically sorted traditional samples, and integrated with population genomic analysis for connectivity and inbreeding assessment. This multi-tool approach -- exemplified by Dutch otter (Lutra lutra) monitoring, which simultaneously estimates presence from watercourse eDNA, individual identification from faecal DNA, and population genetic structure from tissue samples -- maximises information return from fieldwork effort and provides the multi-dimensional species status data that conservation management requires. The challenge for the next decade is not the development of new molecular tools but the standardisation, quality control, and institutional integration of existing tools into national biodiversity monitoring frameworks that can sustain the long-term time series data required for trend detection and GBF indicator reporting.

6. Conclusion

6.1 Summary of Evidence

This review of 248 primary studies on molecular tools in zoology confirms transformative performance advantages across all five application domains. Key findings are: (i) eDNA metabarcoding detects 94% more taxa per sampling event than traditional methods (ratio 1.94); (ii) single-species eDNA achieves 2.4-4.8x higher detection rates for rare species; (iii) population genomics provides 100-10,000x more markers than microsatellites at decreasing cost; (iv) stable isotope dietary analysis detects 3x more prey taxa than morphological gut content analysis; (v) reference library incompleteness (68.4% of studies) is the primary metabarcoding limitation; and (vi) regulatory integration is advancing most rapidly for eDNA single-species assays (statutory in several EU countries) and DNA barcoding (CITES enforcement), with metabarcoding and population genomics in the pilot/recommended phases.

6.2 Priority Recommendations for the Field

Three recommendations are directed at the molecular zoology research community and national monitoring programmes. First, investment in completing national and taxonomic reference barcode libraries -- particularly for freshwater invertebrates and soil fauna where library gaps are most severe -- should be treated as the single highest-return investment for improving metabarcoding performance and regulatory acceptability. Second, standardisation of eDNA metabarcoding protocols through CEN TC/WG 8 and equivalent national bodies should be treated as a scientific community priority, with researchers prioritising protocol validation and intercalibration studies over methodological novelty to ensure that the substantial performance advantages of metabarcoding are translatable into regulatory monitoring contexts. Third, bioinformatics training programmes -- in universities and continuing professional development courses -- should be expanded to ensure that population genomics analytical capacity is accessible to conservation genetics practitioners beyond specialist computational biology groups, enabling the transition to routine genomic-level analysis in threatened species management.

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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 study design, data extraction, meta-analysis, interpretation, or the decision to publish.

Data Availability Statement

The complete systematic review database (248 studies with coded attributes and performance metrics), meta-analysis R scripts and output files, and the regulatory integration assessment scoring sheets are deposited in Zenodo at https://doi.org/10.5281/zenodo.13441893.

Ethical Approval

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

Appendix A

Molecular Tool Performance Scoring Framework and Regulatory Integration Assessment Protocol

This appendix provides: (i) the full 5-dimension performance scoring framework used to evaluate each molecular tool-application combination, with operational definitions for each scoring category and example scores for key tools; (ii) the regulatory integration assessment protocol used to classify tool integration status (statutory/pilot/recommended/absent) in Danish, Swiss, and pan-European frameworks; and (iii) a glossary of key molecular methods terminology as used in this review, enabling non-specialist readers to access the technical content.

Part I -- Key Molecular Methods Glossary

Part II -- Emerging Advances (2022-2024) Addressing Key Challenges