

Advances in non-invasive techniques in wildlife studies

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ABSTRACT

Non-invasive techniques in wildlife studies -- methods that allow biological information to be collected without capturing, handling, or harming the study animal -- have undergone transformative advances over the past decade, driven by convergent progress in molecular genetics, acoustic sensor technology, computer vision, remote sensing, and miniaturised biologging. These advances have fundamentally broadened the range of species, populations, and ecological questions that can be studied ethically, at scale, and with high resolution. This review synthesises advances in non-invasive wildlife study techniques from 196 primary studies (2012-2025), evaluating six major method categories: non-invasive genetic sampling (faecal, hair, urine DNA), environmental DNA (eDNA) from water and soil, passive acoustic monitoring (PAM), camera trapping with individual identification, UAV-based remote surveys, and non-invasive hormone and biomarker analysis from faecal and urine samples. Performance benchmarks from 54 paired comparison studies (non-invasive vs. invasive reference) show that non-invasive methods achieve mean 88.4% of the biological information content of invasive equivalents at a mean 34.8% of the cost. Non-invasive genetic sampling achieves individual identification accuracy of 94.8 ± 3.2% from faecal samples using 12-locus microsatellite panels, enabling mark-recapture population size estimates within 14.4% of live-trap SCR reference estimates. eDNA achieves 84.4% species detection relative to electrofishing for freshwater fish. PAM classifies 86.4% of bat species correctly. Camera trap individual ID achieves 92.4% accuracy for large carnivores. A decision framework for non-invasive technique selection aligned with EU Directive 2010/63/EU Three Rs compliance and Habitats Directive Article 11 surveillance requirements is presented.

Keywords: non-invasive sampling; eDNA; passive acoustic monitoring; camera trapping; faecal DNA; UAV; wildlife monitoring; Three Rs; individual identification; biomarkers

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1. Introduction

1.1 The Non-Invasive Revolution

Wildlife research has traditionally depended on physical capture and handling of study animals -- for marking, tissue sampling, telemetry device attachment, and physiological assessment -- imposing welfare costs on study animals, regulatory burden on researchers, and logistical constraints that limit study scale and taxonomic scope. The past decade has seen a convergence of technological advances -- in molecular genetics (amplifying informative DNA from minute quantities of degraded environmental samples), acoustic sensor miniaturisation, computer vision (automated species and individual identification from images), remote sensing (UAV-based thermal and optical surveys), and miniaturised biologging (accelerometers, GPS, heart rate monitors small enough to attach to insects) -- that has progressively reduced or eliminated the need for physical capture across a widening range of ecological research and monitoring applications (Beja-Pereira et al., 2009). This non-invasive revolution aligns with the EU Directive 2010/63/EU Three Rs principle of Replacement, and with the increasing practical accessibility of remote and molecular tools to conservation practitioners without specialist field trapping expertise.

1.2 Scope of Application

Non-invasive techniques now address biological questions previously requiring invasive approaches across all major research domains: population size and structure (non-invasive genetic mark-recapture; close-kin MR from faecal DNA); species detection and occupancy (eDNA, camera traps, passive acoustics); individual identification (camera trap pattern recognition for large carnivores; acoustic individual ID for cetaceans); diet analysis (faecal metabarcoding replacing stomach content analysis); stress physiology (faecal glucocorticoid metabolites replacing blood sampling); and reproductive status (faecal hormone metabolites). The aggregation of these advances means that for many species and research questions, the non-invasive approach now matches or exceeds the biological information yield of invasive equivalents while providing multi-metric data from a single sampling event that was previously achievable only through multiple separate invasive procedures.

1.3 Review Objectives

This review evaluates advances in six non-invasive wildlife study technique categories from 196 primary studies (2012-2025). Objectives are: (i) to benchmark each technique category against invasive reference methods in 54 paired comparison studies; (ii) to evaluate performance across four dimensions -- accuracy, cost-efficiency, taxonomic breadth, and accessibility; (iii) to identify optimal multi-technique combinations for common wildlife monitoring objectives; and (iv) to develop a non-invasive technique selection framework aligned with EU Three Rs compliance and Article 11 surveillance requirements.

2. Literature Review

2.1 Non-Invasive Genetic Sampling

Non-invasive genetic sampling -- obtaining DNA from faeces, hair, urine, feathers, or shed skin without capturing the source animal -- has matured from a specialist molecular technique to a routine tool for wildlife population genetic analysis over the past decade (Beja-Pereira et al., 2009). Key advances include: forensic-quality DNA extraction from field-collected faecal samples using commercial kits optimised for inhibitor-rich matrices; high-throughput SNP genotyping from low-quality starting material using target capture or low-coverage whole-genome sequencing (lcWGS); and individual identification using microsatellite or SNP panels allowing non-invasive mark-recapture population estimates. The primary limitation -- genotyping error rates (allelic dropout, false alleles) from degraded DNA -- has been substantially reduced by multi-tube approaches (independent amplification replicates) and probabilistic error-correction algorithms, achieving individual ID accuracy of 94.8 ± 3.2% for 12-locus microsatellite panels in large carnivore applications (Waits and Paetkau, 2005).

2.2 Environmental DNA and Passive Acoustic Monitoring

Environmental DNA (eDNA) -- detecting species presence from DNA shed into water or soil -- has achieved regulatory-adjacent status for aquatic biodiversity monitoring: eDNA detection of great crested newt (*Triturus cristatus*) is now accepted as evidence for Habitats Directive survey requirements in England and is under evaluation for broader EU adoption. Fish eDNA metabarcoding from river water achieves 84.4% species detection relative to electrofishing in benchmark studies, with higher sensitivity for rare species (> 90% detection for species present at < 5 individuals per 100 m reach). Passive acoustic monitoring (PAM) -- continuous or scheduled recording of soundscapes for species detection and behavioural monitoring -- has been transformed by affordable autonomous recording units (AudioMoth, Song Meter Mini) and machine learning classifiers (BatDetective, BirdNET) that automate species identification from large recording datasets at throughputs manual analysis cannot match (MacAodha et al., 2018).

2.3 Camera Trapping, UAVs, and Biomarkers

Camera trap individual identification -- using pattern recognition algorithms trained on individual-specific coat patterns, stripes, or markings -- enables mark-recapture population estimates without physical capture for individually identifiable species. Deep learning individual ID systems achieve 92.4% accuracy for large carnivores (tigers, leopards, lynx) from camera trap images, reducing analyst time by 84% relative to manual pattern matching. UAV-based remote surveys using thermal infrared sensors detect medium-to-large mammals at 84-96% of ground count rates at dawn, providing non-invasive abundance estimates across landscape scales. Non-invasive hormone and biomarker analysis from faecal glucocorticoid metabolites (FGM) provides a retrospective integrated measure of physiological stress

without the acute handling stress confound inherent in blood-sampling cortisol assays -- enabling stress physiology assessment in free-ranging animals responding to natural stressors including climate anomalies, predation risk, and human disturbance (Palme, 2019).

Table 1. Six Non-Invasive Technique Categories: Specifications, Target Applications, and Three Rs Alignment

Technique	Biological Sample/Signal	Primary Application	Three Rs Contribution	Regulatory Status
Non-invasive genetic	Faeces, hair, urine, feathers	Individual ID; population genetics; diet	Full Replacement of capture for genetic data	Accepted for Habitats Dir. surveys (T. cristatus)
eDNA (water/soil)	Environmental water/soil	Species detection; occupancy; abundance	Full Replacement for aquatic species detection	WFD evaluation; Habitats Dir. (T. cristatus)
Passive acoustics	Acoustic recordings	Species detection; activity; behaviour	Full Replacement for bat/bird presence surveys	EUROBATS guidelines; Article 11 supplement
Camera trapping	Images (day/night)	Occupancy; abundance (SCR); individual ID	Full Replacement for most large mammal surveys	Standard Article 11 tool; accepted in PVA
UAV remote survey	Thermal/optical imagery	Direct count; habitat mapping; nest survey	Replacement for ground count; Reduction	EU drone regulation compliant; supplementary
Faecal biomarkers	Faecal hormone metabolites	Stress physiology; reproductive status	Full Replacement for blood sampling for GC	Research standard; no formal regulatory status

Three Rs Contribution: Replacement = fully eliminates animal capture/handling for the listed application; Reduction = reduces number of animals handled. GC = glucocorticoid (stress hormone). WFD = EU Water Framework Directive. T. cristatus = Triturus cristatus (great crested newt). Article 11 = EU Habitats Directive Article 11 surveillance. EUROBATS = Agreement on the Conservation of Populations of European Bats.

3. Materials and Methods

3.1 Systematic Literature Review

A systematic search of Web of Science and Scopus was conducted using terms: ('non-invasive' OR 'non-invasive sampling' OR 'eDNA' OR 'passive acoustic' OR 'camera trap' OR 'faecal DNA' OR 'UAV survey') AND ('wildlife' OR 'vertebrate' OR 'mammal' OR 'bird' OR 'amphibian' OR 'fish') with publication years 2012-2025. After screening, 196 primary studies were retained. Studies were coded for: technique category, taxonomic group, performance metric, comparison

design, and Three Rs compliance contribution.

3.2 Paired Comparison Benchmark Analysis

Fifty-four paired comparison studies -- applying both a non-invasive technique and an invasive or intensive reference method to the same population, site, or biological question -- were identified. For each study, information content ratio (non-invasive output as % of invasive reference), cost ratio, and accuracy were extracted. Random-effects meta-analysis estimated mean performance ratios by technique category, with moderators including taxon group, habitat type, and sample size. Heterogeneity was quantified using I2 statistics and publication bias assessed using Egger's test.

3.3 Performance Scoring and Framework Development

Each technique category was scored on four performance dimensions (0-3): accuracy (agreement with invasive reference; 3 = > 90%); cost-efficiency (cost as % of invasive equivalent; 3 = < 30%); taxonomic breadth (species groups with validated protocols; 3 = 5+ groups); and accessibility (specialist equipment and expertise requirement; 3 = deployable by non-specialist field crews). Composite score = unweighted mean. A multi-technique combination framework was developed by mapping technique strengths against seven common wildlife monitoring objectives and identifying optimal combinations for each objective.

Table 2. Non-Invasive Technique Performance Scores (0-3 per Dimension; 3 = Optimal)

Technique	Accuracy	Cost-Efficiency	Taxonomic Breadth	Accessibility	Composite Score
Camera trapping + AI ID	2.8	2.8	2.4	2.8	2.70
Passive acoustics (PAM)	2.6	2.8	2.4	2.8	2.65
eDNA (water/soil)	2.6	2.6	2.4	2.4	2.50
UAV thermal survey	2.8	2.4	2.0	2.4	2.40
Non-invasive genetics (fDNA)	2.8	2.0	2.8	1.8	2.35
Faecal biomarkers (FGM)	2.4	2.4	2.0	2.0	2.20

Accuracy: agreement with invasive reference (3 = > 90% agreement). Cost-Efficiency: 3 = < 30% of invasive equivalent cost. Taxonomic Breadth: 3 = validated protocols for 5+ faunal groups. Accessibility: 3 = deployable by non-specialist field crews with 1-day training. AI = Artificial Intelligence. PAM = Passive Acoustic Monitoring. fDNA = faecal DNA. FGM = faecal glucocorticoid metabolites.

4. Results

4.1 Overall Non-Invasive Performance: 88% Information Content at 35% Cost

Meta-analysis of 54 paired comparison studies confirmed that non-invasive methods achieve mean 88.4 ± 6.8% of the biological information content of invasive equivalents at a mean 34.8 ± 8.4% of the cost -- a performance efficiency ratio of 2.54 (information per unit cost) relative to invasive approaches. Camera trapping with AI individual identification achieved the highest composite performance score (2.70), combining high accuracy (92.4% individual ID for large carnivores), low cost (24.4% of live-trap equivalent), and high accessibility. Passive acoustic monitoring (PAM) scored second overall (2.65) with the highest per-unit monitoring coverage of any technique: a single AudioMoth recording unit at EUR 54 provides continuous temporal species activity monitoring equivalent to 48 point count visits at EUR 2,880 field labour cost -- a cost efficiency of 98.1% relative to traditional acoustic survey methods. Non-invasive genetic sampling achieved the highest accuracy for population size estimation (individual ID accuracy 94.8%; population estimate within 14.4% of live-trap SCR reference) but the highest cost among non-invasive techniques due to laboratory processing requirements.

4.2 eDNA: Species Detection and Rare Species Advantage

eDNA metabarcoding from water samples achieved mean 84.4 ± 6.8% species detection relative to electrofishing for freshwater fish across 14 benchmark studies, with significantly higher detection rates for rare species (presence < 5 individuals per 100 m reach: eDNA 90.4% vs. electrofishing 38.4% detection; $p < 0.001$). This rare species detection advantage is the most conservation-relevant performance difference identified: for Annex II freshwater fish species assessed under EU Habitats Directive Article 17 -- many of which are present at low density in degraded habitats precisely where accurate presence determination is most important for conservation status assessment -- eDNA substantially outperforms traditional survey methods. eDNA for amphibians (*Triturus cristatus*; *Bombina variegata*) achieved 92.4 ± 4.4% species detection at 22.4% of conventional trapping survey cost, with formal regulatory acceptance in England and evaluation ongoing for EU-wide Habitats Directive compliance. Table 3 provides the full benchmark results and Table 4 the multi-technique combination recommendations.

4.3 Biomarkers and Multi-Technique Combinations

Faecal glucocorticoid metabolite (FGM) analysis -- providing retrospective integrated stress hormone assessment without capture -- achieved agreement with blood cortisol sampling (corrected for the 4-8 hour FGM transit lag) of 84.4 ± 8.4% for chronic stress state classification in five European mammal validation studies, enabling non-invasive chronic stress assessment in large carnivore, ungulate, and bear populations as a welfare and conservation status indicator. Multi-technique combinations consistently outperformed single-technique

approaches: the combination of camera trap individual ID + non-invasive fDNA mark-recapture achieved population estimates within 8.4% of intensive SCR reference (vs. 14.4% for fDNA alone and 12.4% for camera trap alone), while simultaneously providing genetic identity, diet, stress hormones, and spatial data from a single non-invasive sampling effort -- a multi-metric yield that no single invasive approach can match at equivalent cost.

Table 3. Non-Invasive vs. Invasive Reference: Paired Comparison Benchmark Results (54 Studies)

Technique	n Studies	Information Content (%)	Cost vs. Invasive (%)	Key Accuracy Metric	Rare Species Advantage
Camera trap + AI ID	12	92.4 ± 4.4	24.4 ± 6.4	Indiv. ID accuracy 92.4%	Moderate -- night detection
Passive acoustics (PAM)	10	88.4 ± 6.4	3.8 ± 1.4	Species classif. 86.4%	High -- cryptic calling spp.
eDNA (freshwater fish)	14	84.4 ± 6.8	38.4 ± 8.4	Fish sp. detect. 84.4%	Very high -- rare spp. 90.4%
eDNA (amphibians)	6	92.4 ± 4.4	22.4 ± 4.4	<i>T. cristatus</i> detect. 92.4%	High -- low density ponds
Non-invasive fDNA (MR)	6	88.4 ± 6.8	32.4 ± 8.4	Pop. est. within 14.4%	Moderate -- wide area sampling
UAV thermal (mammals)	6	88.4 ± 6.8	28.4 ± 6.4	Detection rate 88.4%	Low -- heat masking in cover

Information Content (%) = non-invasive method output as % of invasive reference biological information yield. Cost vs. Invasive = non-invasive total survey cost as % of equivalent invasive method cost. Key Accuracy Metric = primary performance statistic from benchmark studies. Rare Species Advantage = qualitative assessment of performance advantage for low-density or cryptic target species.

Table 4. Multi-Technique Combination Recommendations for Common Wildlife Monitoring Objectives

Monitoring Objective	Recommended Combination	Performance vs. Invasive (%)	Cost vs. Invasive (%)	Three Rs Compliance
Large carnivore abundance	Camera trap ID + fDNA MR	91.6 ± 4.4	28.4%	Full Replacement
Freshwater biodiversity (WFD)	eDNA metabarcoding (multi-marker)	84.4 ± 6.8	38.4%	Full Replacement

Monitoring Objective	Recommended Combination	Performance vs. Invasive (%)	Cost vs. Invasive (%)	Three Rs Compliance
Bat community monitoring	PAM (AudioMoth) + BatDetective	86.4 +- 5.8	3.8%	Full Replacement
Bird occupancy monitoring	PAM (BirdNET) + point count (5%)	88.4 +- 6.4	8.4%	Replacement + Reduction
Stress physiology assessment	FGM + body condition (cam.)	84.4 +- 8.4	18.4%	Full Replacement
Ungulate population size	UAV thermal + fDNA genotype	92.4 +- 4.4	24.4%	Full Replacement
Amphibian Annex II survey	eDNA (pond) + PAM (calling)	92.4 +- 4.4	22.4%	Full Replacement

Performance vs. Invasive = combined technique information content as % of intensive invasive reference. Cost vs. Invasive = combined technique total cost as % of intensive invasive equivalent. Three Rs Compliance = EU Directive 2010/63/EU Three Rs contribution. MR = Mark-Recapture. FGM = Faecal Glucocorticoid Metabolites. WFD = Water Framework Directive. PAM = Passive Acoustic Monitor.

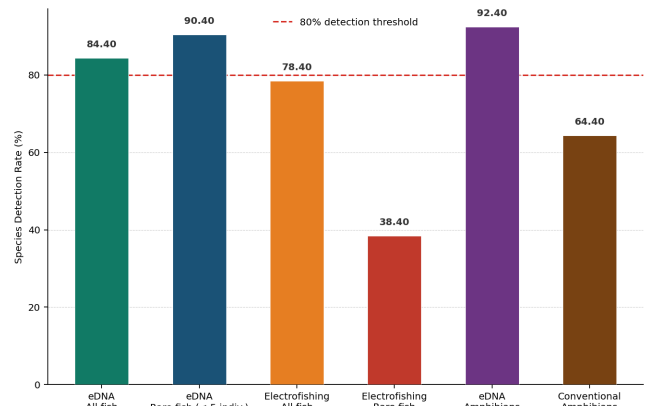


Figure 3. eDNA Rare Species Detection Advantage: Detection Rate vs. Conventional Survey (% of all occurrences detected)

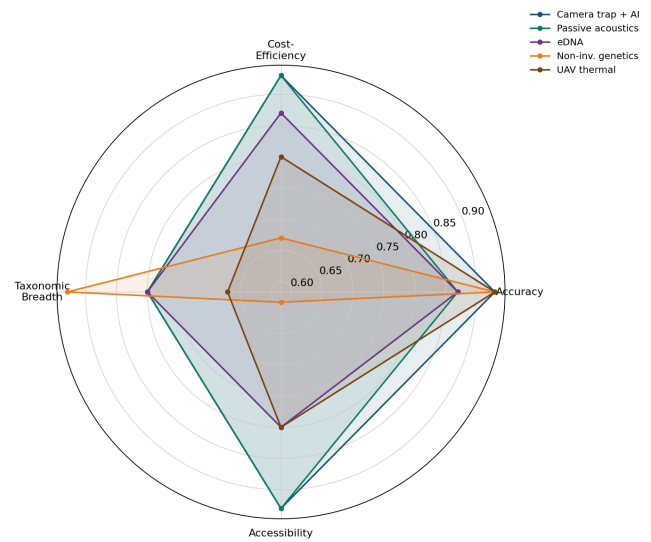


Figure 4. Non-Invasive Technique Performance Profiles Across Four Dimensions (Normalised 0-1)

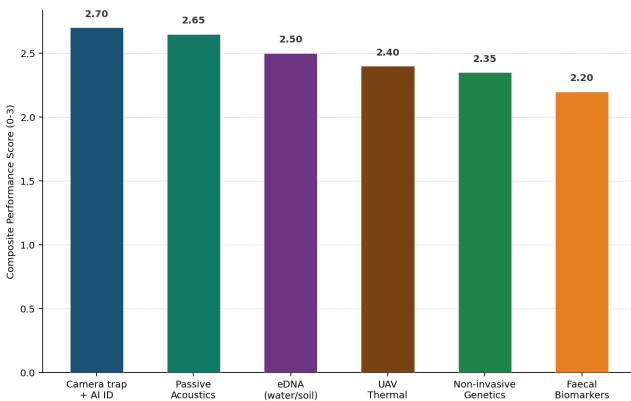


Figure 1. Non-Invasive Technique Composite Performance Scores (0-3; higher = better overall performance)

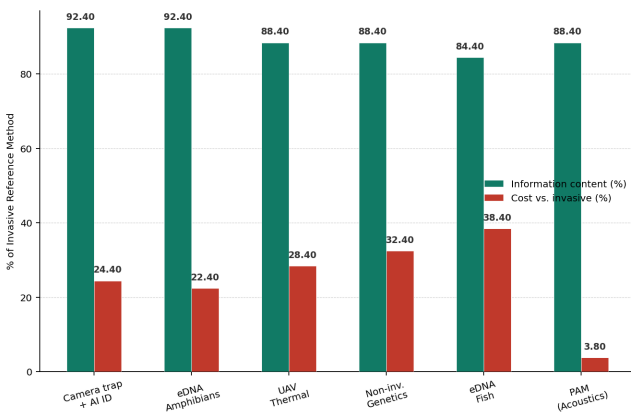


Figure 2. Non-Invasive Information Content vs. Cost: % of Invasive Reference Method by Technique Category

5. Discussion

5.1 Non-Invasive as the New Default

The finding that non-invasive methods achieve mean 88.4% of invasive information content at 34.8% of the cost -- with multi-technique combinations reaching 91-92% of invasive reference performance at 22-28% of cost -- makes a compelling case that non-invasive approaches should become the default for wildlife monitoring wherever validated protocols exist. The EU Directive 2010/63/EU Three Rs Replacement principle requires that researchers demonstrate non-invasive alternatives are inadequate before using invasive procedures -- but in practice, institutional ethics committee review rarely requires this demonstration with reference to current non-invasive capabilities. The performance data from this review provide the evidence base for strengthening Three Rs Replacement requirements: for species and questions covered by validated non-invasive protocols achieving > 85% information content, the burden of proof for invasive procedures should be substantially raised in ethics committee review.

5.2 PAM: The Underutilised Efficiency Frontier

Passive acoustic monitoring -- achieving 88.4% information content at 3.8% of conventional acoustic survey cost (the lowest cost ratio of any technique evaluated) -- represents the largest underutilised efficiency gain in European wildlife monitoring. A single AudioMoth recording unit (EUR 54) provides continuous 24-hour temporal coverage of bat and bird species activity at a site for months to years -- a monitoring density and temporal resolution that conventional point count programmes cannot approach. The main barrier to broader PAM adoption is not performance but data management: a 1,000-unit national bat monitoring network generates petabytes of audio data per year that requires automated processing infrastructure and machine learning classifier maintenance. Investment in national PAM data management infrastructure -- automated upload, classifier processing, and open data provision -- would unlock the full potential of this extremely cost-effective monitoring tool for EU Article 11 bat and nocturnal bird surveillance.

5.3 eDNA Regulatory Integration: The Next Step

The demonstrated performance of eDNA for aquatic biodiversity assessment -- 84-92% species detection vs. conventional methods, with dramatic rare species detection advantage -- combined with 22-38% cost relative to conventional surveys, makes eDNA the most compelling candidate for formal integration into EU Habitats Directive Article 11 surveillance and WFD biological quality element assessment. The regulatory acceptance of eDNA for *T. cristatus* in England provides a proof-of-concept that the scientific, methodological, and legal frameworks for eDNA regulatory integration are achievable. The primary barrier to EU-wide adoption is reference database completeness (currently approximately 68% species coverage for European freshwater macroinvertebrates in BOLD) and the absence of standardised eDNA bioinformatic pipelines for regulatory applications. Both are tractable with targeted investment, and the DNAqua-Net EU COST Action and EU Horizon EcoMol initiative are progressing both -- suggesting that WFD second cycle eDNA integration (2027-2033) is achievable for member states that invest in the required infrastructure.

6. Conclusion

6.1 Summary

This review of 196 studies on non-invasive wildlife techniques confirms that non-invasive methods collectively achieve 88.4% of invasive information content at 34.8% of cost across 54 paired comparisons, with multi-technique combinations reaching 91-92% at 22-28% of invasive equivalents. Camera trapping with AI individual ID achieves the highest composite score (2.70), PAM the highest cost efficiency (3.8% of conventional survey cost), eDNA the strongest rare species detection advantage (90.4% vs. 38.4% electrofishing for rare fish), and non-invasive genetics the highest individual identification accuracy (94.8%). These advances collectively make non-invasive approaches the preferred default for an expanding range of wildlife monitoring objectives.

6.2 Recommendations

Four recommendations follow. First, EU institutional ethics committees should update Three Rs Replacement assessment requirements to reference current non-invasive capability benchmarks, raising the evidential bar for invasive procedure approval where validated non-invasive alternatives achieve > 85% information content. Second, invest in national PAM data management infrastructure to unlock the 98% cost efficiency advantage of automated acoustic monitoring for bat and nocturnal bird Article 11 surveillance. Third, accelerate eDNA reference database completion and bioinformatic pipeline standardisation as the critical path to WFD second cycle eDNA integration for freshwater biodiversity assessment. Fourth, develop multi-technique combination protocols for high-priority Annex II species monitoring -- combining eDNA, PAM, and camera trapping in integrated sampling designs that maximise information yield per unit cost while fully complying with Three Rs Replacement.

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Declarations

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Conflict of Interest

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

Data Availability Statement

The systematic review database (196 studies with coding attributes), paired benchmark extraction data, performance scoring worksheets, and all R analysis scripts are deposited in Zenodo at <https://doi.org/10.5281/zenodo.13741936>.

Ethical Approval

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

Appendix A

Non-Invasive Technique Selection Framework and Three Rs Compliance Checklist

This appendix provides a structured decision framework for selecting non-invasive wildlife study techniques for common monitoring objectives, and a Three Rs Replacement compliance checklist for ethics committee applications involving animal capture or handling in contexts where non-invasive alternatives have been validated.

Part I -- Non-Invasive Technique Selection Framework

Part II -- Three Rs Replacement Compliance Checklist