Gone with the Wind: Airborne Environmental DNA for Avian Biomonitoring

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Abstract

Global avian populations are declining precipitously, and an innovation in biodiversity monitoring – which fuels conservation with statistics and precise diagnoses of problems – is paramount to conserve avian biodiversity and ecosystem services. Traditional bird survey methods such as point counts and call-backs are labor-intensive, expertise-dependent, time-intensive, and subject to imperfect detection despite being informative on abundance and range. This paper aims to evaluate the potential of airborne environmental DNA to become a reliable technique for avian biomonitoring. A critical review of the literature revealed the scalability, accessibility, and non-invasive nature of airborne environmental DNA. This paper further discusses the method's sensitivity and spatial reach, as well as how researchers can overcome the challenge of quantifying abundance by accounting for PCR amplification bias, environmental stochasticity, and shedding variability. The paper presents a set of next steps for the field of aeDNA, including protocol standardization, citizen science engagement, and creating shedding-rate and detection probability databases to develop aeDNA into a key biomonitoring tool of the future, working in tandem with traditional methods. Airborne environmental DNA has the potential to overcome current limitations and mature into a comprehensive avian biomonitoring technique, providing policymakers and researchers with reliable data to monitor species and ecosystems, guiding avian conservation.

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Avian Conservation Status

Birds are among the most intensively studied and ecologically important vertebrate groups, holding important roles in ecosystems such as dispersing seeds, killing pests, reducing disease through scavenging, pollinating, and connecting ecosystems across vast spatial scales (Heleno et al., 2011; Mainwaring, 2017; Ramsey, 1988; Whelan et al., 2008). Yet, globally, 48% of bird species are known or suspected to be declining in contrast to only 6% increasing (Lees et al., 2022). This trend is particularly poignant in North America, where nearly 3 billion birds, or 29% of the continental populations, have been lost since 1970, across all major breeding biomes except wetlands (Fig. 1). This catastrophe results from the culmination of a multitude of issues, including habitat loss and fragmentation, resulting in the loss of 90% of native grasslands; agricultural intensification, leading to a 57% decline in farmland birds in Europe since 1980; invasive species, pollution, and urbanization (Lees et al., 2022; Rosenberg et al., 2019).

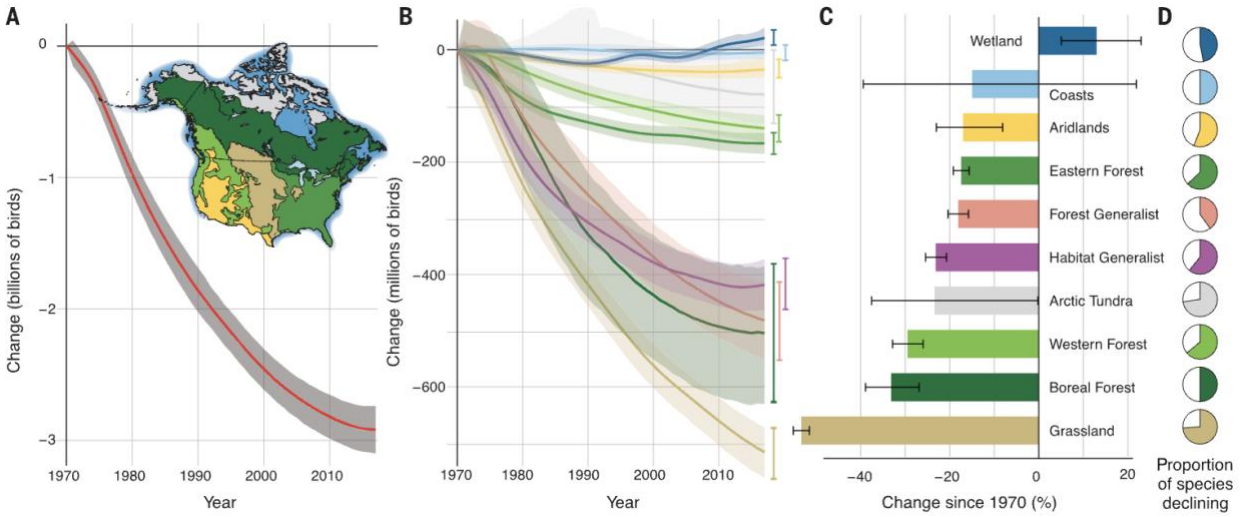

Figure 1: Net population change in North American Birds. (A) Net loss of 2.9 billion birds across 529 species. Gray shading represents the 95% credible interval (CI). (B) Net abundance trajectories across all major breeding biomes. (C) Proportional net population change since 1970. (D) Proportion of declining species in each biome (Rosenberg et al., 2019).

Biomonitoring is the systematic tracking of ecosystems and biological functions, analyzing the presence and abundance of organisms in their communities, assessing the health of their ecosystems, and the state of biodiversity (Makiola et al., 2020). Birds are often used as biomonitors, as shifts in avian populations are often early indicators of problems, allowing the use of biomonitoring to help researchers and policy-makers identify ecosystem degradation before it has escalated to a large scale (Moussy et al., 2021; Fig. 2). For example, a declining insectivore population may signal pesticide overuse, and subtle changes in regional biodiversity can signal habitat fragmentation and extensive urbanization. Therefore, in our current crisis of avian biodiversity decline, biomonitoring provides the data necessary to understand and respond to every issue. The detection of species-specific abundance and range, alongside their

spatial patterns and temporal trends, are key biomonitoring indicators to provide a comprehensive picture of the target population's ecological state.

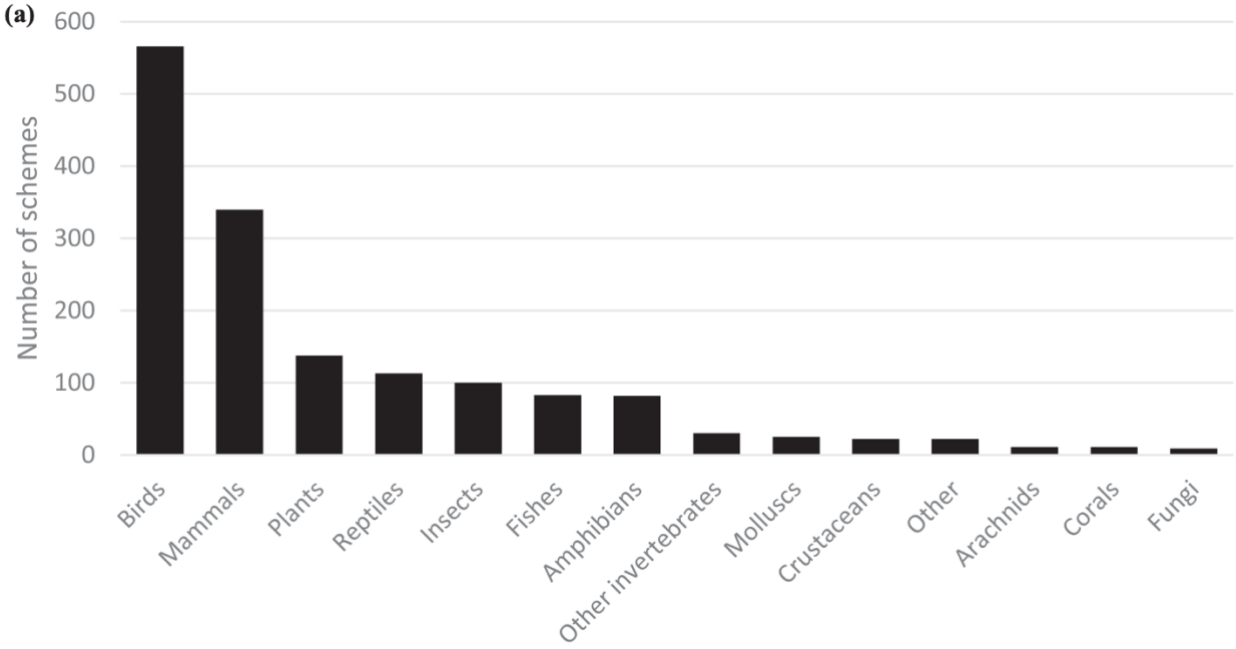

Figure 2: Total number of species monitoring schemes for all taxonomic groups, out of 1168 schemes (some schemes cover more than one taxonomic group, so the combined total is >1168 (Moussy et al., 2021).

Challenges of Traditional Avian Biomonitoring

Traditional methods of surveying birds include point counts in which observers/cameras record all birds seen or heard from a fixed point during a set amount of time, call-back surveys, where researchers play birdcalls and records whether the birds call back, or area searches, where observers search a set area for all birds (Pascoe et al., 2019). While traditional bird surveying techniques have been paramount in providing essential biomonitoring statistics, they come with many fundamental statistical and logistical flaws.

All traditional surveying methods are prone to imperfect detection (when taxa at a site go unrecorded), making the survey an underdetection of overall biodiversity. Kéry & Schmidt (2008) illustrate this issue in a two-step process. For a species to be detected, an individual must be present and available for observation (i.e., the individual must be visible or vocalizing), and they must be perceived by the observer. Even the most robust surveys are prone to error, as any bird may be resting or hidden during the survey, and the observer is prone to human error, favoring charismatic and active species. This shows that species detection is only a probabilistic reflection of an individual's true presence. Boulinier et al. (1998) quantified this margin of error in their analysis of the Breeding Bird Survey, the largest and most comprehensive in North America, showing an average 10-30% underdetection of species richness. Analyses of the survey and over 170,000 additional records show that detection rates between species are also

highly heterogeneous. They fluctuate due to differences in behavior, vocalization rates, habitat, and charisma, resulting in the distortion of the seemingly standardized measure of relative abundance between species in bird surveys, making it challenging to quantify and compare abundance accurately (Boakes et al., 2010; Boulinier et al., 1998).

Traditional surveys face equally challenging logistical issues, being labor-intensive and requiring a high level of expertise. Area searches, the most accurate form of manual survey, require observers to comprehensively know the local avifauna and be completely alert for hours, a highly demanding task (Pascoe et al., 2019). Budka et al. (2022) found that despite the high demand for labor and expertise, manual surveys only detected 41-54% of species identified through acoustic recorders, putting the accuracy of manual surveys into question, too. Though acoustic surveys are shown to be more effective than manual surveys, they cannot currently provide any data on abundance, further illustrating the need for a new avian biomonitoring technique that is more precise and effective.

Overview of Airborne Environmental DNA (aeDNA)

Environmental DNA (eDNA) sampling is a growing biomonitoring method to address the challenges of traditional bird surveys. eDNA refers to genetic material that animals, plants, bacteria, fungi, and archaea shed into the environment, such as skin cells, spores, feather fragments, saliva, or waste. This DNA can be collected from soil, water, or air using various sampling techniques and then extracted from cells using lysis (Yamahara et al., 2019). Next, a specific DNA segment (genetic marker) is selected, amplified by polymerase chain reaction (PCR), and digitally sequenced, detecting the presence or absence of a taxon, with further iterations showing abundance.

There are two directions to follow with eDNA sequencing based on the choice of the primer and PCR amplification region. For surveys focusing on a single taxa (results will not show which subtaxa are present), researchers can target a unique DNA segment using a custom assay to identify the presence and abundance of a taxon. For more general surveys aimed at identifying the presence of multiple, separate taxa within a community, metabarcoding can be used to target a region of DNA that is variable between species, but surrounded by DNA that is mostly shared. The primer attaches to the shared DNA, and the variable “barcode” region is amplified by PCR. The “barcodes” can then be matched with a reference library such as GenBank to identify multiple species in a mixed environment (Seymour, 2019). The steps of DNA metabarcoding are illustrated in Fig. 3. Metabarcoding is not only used for conservation, but for microbiome analysis, disease monitoring, and more.

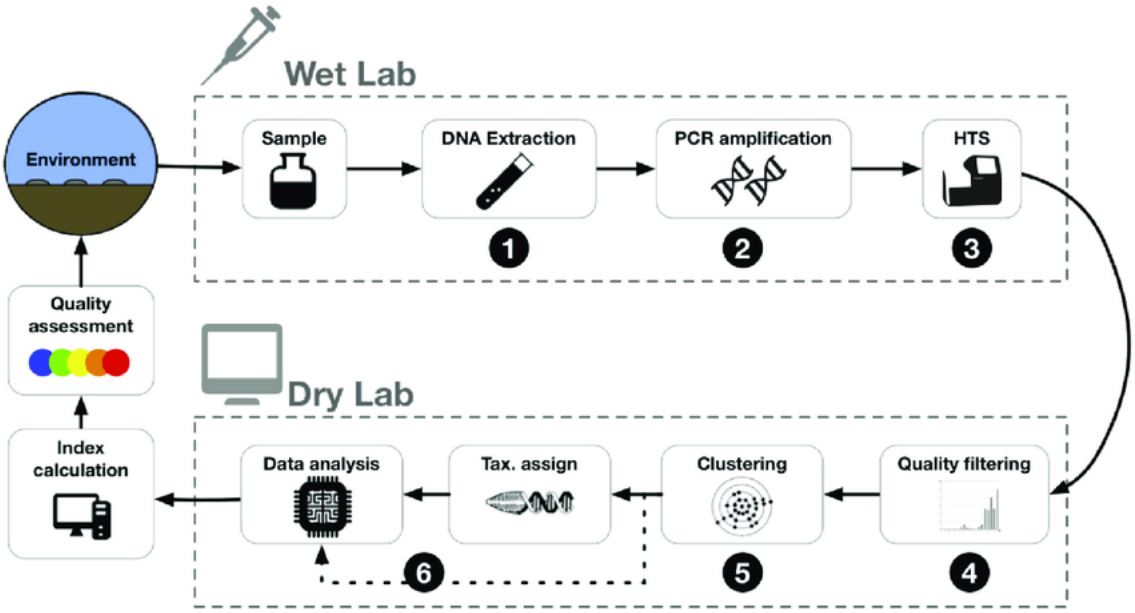

Figure 3: Schema of key steps in eDNA and metabarcoding (Pawlowski et al., 2018).

Most current eDNA biomonitoring methods focus on extracting eDNA from water and soil, as eDNA is more concentrated and less easily relocated. While water- and soil-eDNA has shown efficacy in some avian surveys, it fails to reliably detect many passerines and canopy dwellers that do not frequent water bodies (Day et al., 2019; Tetzlaff et al., 2024). Airborne eDNA (aeDNA) holds promise as a new biomonitoring method to extract the eDNA birds shed into the air through air filters without needing to sample water or soil.

Survey Methodology

Sampling

aeDNA sampling methodologies involve using air filters in different ways depending on the context. Lynggaard et al. (2022) tested three air sampling systems: a water vacuum, drawing air through a bottle of sterile water using vacuum, and two F8 particulate filters setup with a high and low airflow fan, allowing for different sample time-scales. The high airflow setup detected the most species consistently, making it ideal for speed and precision, while the low airflow setup detected slightly lower numbers while being quieter, more portable, and taking a longer sample time, making it ideal for quiet environments or overnight sampling. This study underscores the need to match sampler design to field conditions and conservation goals.

In contrast, Métris & Métris (2023) mounted a stainless-steel probe onto a light aircraft, filtering up to 2,175 L of atmospheric air per sample using a vacuum pump and sealed filter chamber (Fig. 4). The methodology was highly effective, allowing the researchers to detect DNA from vertebrates, plants, and bacteria from altitudes of 300 to 2,500 m. However, the study's

relatively low air volumes, short flight duration, and lack of ground-truth validation limited its ability to assess detection sensitivity or accuracy.

Figure 4: High-integrity steel probe in action during aeDNA survey (Métris & Métris, 2023).

Material and Expertise

One of the driving factors for the development of eDNA is that the eDNA methodology requires less manpower and expertise than traditional methods, making it accessible and scalable. However, its successful development in all types of habitats across the globe still demands considerable technical expertise and advanced facilities. As opposed to a need for experts to spend vast amounts of time in the field to identify species, the eDNA workflow requires an advanced understanding of molecular biology and statistics for the development of primers, assays, and statistical models, but can be readily used to biomonitor by professionals with understandings of anti-contamination protocols and proper sampling techniques. eDNA is labor- and expertise-demanding in the laboratory, as every basic eDNA sample involves the multistep process described in the “Introduction to eDNA” section, where a slight mishap at any step can result in the contamination of the entire sample. The impact of contamination is especially amplified in aeDNA due to low overall DNA concentration, relatively high environmental noise, and the stochastic nature of airborne eDNA (Mathieu et al., 2020; Rourke et al., 2022). Therefore, the effective interpretation of aeDNA data requires not only technical and statistical knowledge, but also ecological judgment tailored to species behavior and landscape context (Lynggaard et al., 2022; Tournayre et al., 2025).

Detection Dynamics and the Challenge of Abundance Quantification

Sensitivity and Reach

aeDNA surveys can track rare, elusive, or nocturnal species that traditional methods miss, as even a minuscule amount of eDNA floating in the air can be detected. However, high sensitivity can result in secondary DNA transfer, where DNA from org not present on site can be detected. For example, Johnson et al. (2023) detected cattle DNA at natural sites 800+ meters away from the nearest cattle, showing aeDNA drift from anthropogenic sources. This secondary DNA transfer may mask and suppress the amplification of low-abundance DNA during PCR, making the survey less accurate and precise.

aeDNA surveys can capture DNA from an 80 km radius, with closer signals more likely to be present, allowing for fewer physical detection sites to sample the eDNA of an entire habitat. This high spatial reach also means that there is less geographical certainty on the location of each eDNA detection, with a chance of it having come from anywhere within an 80 km radius. This represents an increase in the likelihood of false positives, as detections may not reflect an actual presence at the sampling site (Tournayre et al., 2025).

Quantification of Abundance

The Challenge

The most important challenge that aeDNA, as well as all eDNA surveys, face in becoming the most comprehensive biomonitoring tool is the quantification of abundance. The key data point to quantify abundance from eDNA surveys is DNA concentration/DNA copy numbers, which show how much of a species' DNA is present in the survey. aeDNA faces a two-pronged challenge to the quantification of abundance in the form of environmental and internal factors.

Environmentally, aeDNA faces stochastic detection due to wind direction, shedding rates, and degradation, which aeDNA is more prone to than water or soil eDNA. Different species also shed DNA at different rates due to their size, metabolism, and various unpredictable factors, making it challenging to compare the abundance of species using their respective DNA concentrations. For example, a piece of feather from kilometers away could fly into the filter by chance and skew the concentration of DNA (Mathieu et al., 2020).

Methodologically, aeDNA faces the challenge of PCR amplification bias, amplifying the DNA of some species more than others. The metabarcoding process uses a universal primer that bonds a shared piece of DNA between target species in the survey. However, the bonding sections of the species' DNA are not exactly identical, with some small mismatches occurring between the primer and the gene. The primer will still bond to all species with this similar gene, but it will

have a weaker bond to the genes with mismatches, and thus amplify them less (Elbrecht & Leese, 2015; Rourke et al., 2022).

These challenges were illustrated empirically in Polling et al. (2024)'s 31-day aeDNA time-series study. Though the study successfully identified 16 vertebrate taxa, the authors found significant variability in detection frequency and the lack of a correlation with abundance. Therefore, to quantify abundance, researchers must figure out whether the reason a species has a higher DNA concentration is truly due to a higher abundance, the species sheds more eDNA, the DNA was amplified with bias, or that a large piece of DNA reached the detection site by chance. The solutions to most of these points are possible with current technology.

The Solutions

Quantification methods like digital droplet PCR (ddPCR), which encapsulates DNA copies in droplets and counts the droplets, and quantitative PCR (qPCR), which amplifies the target gene while estimating DNA copy numbers based on the intensity of a fluorescent dye, can provide DNA copy numbers from raw sequencing reads. They also utilize negative and positive controls by sampling the fluorescence of an empty sample and by comparing the results to known DNA detection regressions to lower the rate of contamination and false positives. However, they do not account for PCR amplification bias.

The qMiSeq method, a quantitative eDNA metabarcoding technique, was developed to allow researchers to convert raw sequencing reads into DNA copy numbers and concentration while accounting for PCR amplification bias. Researchers add synthetic DNA fragments to the sample as reference points for PCR amplification, known as internal standard DNAs. These DNAs are added at precisely known quantities of DNA copies, serving as reference points that allow researchers to account for PCR amplification bias to estimate DNA copy numbers (Tsuji et al., 2022). The method has been verified by Tsuji et al. (2022) in fish communities, though further research is needed for airborne field-testing.

To address the problem of environmental stochasticity in detection, Allen et al. (2024) advocate for using occupancy modeling frameworks. These models use replicate surveys at each site to statistically separate true presence from imperfect or chance detections, allowing researchers to estimate the extent of an organism's presence in an area. For instance, if a piece of feather from a distant bird were to randomly drift into the detection apparatus during one trial, it is still extremely unlikely that the event would occur for every replicated survey at the same location. By modeling detection probability, occupancy models reduce the influence of such events, allowing researchers to more confidently detect whether a species is truly occupying a site.

With the qMiSeq technique and occupancy models, researchers can rule out PCR amplification bias and environmental stochasticity as limiting factors to the quantification of abundance.

However, researchers cannot confidently quantify and compare the abundance between species with the glaring issue of different shedding rates between species (Elbrecht & Leese, 2015).

Conservation Applications and Strategies

aeDNA biomonitoring can effectively track avian abundance and range across temporal and spatial scales, allowing researchers to passively track avians over a set area, including elusive species that were difficult to monitor or uncharismatic species that were often misidentified or biased against in traditional surveys.

Large-scale Biodiversity Surveys

aeDNA can be used to conduct large scale biodiversity surveys with more reach and fewer hurdles than any other biomonitoring tool. Tournayre et al. (2025) conducted the first-ever national-scale aeDNA biodiversity audit by repurposing PM10 air pollution samplers from the UK's preexisting air quality monitoring network. The researchers used 15 sites that spanned the country, collecting eDNA in weekly cycles for over a year (Fig. 5). They used 5 metabarcoding primers, detecting over 1100 taxa and demonstrated that aeDNA surveys of this vast spatial scale are plausible and effective, monitoring common and rare species alike. This survey underscores the future of scalable, cost-effective biodiversity monitoring using pre-existing infrastructure, though improvements can be made in future iterations to improve spatial clarity using more surveying sites. A variety of methods, including airplane flybys as explained earlier, can be used to achieve a similar result of large-scale biomonitoring (Métris & Métris, 2023).

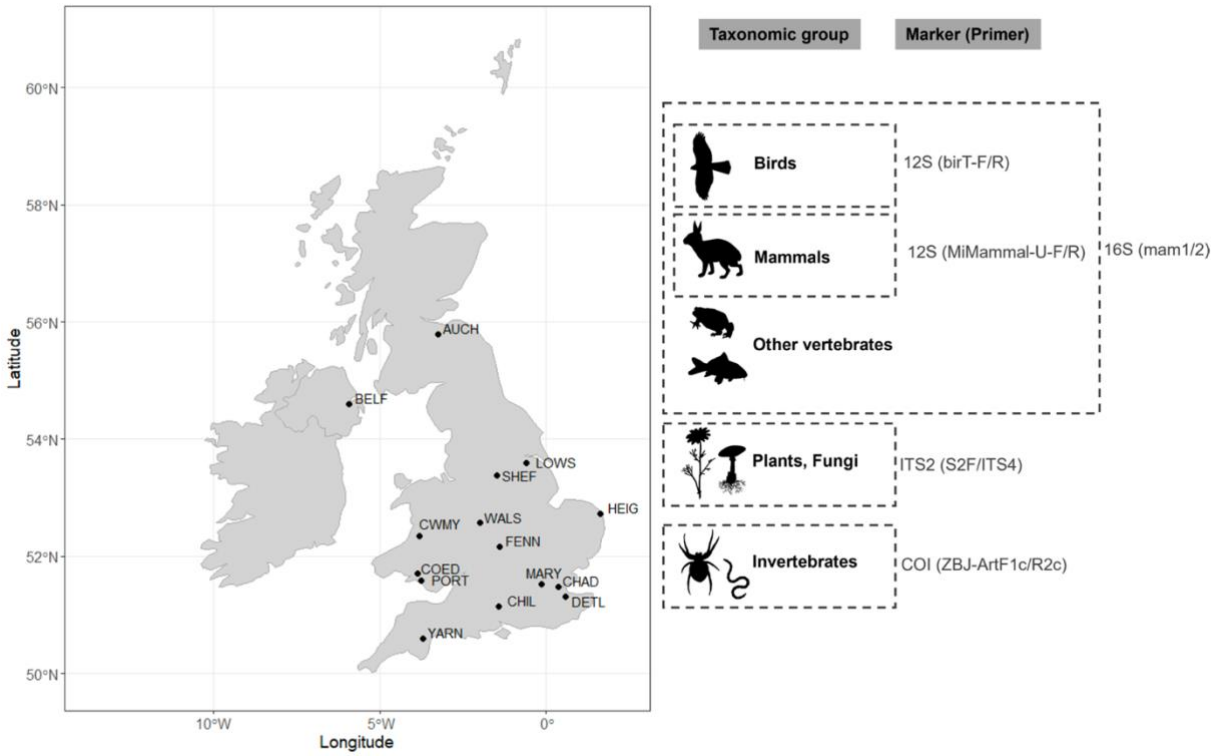

Figure 5: Map of 15 sampling sites across the UK heavy metals monitoring program and the primers used to sequence different taxa (Tournayre et al., 2025).

Species-specific Tracking & Monitoring

aeDNA can also be used to monitor the abundance and ranges of rare, elusive, invasive, and threatened species. Researchers have previously used water-based eDNA in a specific case to detect the endangered Gouldian finch (*Erythrura gouldiae*) by sampling its watering holes in its arid Australian habitat. As the finch must drink water daily due to its granivorous diet, and as water becomes scarce during the dry season, gouldian finches reliably deposit their DNA into a few shared water sources, making it possible to detect them with water-based eDNA (Day et al., 2019). While this study shows the possibility of tracking the ranges of endangered birds using eDNA, it represents a best-case scenario for water-based eDNA as the species' predictable drinking patterns allow for a direct pathway for the detection of its DNA. The water-based methodology will struggle with detecting birds with large home ranges or with sporadic drinking patterns. In these cases, aeDNA will be much better at tracking the birds' abundance and ranges. The white-headed vulture is a prime example of the types of birds that aeDNA could target.

The white-headed vulture (*Trigonoceps occipitalis*) is a solitary-nesting savannah raptor with a population of roughly 5500 individuals. Even in known breeding territories, the raptor proved remarkably difficult to detect, with Murn & Holloway (2016) only detecting it on 72 occasions

across 359 visits to 17 confirmed locations, an average per-visit detection rate of just 0.207. Therefore, 13 visits must be conducted to infer absence at a site in the future (95% CI), making it difficult to accurately map *T. occipitalis'* range. With home ranges between 20 and 100 km^2, both conventional detection and water eDNA are not fit to accurately judge the species' range and abundance. aeDNA can assess the vulture's presence at a site and map its range. Depending on the budget, the researchers could set up overnight sampling stations at any sites they visit, following Lynggaard et al. (2022)'s methodology, or they could follow Métris & Métris (2023)'s methodology by using a plane and probe to fly over sites one by one, detecting and mapping the vultures' presence.

aeDNA in Tandem with Traditional Surveys

The value of aeDNA as a biomonitoring tool is highest when it is used in combination, not in competition with traditional surveys. A vast majority of current aeDNA studies contain ground-truthing using traditional methods or known elements. For example, studies like Lynggaard et al. (2022) and Polling et al. (2024) show the validity of aeDNA by ground-truthing their detections through camera traps and by sampling in an environment with a known quantity and species of animals (zoo). Studies like Murn & Holloway (2016), on the other hand, provide sighting rates for key species with traditional surveys, which allows the construction of occupancy models to increase the accuracy of aeDNA surveys. This is especially important as aeDNA is in its developmental stage and requires extensive confirmations of its validity, with follow-up traditional surveys serving as a tested method to do so. Standardized traditional surveys can become part of the larger system of aeDNA development, serving as an anchor and reference to the truth to guide and calibrate aeDNA surveys. This will allow the new method to grow and eventually be able to accurately and reliably provide extensive data on natural phenomena and species range and abundance.

Next Steps

In the short term, the continued development of aeDNA as a biomonitoring tool requires the standardization of field protocols. Studies to date have used a variety of air sampler types, filter types, fans, probes, and sampling times. Establishing standardized protocols for equipment and sampler design, with variations depending on the purpose of the survey (general biodiversity monitoring, elusive species tracking, etc.) will improve data reliability and allow for cross-referencing of data between studies. This will also support the development of detection probability databases and shedding rate databases. Nations, states, provinces, or even cities with developed air quality management systems should follow Tournayre et al. (2025)'s proof of concept and repurpose parts of their air quality systems for a short amount of time and for a low cost to conduct a large-scale biodiversity survey. On a smaller scale, citizen science projects can be developed, to distribute DIY filter kits to experienced birders and compare the aeDNA results with citizen-observed eBird lists to evaluate the concept of citizen-eDNA.

Looking forward, the long-term dependability of aeDNA as a comprehensive biomonitoring tool will depend on the development of analytical frameworks to understand more from aeDNA data. A species-specific DNA shedding rates database is paramount to allow for the quantification of abundance from aeDNA results. Researchers can survey the aeDNA at zoos or aviaries where the quantity and species of birds are known to begin building a statistical model that can account for the shedding rates of different species in detection, allowing for the solving of the last environmental factor needed to quantify abundance. Building a more comprehensive global detection probability database for as many species as possible is necessary, too, linking sampling methods to species detection rates across habitats, allowing researchers to use this data in occupancy models and statistically rule out false negatives and infer species presence more accurately. Reference libraries must also be continually expanded for more barcode regions and more species to allow for more reliable and comprehensive sequencing.

Conclusion

Airborne environmental DNA could be the future of avian biomonitoring, offering novel solutions to the enduring limitations of traditional surveys. While aeDNA cannot yet provide interspecific abundance estimates due to variable shedding rates, this issue will be solved only in a matter of time, with the development of new databases and statistical frameworks to model shedding rates. aeDNA can be applied in a myriad of methods, showing potential to track the ranges of endangered or invasive species at landscape scales. Its passive, scalable nature makes it suited for integration into preexisting infrastructure and citizen science projects, with potential to democratize and upscale biomonitoring efforts. Future work must focus on building the tools required for robust interpretation, standardizing field protocols, species-specific shedding rates and detection probabilities, and expanding genetic reference libraries. By addressing these challenges, aeDNA can evolve into a comprehensive and accessible tool – the future of biomonitoring.

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