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A Simple Passive Sampler Reveals Airborne eDNA Sampling and Processing Trade-Offs

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ABSTRACT

Airborne environmental DNA (eDNA) is an emerging tool for biodiversity assessment, enabling the detection of organisms from biological particles suspended in the air. Recent studies have demonstrated its potential for detecting diverse taxonomic groups, with applications in conservation, biosecurity, and ecological monitoring. However, given the early stage of development and the diversity of collection approaches, it remains unclear how key sampling design parameters influence airborne eDNA collection, detection, and ecological inference. Here, we use a simple, low-cost, 3D-printable passive collection device to systematically evaluate the effects of filter surface area, sampling duration, and post-collection storage on target-species detection and community recovery. Using species-specific qPCR targeting European rabbit (*Oryctolagus cuniculus*), shingle-back lizard (*Tiliqua rugosa*), and tiger (*Panthera tigris*), alongside general vertebrate 12SV5 and mammal-specific Mam1/2 metabarcoding, we found that smaller filters generally improved detection sensitivity, recovered signal increased rapidly over short deployment periods before showing diminishing gains, and dry storage for 28 days had only modest effects on detection outcomes. Zoo deployments recovered ecologically plausible signals, although zoo-held taxa were detected inconsistently across replicate filters. Together, these findings demonstrate that sampling design can substantially influence detection within passive airborne eDNA workflows and provide practical guidance for developing scalable and reproducible monitoring strategies.

1 | Introduction

Airborne environmental DNA (eDNA) has rapidly emerged as a promising new non-invasive approach for terrestrial biodiversity monitoring, enabling detection of organisms from biological material suspended in the air (Johnson and Barnes 2024; Tulloch et al. 2025). While eDNA methods are now widely used in aquatic systems, extending these approaches to air sampling has opened new avenues for broad-scale terrestrial ecosystem surveillance.

Airborne eDNA is derived from bioaerosols, which are particles of biological origin that encompass a variety of organic materials such as microorganisms, pollen, spores, and cellular debris

from various macroorganisms (Després et al. 2012). The potential of airborne eDNA lies in its ability to monitor the diversity of biological communities across different taxa and landscapes, allowing for the detection of rare, elusive, or nocturnal species that might otherwise go unnoticed (Garrett, Watkins, Simmons, et al. 2023; Garrett, Watkins, Francis, et al. 2023; Lynggaard et al. 2024; Métris and Métris 2023). It also offers opportunities to improve our understanding of community composition dynamics in greater depth than can be achieved using traditional field methods alone (Johnson, Cox, et al. 2021; Johnson, Fokar, et al. 2021). In terms of practical applications, airborne eDNA has shown promise in conservation monitoring, where it can be used to detect invasive species and enhance eco-restoration

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and biosecurity efforts (Johnson, Fokar, et al. 2021; Sanders et al. 2023; Trujillo-Gonzalez et al. 2022).

Recent studies have demonstrated the feasibility of airborne eDNA detection in both controlled and natural environments. In early experiments conducted under controlled conditions, vertebrate DNA was successfully recovered from enclosed animal habitats, demonstrating that airborne genetic material could be consistently captured and identified (Clare et al. 2021). Building on these findings, subsequent research has extended airborne eDNA sampling to settings including zoo enclosures, natural forested landscapes, and urban locales (Clare et al. 2022; Johnson et al. 2023; Lynggaard et al. 2022; Roger et al. 2022; Runnel et al. 2024). Collectively, this work points to a growing and versatile toolkit for ecological sampling that complements conventional methods and expands the scope of biodiversity monitoring.

Airborne eDNA can be collected using a variety of sampling methods, broadly grouped into active and passive approaches. Active samplers typically use powered vacuum pumps or fans to draw air through filters or other collection media at controlled flow rates, enabling standardized air volume sampling (Brennan et al. 2019; Garrett, Watkins, Simmons, et al. 2023; Lynggaard et al. 2022; Roger et al. 2022). However, their reliance on external power sources and mechanical components can limit deployment flexibility, particularly in remote or rugged environments. Passive samplers, by contrast, operate without powered airflow and collect biological material through ambient transport and deposition, using designs ranging from directly exposed filters, sticky tapes, or settling plates to structures that orient or concentrate ambient airflow across a collection surface without generating a controlled airflow or defined sampling volume (Johnson et al. 2023, 2019; Runnel et al. 2024; Schlegel et al. 2024). Related approaches use naturally occurring surfaces as passive collectors, recovering eDNA by swabbing leaves or collecting spider webs (Berard et al. 2025; Bodawatta, Madsen, Lynggaard, and Bohmann 2026; Gregoric et al. 2022; Lynggaard et al. 2023; Newton et al. 2024). Compared to active samplers, purpose-built passive devices are generally inexpensive, lightweight, and easily deployed at scale, although they lack defined sampling volumes and may be influenced more strongly by local air currents, weather, and physical particulate characteristics. Comparative studies between passive and active sampling remain limited, but initial evidence suggests passive detection methods can be at least as effective (Dillon et al. 2023; Jager et al. 2026; Yamamoto et al. 2011).

Despite recent advances in airborne eDNA methods, uncertainty remains about the factors influencing detection sensitivity and ecological inference (Tulloch et al. 2025). Detection outcomes may reflect interacting effects of environmental conditions, target-species biology, and sampling design. Environmental factors such as temperature, humidity, wind speed, and UV exposure are likely to affect airborne eDNA persistence, much as they do in other environmental matrices (Tulloch et al. 2026). Studies in aquatic (Barnes et al. 2014; Jo and Minamoto 2021; Joseph et al. 2022; McCartin et al. 2022) and soil (Guthrie et al. 2024; Sirois and Buckley 2019) systems have demonstrated the influence of these variables on eDNA persistence and

detectability. While airborne particles can include both local and regionally transported material, such as pollen and fungal spores, recent studies suggest that animal-derived airborne eDNA is often spatially localized, reflecting organisms near the sampler and showing catchment patterns over hundreds of meters (Lynggaard et al. 2022; Roger et al. 2022; Stewart et al. 2026; Tournayre et al. 2025).

Target species biology plays a crucial role in eDNA detection probabilities. Studies in aquatic systems have shown that shedding rates vary widely among taxa, influenced by physiology, metabolic rate, and behavior (Dowell et al. 2024; Klymus et al. 2015; Maruyama et al. 2014; Pilliod et al. 2014). Emerging evidence from airborne eDNA studies suggests that similar biological factors may influence detection in air. Zoo-based studies have found that animal biomass is positively associated with both airborne eDNA detection probability and sequencing read abundance (Jager et al. 2026; Lynggaard et al. 2022), supporting the idea that source population characteristics and shedding dynamics shape airborne eDNA profiles. However, further research is needed to clarify how particle form, shedding rate, and behavioral patterns interact with atmospheric conditions to influence detectability in natural systems.

Many environmental and biological influences are intrinsic to the system being sampled, and while they can be accounted for, are not readily controllable in field applications. By contrast, sampling design can be deliberately optimized to improve detection sensitivity, reproducibility, and ecological interpretation. Factors such as deployment height, sampling duration, airflow rate, filter material, and spatial replication likely shape the volume and composition of airborne eDNA captured. Previous studies have found context-dependent outcomes across sampler types and sampling durations (Lynggaard et al. 2022), taxon-dependent gains from additional replication (Garrett, Watkins, Francis, et al. 2023), and continued accumulation of vertebrate richness across passive deployments from 6 to 96 h (Jager et al. 2026). However, the generality of these effects across sampler designs and environmental settings remains poorly resolved, limiting our ability to interpret detections, compare approaches across studies, and design effective monitoring workflows.

This study aimed to systematically evaluate how key sampling design parameters influence airborne eDNA detection outcomes, using a simple, low-cost passive sampling platform to enable controlled comparisons. The platform was developed to support robust replication and repeatable processing workflows, with low construction cost enabling larger sample sizes, a removable filter system reducing direct sample handling during collection and extraction, and a modular design facilitating systematic testing of individual sampling parameters. We focused on evaluating three core variables likely to affect detection outcomes: filter surface area, sampling duration, and post-collection storage time prior to extraction. Each of these parameters represents a common point of variation in field protocols, and understanding their influence is important for designing accessible and reproducible sampling approaches. Larger filters may capture more airborne material, but increased handling requirements risk introducing background

contaminants and pose challenges for high-throughput DNA extraction workflows. Extended sampling periods may increase recovered DNA signal but may also increase exposure to degrading environmental conditions such as heat, humidity, or UV. Similarly, filter storage prior to downstream processing may enable sampling flexibility but may affect DNA integrity and detection sensitivity. Together, these variables encompass key stages of the airborne eDNA sampling workflow, from particle capture to downstream processing.

To evaluate these factors, we paired species-specific qPCR assays for focal taxa with metabarcoding to assess broader community recovery. We also characterized background signal across control types and filter-preparation workflows and evaluated sampling outcomes in a zoo setting to assess ecological plausibility and spatial coherence under real-world conditions.

2 | Methods

2.1 | Study Overview and Experimental Framework

We conducted three controlled field experiments evaluating the effects of filter surface area, sampling duration, and post-collection storage on airborne eDNA detection, together with zoo deployments as a biologically complex test case with known species context (Tables 1 and S1). We used species-specific quantitative PCR (qPCR) to assess focal taxon detection and signal strength, and vertebrate metabarcoding to assess detection frequency, taxonomic richness, and community composition.

2.2 | Sampling Platforms and Device Design

2.2.1 | Filter Surface Area Trial Platform

For the filter surface area experiment, we used a custom sampling platform that mounted equal numbers of small and large filters vertically at approximately 1.5m above the ground, beneath a basic rain cover and with both faces exposed to ambient airflow (Figure 1A,B). Full construction details are provided in Appendix S1.

2.2.2 | 3D-Printed Passive Air Sampler

We designed a portable passive sampler consisting of a durable 3D-printed PETG housing and four modular filter cartridges (Figure 1C–F). The open-sided housing positions the cartridges beneath a protective roof while leaving both faces of each filter exposed to ambient air. Each cartridge securely holds a single circular filter that passively intercepts airborne particles. The cartridges are designed to fit into a standard 50-mL tube after retrieval, reducing direct handling during transport and processing. Integrated mounting slots allow the sampler to be secured to trees, posts, or other vertical structures. This device is registered with IP Australia (Design No. 202510703), and the printable files are publicly available for non-commercial use (<https://doi.org/10.25919/8mh3-ys76>).

TABLE 1 | Overview of experimental design used to evaluate passive airborne eDNA sampling.

Experiment	Site	Sampler	Experimental comparison	Replication	qPCR target	Metabarcoding loci
Filter surface area	BM	FS	6.25 versus 25-cm ² filters; 7-day deployment	Two deployments; 3 and 9 filters per treatment	Rabbit	12SV5; Mam1/2
Sampling duration	BM	3D	1 h, 1 day, 1 week and 2 weeks	Two deployments; 3 filters per duration per deployment	Rabbit	12SV5; Mam1/2
Post-collection storage	BM	3D	Immediate extraction versus 28 days storage; 1-month deployment	Two deployments; 6 and 8 filters per treatment	Rabbit	12SV5; Mam1/2
Zoo target validation	NZA	3D	Reptile house and tiger enclosure; 7-day deployment	4 filters per location	Shingleback lizard, tiger	—
Zoo community sampling	NZA	3D	Reptile house and three outdoor locations; 7-day deployment	Two deployments; 4 filters per location per deployment	—	12SV5; Mam1/2

Note: Details for each of the five experimental components. Sampling sites include CSIRO Black Mountain campus (BM) and the National Zoo and Aquarium (NZA). Sampler types include the custom filter surface area trial platform (FS) and the 3D-printed passive sampler (3D). Replication values refer to separately processed filters. Rabbit, European rabbit (*Oryctolagus cuniculus*); shingleback lizard (*Tiliqua rugosa*); tiger (*Panthera tigris*). Complete deployment dates, filter preparation conditions, treatment allocations and weather summaries are provided in Table S1.



FIGURE 1 | Passive airborne eDNA sampling devices used in this study. We deployed two passive eDNA collection platforms. (A–B) *Filter surface area trial platform*: (A) full view of the custom-built device constructed from a vertical fence post set in a concrete-filled bucket and fitted with a bin lid for rain protection, with multiple filter holders mounted along the post; (B) close-up of the PVC Mozzie Stoppa trial units with 3D-printed filter holders shown inserted to vertically hold differently-sized filters (2.5 × 2.5 and 5 × 5 cm); (C–F) *3D-printed passive air sampler*: (C) field deployment of the platform with a strap securing the unit to a tree; (D) close-up of the housing showing four cartridge insertion points, including the internal locking mechanism to prevent cartridge displacement; (E) assembled filter cartridge showing the recessed channel that engages with the locking mechanism; (F) disassembled filter cartridge illustrating placement of the circular filter within the cartridge cap.

2.2.3 | Filter Material and Preparation

We collected all airborne eDNA samples using Filtrete 1900 Smart Air Filters (3M), a HEPA-type fibrous filter material previously validated for airborne eDNA collection and shown to perform comparably to class F8 fibrous filters used in other studies (Garrett, Watkins, Francis, et al. 2023; Garrett, Watkins, Simmons, et al. 2023; Lynggaard et al. 2022). For the 3D-printed passive samplers, we used circular filters measuring 2.5 cm in diameter, corresponding to a one-sided surface area of 4.9 cm². For the filter surface area experiment, we used square filters measuring 2.5 × 2.5 cm (Small) and 5 × 5 cm (Large), corresponding to one-sided surface areas of 6.25 and 25 cm², respectively. We used both sterile and non-sterile filter and device preparations to characterize background DNA associated with filter material and handling. For sterile deployments, we autoclaved and UV-irradiated the filters and decontaminated the PETG sampler components using bleach, ethanol, and UV treatment. For non-sterile deployments, we used untreated filters and new, untreated housings and cartridges. Complete filter preparation, decontamination and transport procedures are provided in Appendix S1.

2.3 | Study Sites

We carried out deployments at two sites in Canberra, ACT, Australia: the CSIRO Black Mountain campus and the National Zoo and Aquarium (Figure 2).

2.3.1 | CSIRO Black Mountain Campus

We conducted field experiments in an open grassy area at the base of Black Mountain (approximately 35.273239° S, 149.111278° E), within a mixed-use research precinct adjacent to native eucalypt woodland (Figure 2A).

2.3.2 | National Zoo and Aquarium

We conducted zoo deployments at the National Zoo and Aquarium, Canberra (35.2926° S, 149.0884° E), which houses a diverse assemblage of native and exotic vertebrates. We deployed samplers outdoors near animal enclosures and a central food preparation area, and indoors within an off-exhibit

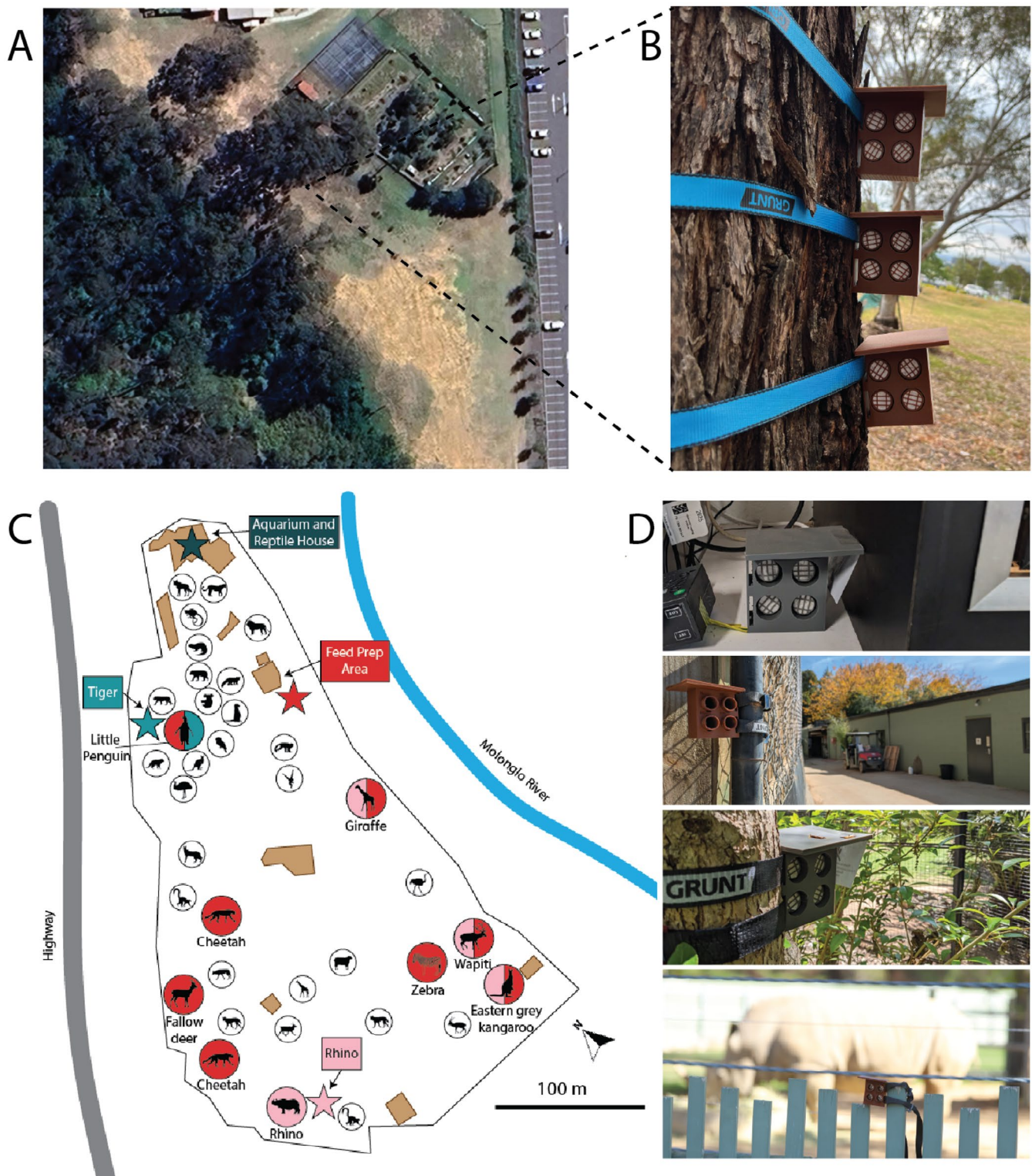


FIGURE 2 | Sampling sites and airborne eDNA sampler deployment locations. Sampling sites used for airborne eDNA collection in Canberra, ACT, Australia. (A) Aerial view of the CSIRO Black Mountain campus showing the open grassy area adjacent to native eucalypt woodland where controlled field experiments were conducted; dashed line indicates the focal sampling area. (B) Simultaneous deployment of three 3D-printed passive samplers mounted to a tree trunk. (C) Schematic map of the National Zoo and Aquarium illustrating the spatial arrangement of animal enclosures. The Molonglo River, major access roads, and scale bar are shown for spatial reference. Color-coded stars denote airborne eDNA sampler deployment locations at the reptile house (dark green), tiger enclosure (teal), food preparation area (red), and rhino enclosure (pink). Detected taxa are shown as enlarged species icons and colored according to the sampler at which they were detected, including ambiguous detections (fallow deer, wapiti, and eastern gray kangaroo). All species icons are publicly available from PhyloPic (<https://www.phylopic.org/>). (D) Sampler deployments at the four locations at the National Zoo and Aquarium at the reptile house, food preparation area, tiger enclosure and rhino enclosure (top to bottom).

reptile facility used by staff for animal husbandry and handling (Figure 2C).

2.4 | Sampler Deployment and Field Handling

We mounted 3D-printed passive samplers on tree trunks and fence posts approximately 1.5 m above ground, with filters oriented vertically (Figure 2B). At the CSIRO campus, we oriented all filters vertically and in the same compass direction within each deployment. Where practical, we scheduled deployments during forecasted dry conditions to reduce weather-related variation among experimental comparisons. Field personnel wore clean gloves during deployment and retrieval. Upon retrieval, we placed retrieved filters or cartridges into sterile 50-mL tubes and transported samples and field blanks to the laboratory under ambient conditions.

2.5 | Experimental Design and Replication

Experimental designs and replication are summarized in Table 1, with complete deployment details provided in Table S1. Across all experiments, each filter constituted an independently processed biological sample for both qPCR and metabarcoding analyses. For the 3D-printed passive samplers, each **device** comprised one housing holding four independent filter **cartridges**. We defined a **deployment** as a continuous exposure period at a given site under a fixed experimental configuration.

2.5.1 | Filter Surface Area Experiment

We compared Small (2.5×2.5 cm; 6.25 cm²) and Large (5×5 cm; 25 cm²) filters during two 7-day deployments at the CSIRO campus. In the first deployment, we exposed three filters of each size concurrently on one platform. In the second deployment, we deployed three platforms each holding three filters of each size (9 filters per size).

2.5.2 | Sampling Duration Experiment

We compared sampling durations of 1 h, 1 day, 1 week, and 2 weeks during two deployments at the CSIRO campus. Each deployment used three 3D-printed samplers, with one cartridge retrieved from each sampler at each time point, providing three filters per duration per deployment.

2.5.3 | Post-Collection Storage Experiment

We deployed filters for 1 month and then **extracted immediately (Day 0)** or stored for **28 days before extraction (Day 28)**. We stored filters for the Day 28 treatment in sterile 50-mL tubes at approximately 22°C in the dark. The two deployments used three and four samplers, respectively, with two filters per sampler assigned to each treatment, providing six and eight filters per treatment.

2.5.4 | Zoo Deployments

We sampled one indoor location (reptile house) and three outdoor locations near the food preparation area, tiger enclosure, and rhinoceros enclosure (Figure 2C,D). We conducted an initial functional test deploying one sampler at the reptile house and one adjacent to the tiger enclosure for 1 week. In two subsequent deployments, we placed one sampler containing four filters at each location. We obtained fecal material from shingleback lizard (*Tiliqua rugosa*) and tiger (*Panthera tigris*) enclosures and used these samples as positive controls for qPCR analyses. A full list of species held at the National Zoo and Aquarium is available in Table S2.

2.5.5 | Field Blanks

For each experiment, we included non-deployed field blanks consisting of prepared filters or cartridges transported to the field but not exposed. These blanks experienced the same handling, transport, storage, extraction, amplification, and sequencing procedures as deployed filters.

2.6 | DNA Extraction From Filter Material

We performed DNA extractions in a pre-PCR laboratory using a DNeasy Blood & Tissue Kit (Qiagen; Cat no. 69506). We extracted all filter material whole, processing circular and small square filters in 2-mL tubes and large square filters in 15-mL tubes with proportionally adjusted reagent volumes. We pooled lysates from each large filter extraction onto a single spin column for purification. We eluted DNA in 200 µL Buffer AE and subsequently concentrated all extracts four-fold using DNA Clean & Concentrator-5 columns (Zymo Research) before qPCR and metabarcoding. We processed non-deployed filter blanks and reagent-only negative extraction controls alongside experimental samples. We stored extracts at −20°C. Complete extraction, concentration, and laboratory decontamination procedures are provided in Appendix S1.

2.7 | Species-Specific Quantitative PCR

We performed species-specific qPCR assays targeting European rabbit (*Oryctolagus cuniculus*) mitochondrial 12S rRNA, shingleback lizard (*Tiliqua rugosa*) ND4, and tiger (*Panthera tigris*) cytochrome *b* (Brown et al. 2023; Liu et al. 2023; Pegels et al. 2013). All assays used SYBR Green chemistry on a Bio-Rad CFX96 Real-Time PCR Detection System with samples analyzed in technical replicate. Each run included no-template controls, extraction and field blanks, and we assessed assay specificity using post-amplification melt curves. We classified reactions with a cycle threshold (C_t) ≤ 40 as positive using a predefined operational detection threshold. We evaluated assay performance using six-point, 10-fold standard curves generated from rabbit gDNA or a synthetic dsDNA fragment containing shingleback and tiger target regions. We calculated amplification efficiency from the slope of the standard curve (C_t versus $\log_{10}$ template concentration) and assessed linearity using the coefficient of determination (R^2) from linear regression. Primer sequences,

reaction components, cycling conditions and standard-curve preparation are provided in Appendix S1.

We processed amplification data using Bio-Rad CFX Manager and conducted statistical analyses in R v4.5.1. We treated each separately processed filter as the unit of observation, and we averaged C_t values across technical replicates before analysis. We classified reactions with $C_t > 40$ or no C_t call as non-detections. We retained these samples when summarizing detection frequency but excluded them from analyses of C_t values. We fitted separate ANOVA models testing the effects of filter surface area, sampling duration, and post-collection storage on qPCR signal, including deployment and replicate unit as blocking terms where appropriate to the experimental design. Where ANOVA results indicated significant main effects, we conducted post hoc pairwise comparisons using Tukey's honest significant difference tests. All raw qPCR C_t data are provided as [Supporting Information](#) (Data S1), formatted with the FAIRe metadata framework for eDNA data reporting (Takahashi et al. 2025).

2.8 | Metabarcoding Wet-Lab Methods

We amplified the general vertebrate 12SV5 marker (Riaz et al. 2011) and mammal-optimized Mam1/2 marker (Taylor 1996) using a two-step PCR workflow. We performed one PCR1 replicate per sample using locus-specific primers containing 7-bp sample-specific inline tags and partial Illumina adapter sequences. Each sample received a unique combination of forward and reverse tags. All PCR1 runs included field blanks, extraction blanks, and PCR no-template controls, which we retained through sequencing. Primer sequences, reaction compositions, and cycling conditions are provided in Appendix S1.

The Australian Genome Research Facility (AGRF) performed PCR2 to add full-length Illumina adapters and dual indices. AGRF processed 12SV5 and Mam1/2 libraries separately, normalizing and pooling equimolarly and sequencing on separate Illumina NextSeq lanes using 2×150-bp paired-end chemistry. AGRF performed demultiplexing of all libraries.

2.8.1 | Metabarcoding Bioinformatic Processing

We processed 12SV5 and Mam1/2 libraries separately in R (v4.5.1) using DADA2 (v1.38.0). We filtered and trimmed reads using locus-specific parameters before error modeling, dereplication, denoising and paired-read merging. We constructed ASV tables and removed chimeras using the DADA2 consensus method. We tracked read retention throughout the pipeline (Data S2). Complete filtering and trimming parameters are provided in Appendix S1.

We assigned taxonomy by querying ASVs against the NCBI nucleotide (nt) database using BLAST via rBLAST and retrieving taxonomic lineages using taxonomizr. We filtered candidate matches by *e*-value, sequence identity and alignment length before classifying ASVs as unambiguous, ambiguous, or poor. We adjudicated ambiguous assignments conservatively using taxonomic and biological context, including collapsing assignments to a higher taxonomic rank where species-level resolution was

unsupported. We excluded poor or clearly spurious assignments. We then combined curated taxonomic assignments with ASV count tables, retained ASVs represented by at least 10 reads in one or more samples, calculated per-sample relative abundance, and aggregated ASVs to curated taxon identities. Detailed filtering and adjudication rules are provided in Appendix S1.

2.8.2 | Metabarcoding Statistical Analysis

We analyzed each experiment independently using curated sample–taxon tables. For the three controlled experiments, we evaluated rabbit (*O. cuniculus*) detection (presence/absence), read abundance among positive samples, and per-sample taxon richness. We compared read abundance and richness among treatments using Wilcoxon rank-sum or Kruskal–Wallis tests, as appropriate. We also fitted binomial generalized linear models (GLMs) for rabbit detection and Poisson or quasi-Poisson GLMs for taxon richness, with deployment included as a fixed blocking factor. For the filter surface area experiment, we additionally modeled log-transformed rabbit read abundance and assessed replicate concordance using pairwise Jaccard similarity. Complete test specifications are provided in Appendix S1.

We used negative controls to contextualize rather than automatically exclude detections, considering control prevalence, consistency across filters and deployments, and coherence with the experimental or ecological setting. We characterized background signal by control type, metabarcoding locus, and filter preparation, including summarizing read abundance, taxon richness, taxon prevalence, and overlap between samples and controls.

For the zoo experiment, we classified taxa as zoo-held, background wildlife, domestic/feed, human, or ambiguous. We defined zoo-held taxa using an independent reference list of species housed at the zoo during sampling (Table S2). We classified ambiguous taxa as those that could represent either zoo-held animals or free-living populations in the surrounding environment (e.g., *Macropus giganteus*, eastern gray kangaroo). We summarized signal composition using within-sample relative read abundance and quantified detection consistency for each taxon and location as the proportion of replicate filters across the two deployments in which it was detected.

3 | Results

3.1 | Metabarcoding Data and Processing Outcomes

We generated 68.4 million and 84.1 million sequencing reads across 137 and 139 samples using the 12SV5 and Mam1/2 markers, respectively. After quality filtering, denoising, and chimera removal, 51.3 million (75.0%) reads were retained for 12SV5 and 58.4 million (69.5%) for Mam1/2. Across both markers, experimental samples yielded an average of 579,627 raw reads per sample, with 425,112 reads (72.5%) retained. Read retention was broadly consistent across control types (field, extraction, and PCR blanks), although PCR blanks yielded substantially fewer retained reads (Data S2).

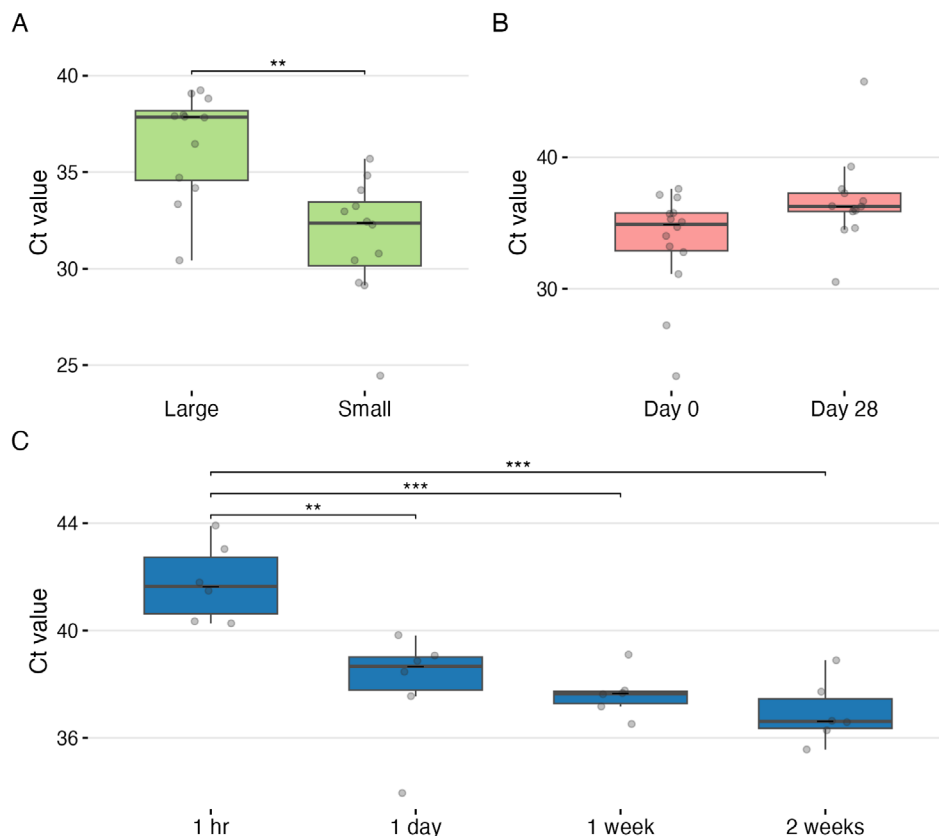


FIGURE 3 | Effects of sampling design and storage on airborne eDNA recovery measured by qPCR. qPCR cycle threshold (C_t) values from assays targeting rabbit (*Oryctolagus cuniculus*). Lower C_t values indicate higher recovered DNA quantity. Boxplots show median values and interquartile ranges, with individual biological samples (one filter) overlaid as jittered points. Panels show distributions across (A) filter surface area (large and small), (B) storage (immediate processing versus 28 days of storage), and (C) sampling duration (1 h, 1 day, 1 week, and 2 weeks). Significant pairwise differences identified by Tukey's honest significant difference are indicated where applicable (** $p < 0.01$; *** $p < 0.001$).

These reads resolved into 6206 and 8427 ASVs for the 12SV5 and Mam1/2 markers, respectively. Following taxonomic assignment, filtering, and ambiguity resolution, we retained 2785 ASVs for the 12SV5 and 3977 for Mam1/2 markers. These curated ASVs represented 75 (12SV5) and 28 (Mam1/2) taxa, yielding a total of 85 unique taxa resolved to at least genus level across both markers, including 69 resolved to species level.

3.2 | Filter Surface Area Influences Detection Sensitivity and Read Recovery

Filter surface area had a strong effect on recovered DNA quantity as measured by qPCR C_t (ANOVA, $F_{1,13} = 14.82$, $p = 0.002$; Figure 3A), with significantly lower C_t values observed for small filters compared with large filters. Neither deployment ($p = 0.54$) nor deployment replicate ($p = 0.64$) significantly affected C_t values. Amplification success was 100% for both filter sizes, indicating that observed differences in C_t values reflect changes in recovered DNA quantity rather than differences in detection failure.

Filter surface area also influenced detection of the target taxon (*O. cuniculus*) in metabarcoding data, but with a different pattern to that observed for qPCR. Detection success was higher in samples collected using small filters than large filters (16/24 versus 7/24 samples, respectively), and this difference was

significant after accounting for deployment (binomial GLM, $p = 0.008$). Among samples in which rabbit DNA was detected, however, significantly higher read abundance was observed in samples collected using large filters (Wilcoxon rank-sum test, $p = 0.01$; Figure 4A). This result was likewise retained after accounting for deployment (linear model, $p = 0.045$).

Species richness varied among samples and showed a consistent directional trend between filter sizes, although this difference was not statistically significant (Wilcoxon rank-sum test, $p = 0.075$; Figure 4B). Samples collected using small filters tended to recover higher species richness than those collected using large filters. Overall richness differed substantially between deployment events (March: median richness 7 taxa, IQR = 3.25, range = 2–9; June: median richness 9 taxa, IQR = 11, range = 1–23), and sensitivity analyses accounting for deployment likewise found no significant effect of filter surface area (quasi-Poisson GLM, $p = 0.29$). Assessment of replicate consistency under controlled conditions (sterile filters used in the June deployment) showed no difference in pairwise community similarity between filter sizes (Jaccard similarity; Wilcoxon rank-sum test, $p = 0.38$).

Together, qPCR and metabarcoding results indicate that smaller filters produced stronger qPCR signal and higher detection frequency, while larger filters yield higher target read abundance when detection occurs.

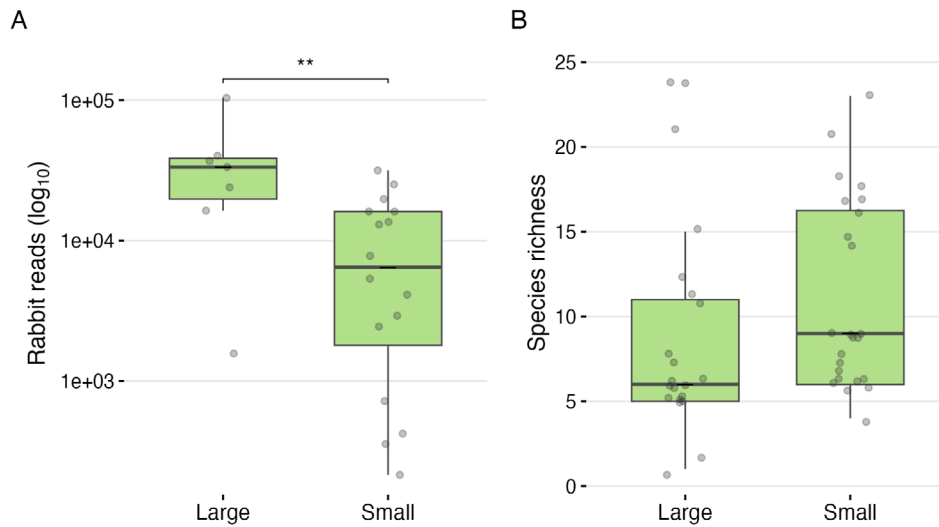


FIGURE 4 | Effect of filter surface area on airborne eDNA recovery from metabarcoding data. Metabarcoding-derived measures of airborne eDNA signal and diversity are shown for large and small filters. Boxplots show median values and interquartile ranges, with individual samples overlaid as jittered points. (A) Read abundance for rabbit (*Oryctolagus cuniculus*) among samples in which the target taxon was detected, shown on a log₁₀ scale. (B) Species richness recovered per sample based on presence–absence metabarcoding data following taxonomic filtering. Statistical significance was assessed using Wilcoxon rank-sum tests and is indicated as follows: ** $p < 0.01$.

3.3 | Post-Collection Storage Weakly Reduces Sensitivity Without Altering Community Recovery

Extended post-collection storage had a modest but non-significant effect on recovered DNA quantity as measured by qPCR C_t (ANOVA, $F_{1,17} = 3.55$, $p = 0.077$, Figure 3B). Mean C_t values increased between samples processed immediately and those processed after 28 days of storage (Day 0: 33.6 ± 1.07 SE; Day 28: 36.7 ± 0.94 SE; Figure 3B), consistent with a trend towards reduced DNA yield following storage. Neither deployment ($p = 0.91$) nor deployment replicate ($p = 0.95$) significantly influenced C_t values. Detection frequency via qPCR remained high under both storage conditions but declined modestly following extended storage (Day 0: 14/14 filters, 100%; Day 28: 13/14 filters, 92.9%), consistent with the observed shift towards higher C_t values. Together, these results indicate that storage duration may slightly reduce DNA yield, although effects were small and not statistically significant under the conditions tested.

Rabbit DNA was detected more frequently in samples processed after 28 days of storage than in those processed immediately. After accounting for deployment, however, this trend approached but did not reach statistical significance (binomial GLM, $p = 0.052$), suggesting that the apparent increase in detection likely reflects stochastic detection at low template concentrations rather than a true biological increase in signal following storage. This pattern was observed across both deployments and at both loci, although detection rates were consistently higher for the 12SV5 marker than for Mam1/2 (Table 2). Among samples in which we detected rabbit DNA, read counts did not differ significantly between storage conditions (Wilcoxon rank-sum test, $W = 106$, $p = 0.77$), indicating that storage effects were expressed primarily as differences in detection frequency rather than read abundance.

Community-level metrics were largely insensitive to storage duration. Taxonomic richness did not differ significantly between

TABLE 2 | Detection frequency of rabbit DNA from metabarcoding following immediate processing and 28-day storage.

Deployment number	Locus	Proportion positive detections	
		Day 0	Day 28
1	12SV5	0.83 (5/6)	1 (6/6)
1	Mam1/2	0.33 (2/6)	0.67 (4/6)
2	12SV5	0.38 (3/8)	0.75 (6/8)
2	Mam1/2	0.13 (1/8)	0.25 (2/8)

Note: Values indicate the proportion of individual filter samples in which rabbit DNA (*Oryctolagus cuniculus*) was detected (≥ 10 reads following quality filtering) for each deployment within the storage effect experiment. Detection frequencies are shown separately for the 12SV5 and Mam1/2 loci. Values are presented as proportions with corresponding counts of positive detections over total samples.

immediately processed samples and those stored for 28 days (Wilcoxon rank-sum test, $W = 311$, $p = 0.19$), and this conclusion was unchanged after accounting for deployment (quasi-Poisson GLM, $p = 0.29$). The number of native taxa detected varied between deployments, with no consistent directional change between Day 0 and Day 28 (12 vs. 8 taxa in Deployment 1; 6 vs. 14 in Deployment 2). Together, these results indicate that storage at ambient temperature for 28 days did not systematically alter background biodiversity signal recovery.

3.4 | Sampling Duration Increases Detection Frequency but Plateaus in Signal Strength

Sampling duration had a strong effect on DNA signal strength as measured by qPCR C_t (ANOVA, $F_{3,17} = 13.51$, $p < 0.001$), driven by substantially higher C_t values in 1-h deployments relative to all longer sampling durations (Tukey HSD, all

$p < 0.01$; Figure 3C). In contrast, we observed no significant differences among 1-day, 1-week, and 2-week deployments (Tukey HSD, all $p > 0.2$).

Neither device ($p = 0.34$) nor deployment event ($p = 0.32$) significantly influenced C_t values. Rabbit locus amplification success was 100% across all sampling durations, with successful detection in all biological samples, indicating that differences in C_t values reflected variation in DNA quantity rather than differences in detection frequency.

Metabarcoding analyses revealed a weak and non-monotonic effect of sampling duration on the probability of detecting the target taxon (*O. cuniculus*). Rabbit DNA was detected in 25% of 1-h samples (3/12), compared with 8% of 1-day samples (1/12), 17% of 1-week samples (2/12), and 58% of 2-week samples (7/12) (Table 3). Overall, detection frequency showed no consistent pattern across the shorter sampling durations but was higher after 2 weeks of deployment compared to 1 h (binomial GLM, $p = 0.027$ for 2 weeks relative to 1 h). Among samples in which rabbit DNA was detected, pooled read counts across loci (12SV5 and Mam1/2) varied across sampling durations, but differences in read abundance were not statistically significant (Kruskal–Wallis test, $\chi^2 = 2.93$, $df = 3$, $p = 0.40$; Table 3). Thus, once rabbit DNA was detected, longer deployments did not consistently yield higher read abundance.

Taxonomic richness did not differ significantly among sampling durations when analyzed within deployment events (Kruskal–Wallis tests; $p = 0.914$ and $p = 0.651$) or when pooled across deployments ($\chi^2 = 1.22$, $df = 3$, $p = 0.75$). This result was unchanged after accounting for deployment in a Poisson generalized linear model (all $p > 0.29$). Similarly, the number of unique native taxa detected varied across sampling durations and deployments but showed no consistent increase with longer sampling durations (typically 3–8 taxa per deployment across durations).

3.5 | Zoo Deployments Recover Ecologically Plausible Vertebrate Signals

Deployments at the National Zoo and Aquarium in Canberra evaluated target species detection, composition and spatial

TABLE 3 | Effect of sampling duration on rabbit detection frequency and read magnitude.

Sampling duration	Detection frequency (n/N)	Median read count (n positive samples)
1 h	0.25 (3/12)	10,143
1 day	0.08 (1/12)	218
1 week	0.17 (2/12)	2112
2 weeks	0.58 (7/12)	14,994

Note: The proportion of samples positive for European rabbit (*Oryctolagus cuniculus*) DNA and the median read counts among positive detections are shown for four sampling durations (1 h, 1 day, 1 week, and 2 weeks). Detection frequency is defined as the proportion of samples with ≥ 10 reads assigned to *O. cuniculus*, combined across metabarcoding loci (12SV5 and Mam1/2). Read counts represent the median number of curated read counts assigned to *O. cuniculus* per sample, summed across loci, among positive detections only.

distribution of vertebrate detections, and consistency across replicate filters and deployments.

3.5.1 | Initial Target Species Tests

All four filters from each site produced amplification, while no-template controls showed no amplification. Standard curves generated using synthetic gBlock targets spanning six 10-fold dilutions run in triplicate showed strong linearity across the tested range ($R^2 > 0.98$), with PCR efficiencies of 98% and 93% for the shingleback and tiger assays, respectively.

Using shingleback-specific primers on filters collected from the reptile house, all samples amplified below a conservative cycle threshold of $C_t \leq 40$, with C_t values ranging from 31.9 to 34.1 (mean = 33.1, $SD = 0.71$), indicating consistent recovery of low-abundance target DNA across filters. In contrast, amplification using tiger-specific primers on filters deployed near the tiger enclosure occurred at substantially higher C_t values (range = 37.6–41.4; mean = 38.9, $SD = 1.29$), approaching the lower end of the assay's calibrated detection range. Applying the same $C_t \leq 40$ cutoff, three of four tiger-deployed filters yielded amplification below the threshold, while the remaining filter amplified only above this threshold. Species-specific scat extracts included as positive controls amplified robustly (shingleback: mean $C_t = 11.54 \pm 0.27$; tiger: mean $C_t = 12.80 \pm 0.30$), and dilution series of these controls showed the expected increase in C_t with decreasing template concentration, supporting assay sensitivity.

3.5.2 | Composition of Vertebrate Taxa Detected Across Zoo Environments

Across all zoo deployments, airborne eDNA metabarcoding recovered a diverse assemblage of vertebrate taxa spanning the zoo-held, background wildlife, domestic or feed-associated taxa, human, and ambiguous categories (Data S3). Ambiguous detections included *Cervus* sp., *Dama dama*, *Equus* sp., and *M. giganteus*, which could not be uniquely attributed to zoo-held animals, free-ranging wildlife, or feed sources.

Outdoor samplers located at the food preparation area and near the tiger and rhinoceros enclosures collectively detected 52 unique taxa. These detections were dominated by background local wildlife (24 taxa) and domestic or feed-associated species (19 taxa), alongside a smaller number of zoo-held taxa (five taxa) and four ambiguous taxa.

Indoor sampling within the reptile house recovered a similarly structured but reduced assemblage of vertebrate taxa. Across indoor deployments, 32 taxa were detected, comprising 16 background wildlife taxa, 13 domestic or feed-associated taxa, two ambiguous taxa (*Cervus* sp. and *M. giganteus*), and one zoo-held species (*Paracanthurus hepatus*, blue tang).

Notably, no reptile taxa were detected via metabarcoding across any zoo deployment, including samples collected within the reptile house. Across both indoor and outdoor zoo deployments, vertebrate detections were consistently dominated by domestic/feed-associated and human-associated taxa, with background

wildlife detected at moderate richness and zoo-held taxa forming a smaller component of the assemblage. When outdoor samples were further stratified by sampler location, each deployment independently recovered a broadly similar taxonomic composition, dominated by the same major categories, indicating consistency in overall signal structure across space and time (Table S3).

3.5.3 | Spatial Coherence of Zoo-Held Detections Relative to Sampler Placement

We interpreted outdoor zoo-held detections in relation to sampler placement relative to animal enclosures, shared infrastructure, and the zoo perimeter, recognizing that airborne eDNA may integrate signals across space rather than reflecting only immediately adjacent sources. Zoo-held detections at outdoor sampling locations varied in both composition and spatial plausibility.

The food preparation area yielded the highest number of strictly zoo-held taxa, including *Acinonyx jubatus* (cheetah), *E. quagga* (zebra), *Eudiptula minor* (little penguin), and *Giraffa* sp. (giraffe). Of these, the little penguin and giraffe enclosures are located near the food preparation area, whereas cheetah and zebra are housed at greater distances, suggesting that detections at this site reflect a mixture of proximate sources and more diffuse signals associated with shared infrastructure and animal management activities. Ambiguous detections at this site included *Cervus* sp. (deer), *D. dama* (fallow deer), *M. giganteus* (eastern gray kangaroo), and *Equus* sp. (zebra or horse).

At the rhinoceros enclosure, two zoo-held taxa (rhinoceros and giraffe) and two ambiguous taxa (*Cervus* sp. and eastern gray kangaroo) were detected. The proximity of giraffe and wapiiti enclosures to the sampler supports a plausible enclosure-adjacent contribution to the observed signal. In contrast, gray kangaroo detections at this site may reflect either zoo-held animals or free-ranging individuals, as the enclosure is situated near the southern boundary of the zoo and adjacent to natural habitat. The sampler located at the tiger enclosure detected *E. minor* (little penguin), which is housed in an adjacent enclosure. Together, these site-specific patterns indicate that zoo-held airborne eDNA detections are spatially structured and ecologically plausible, reflecting a combination of enclosure proximity, shared infrastructure, and broader landscape context.

3.5.4 | Detection Consistency Across Replicates, Deployments, and Loci

Detection consistency across replicate filters varied strongly among taxonomic categories and between metabarcoding loci (Table S4). Zoo-held taxa were detected inconsistently across replicate filters, with a median detection frequency of 0.25 for both markers, indicating that most taxa were detected in only one or two filters per location. In contrast, background wildlife taxa showed moderate consistency with the 12SV5 marker (median = 0.5) but were detected infrequently with Mam1/2

(median = 0.125). Domestic and feed-associated taxa exhibited the highest detection consistency across both markers, with median detection frequencies of 1.0 (12SV5) and 0.875 (Mam1/2), indicating frequent detection across most replicate filters.

3.5.5 | Relative Abundance Patterns Within Airborne eDNA Samples

Across zoo samples, relative read abundance (RA) was consistently dominated by domestic/feed-associated taxa and human DNA (Table S5). Using the 12SV5 marker, domestic/feed taxa accounted for a median of 57% of reads per sample, while human DNA comprised a substantial but variable fraction of the signal (median 31%, range 13%–69%). Background wildlife contributed a smaller component (median 11%). Zoo-held taxa were typically present at low RA, contributing a median of <1% of reads per sample, although occasional higher values were observed in individual samples (range 0%–46%). Patterns were broadly similar but more variable for the Mam1/2 marker. Domestic/feed-associated taxa (median 55%) and human DNA (median 43%) again dominated the recovered signal, often accounting for the majority of reads within individual samples. Background wildlife and zoo-held taxa were frequently absent, with background wildlife contributing only minor proportions when detected (maximum 4%). Zoo-held taxa occasionally comprised a larger fraction of reads in individual samples (maximum 18%), despite being detected infrequently overall.

3.6 | Structure and Interpretation of Background Signal Across Airborne eDNA Workflows

Across all four experiments, negative controls yielded low-level metabarcoding signal dominated by a small number of ubiquitous taxa, including human and domestic species. Signal prevalence and composition differed between control types and metabarcoding loci (Data S3).

3.6.1 | Behavior of Negative Controls

Extraction blanks and non-deployed field blanks showed detectable signal in the majority of samples for both loci. Consistent with their differing taxonomic breadth, the two markers exhibited distinct background profiles across control types. For the 12SV5 marker, we detected signal in all extraction blanks and field blanks (100%), while for Mam1/2, we detected signal in 87.5% of extraction blanks and 93.3% of field blanks. We observed these patterns across both sterile and non-sterile filter deployments.

PCR negative controls (no-template controls, NTCs) showed minimal amplification but contrasting behavior between loci. For 12SV5, we detected low-level signal in 80% of PCR blanks, with comparable read counts across PCR plates. This consistency suggests locus-associated background rather than plate-specific contamination. In contrast, Mam1/2 PCR blanks were largely free of detectable signal, with a single

low-level detection observed across eight NTCs (12.5%), confined to a single PCR plate. Together, these results indicate that background signal was primarily associated with field- and extraction-level processes, while PCR-level contamination was minimal.

Across control types, total read counts were typically dominated by a small number of predictably ubiquitous taxa coupled with substantial variability among samples, indicating that control read abundance alone is a poor indicator of contamination. To quantify how this background signal intersected with biological detections, we assessed taxon overlap between samples and negative controls for each metabarcoding locus. For the broad-range 12SV5 marker, 55.4% of taxa detected in samples were absent from all control types. Where overlap did occur, it was concentrated in field and extraction blanks, with minimal overlap in PCR blanks (4.6%). We observed a similar pattern for the mammal-specific Mam1/2 marker, for which 60% of sample taxa were not detected in any controls and overlap with PCR blanks remained low (8%).

3.6.2 | Background Signal Associated With Sterile and Non-Sterile Filter Preparations

For the broad-range 12SV5 marker, sterile field blanks exhibited reduced background taxon richness relative to non-sterile blanks (median 9.5 taxa versus 11.5), while total reads detected were comparable between conditions. In contrast, for the mammal-specific Mam1/2 marker, background richness was uniformly low under both sterile and non-sterile conditions (median 3 taxa with sterile filters versus 2.5 for non-sterile filters), and total read counts varied substantially among samples. Differences between sterile and non-sterile filter preparations were therefore more apparent for taxon richness than for read abundance and differed between markers.

Across both loci, taxa detected exclusively or more frequently under non-sterile conditions were typically low-prevalence background taxa, whereas commonly detected taxa (including human and domestic mammals) were recovered irrespective of filter sterilization. Sterile filter preparation was associated with reduced background taxon richness, particularly for the broad-range 12SV5 marker, but did not eliminate low-level background signal.

4 | Discussion

This study evaluated how key sampling design parameters influence airborne eDNA recovery within a passive sampling framework. Using a simple modular collection device, we found that filter surface area, sampling duration, and post-collection storage influenced detection outcomes to differing degrees. Species-specific qPCR and metabarcoding provided complementary but distinct perspectives on airborne eDNA dynamics: qPCR detected focal taxa more consistently and resolved treatment-related differences in signal strength, whereas metabarcoding provided broader community-level information but more variable focal-taxon detection. Together, these findings demonstrate that practical sampling and processing choices can substantially

affect the sensitivity, reproducibility, and ecological interpretation of passive airborne eDNA surveys.

4.1 | Filter Surface Area and Recovery Trade-Offs

Filter surface area influenced recovery in contrasting ways. Smaller filters consistently yielded stronger qPCR signals and higher target detection frequency in metabarcoding, despite larger filters recovering higher read counts when detections occurred. However, pairwise community similarity did not differ between filter surface areas in the replicated sterile deployment, providing no evidence that the larger collection surface reduced stochastic variation among filters. Increasing filter surface area therefore did not consistently improve detection sensitivity or replicate concordance within our passive sampling design.

Filter dimensions remain highly sampler-specific across airborne eDNA studies, and no standard format has yet emerged. For example, the filters used in another passive sampling study had a one-sided surface area of approximately 17.3 cm² (Jager et al. 2026), falling between the 6.25- and 25-cm² areas tested here. Under active airflow, Bodawatta, Madsen, Holman, et al. (2026) found greater vertebrate recovery from filters with exposed areas of 36 than 16 cm²; further indicating that the effects of filter surface area depend on sampler configuration and airflow. For the portable sampler designed for this study, we selected the 25-mm circular format to fit within standard 50-mL tubes, facilitating compatibility with routine laboratory workflows and permitting processing without subdivision. Thus, practical filter selection should consider integration across collection, transport and extraction workflows alongside potential gains in particle capture.

Our experimental results illustrate how key workflow considerations may mediate the balance between particle interception and downstream recovery. Although greater filter area may increase particle interception, recovery can be constrained by incomplete lysis arising from limited contact between the filter material and lysis buffer, co-extraction of inhibitors, and losses during extraction and purification (Wong et al. 2020). In airborne eDNA workflows, larger or more complex filters may require subdivision, greater lysis volumes and additional handling, increasing extraction effort and reagent use while potentially introducing further variability (Bodawatta, Madsen, Holman, et al. 2026; Lynggaard et al. 2022). Optimal filter design is therefore likely to be system dependent. Within our passive sampling framework, smaller filters appeared to maximize recoverable target signal while enabling rapid, scalable processing. These relationships may differ in passive sampler architectures that channel or concentrate ambient airflow across the collection surface. Comparing filter surface area and recovery across such platforms would further clarify how filter configuration and passive airflow interact to shape detection outcomes.

4.2 | Sampling Duration and Signal Recovery

Our examination of sampling duration effects revealed a rapid initial increase in recovered signal followed by diminishing net gains. qPCR signal increased sharply between 1 h and 1 day and

then plateaued, while metabarcoding detection frequency increased only modestly with longer deployments, primarily at the longest (2-week) sampling duration. This plateau could reflect a balance among continued deposition, degradation, and loss of material from the filter. Because our measurements represent endpoint recoveries from separate filters, we cannot distinguish these processes from temporal variation or intermittent pulses in airborne DNA input. Future studies combining repeated sampling with direct measurements of DNA persistence and loss from collection surfaces could help resolve their relative contributions.

Recent airborne eDNA studies report more variable effects of sampling duration. For example, Jager et al. (2026) observed continued accumulation of vertebrate richness between 6 and 96 h, with many detections occurring sporadically rather than being consistently retained through time. Considered alongside our broader sampling duration range of 1 h to 2 weeks, these results suggest that airborne eDNA signal recovery changes most rapidly over short timescales (several hours), while longer deployments can integrate additional, temporally variable detections. Taxon-specific responses further complicate this pattern, with Lin et al. (2025) reporting increasing vertebrate richness but declining plant richness over multi-day passive deployments. More broadly, passive eDNA studies show non-linear detection accumulation and context-dependent responses to sampling duration (Bessey et al. 2022; Chen et al. 2022; Sandré et al. 2026; van der Heyde et al. 2023).

Weather conditions may further affect the relationship between deployment duration and signal recovery. Rainfall was negatively associated with vertebrate richness in a year-long passive sampling study, although rainfall may also increase activity and shedding of some taxa (Johnson et al. 2023). Short-term shifts in airborne vertebrate community composition have similarly coincided with changing precipitation and other meteorological conditions (Lynggaard et al. 2024).

Together, these findings indicate that sampling duration should balance rapid signal recovery with temporal integration. Short deployments may maximize efficiency in accessible or high-frequency sampling designs, whereas longer deployments may be appropriate in logistically constrained settings or where integration of temporally variable or low-frequency detections is a priority.

4.3 | Storage Effects and Post-Capture Signal Decay

Storage effects were modest and primarily influenced signal strength rather than community composition. Delayed processing resulted in a small reduction in qPCR sensitivity but had limited impact on metabarcoding-based community patterns, indicating that airborne eDNA signals are relatively robust to short-term post-capture filter storage under cool, dry, and dark conditions. This is consistent with a model of progressive post-capture decay, in which DNA degradation begins immediately following collection but proceeds gradually. Evidence from aquatic systems shows that storage outcomes depend strongly on preservation strategy and filter type. Appropriate preservation can maintain detectable signal over short storage intervals,

whereas poorly preserved filters exhibit reduced recovery (Spens et al. 2017). Preservation method can also influence community composition consistency, with dry or buffer-based approaches outperforming alternatives under field conditions (Majaneva et al. 2018). Similar sensitivities are evident in air-borne eDNA, where dry, frozen storage improves taxon recovery relative to buffer-based methods (Bodawatta, Madsen, Holman, et al. 2026). Together, these findings suggest that while immediate processing remains optimal, short delays are unlikely to compromise ecological inference if storage conditions minimize post-capture degradation. This tolerance to delayed processing expands the feasibility of remote or logistically constrained deployments and supports the potential utility of archived or retrospectively analyzed eDNA samples, where preservation conditions can be controlled but immediate processing is not possible.

4.4 | Zoo Deployments and Environmental Context

Zoo deployments provided a practical validation of sampling design outcomes under real-world conditions. Using 32 passive filter samples collected across four locations and two 7-day deployments, we detected six taxa uniquely attributable to animals held at the zoo, together with four ambiguous taxa that could represent either zoo-held animals or free-living populations. This recovery was lower than in previous zoo-based active-sampling studies, which detected 17 resident species at Hamerton Zoo and 38 zoo-held species at Copenhagen Zoo using 72 and 40 samples, respectively (Clare et al. 2022; Lynggaard et al. 2022). Recent passive-sampling studies demonstrate that recovery also varies substantially with sampling design and effort. At Rotterdam Zoo, Jager et al. (2026) recovered 20 resident species using an intensive design comprising 90 passive samples collected across multiple locations, durations and sampling weeks. By contrast, a matched 48-h comparison across 20 sites at Perth Zoo recovered lower richness from exposed passive filters than from active samplers, although the passive filters still detected 46 vertebrate taxa compared with 83 using active sampling (Newton et al. 2026). While these detection rates are not directly comparable due to differences in study design and species assemblages, they nevertheless show that substantial recovery is possible with passive sampling. The lower zoo-held recovery in our study is consistent with its more limited spatial sampling density. Differences in enclosure openness, airflow, climate, and husbandry practices may also influence DNA release, transport, and persistence across zoo environments.

Despite our relatively limited sampling effort, zoo-held taxa were detectable but inconsistently recovered across replicate filters. Most taxa occurred in only one or two of the eight replicate filters per location, consistent with the stochastic recovery of low-abundance eDNA. In contrast, background wildlife and domestic or food-associated sources were detected more consistently, reflecting more widespread or persistent DNA sources within the environment. Newton et al. (2026) similarly found that airborne eDNA assemblages at Perth Zoo were dominated by background local wildlife, demonstrating that zoo sampling integrates broader environmental signals. Notably, we failed to detect any reptiles by metabarcoding, despite

consistent species-specific qPCR detection of shingleback lizard DNA in the reptile house samples. Reptile eDNA research remains comparatively limited, but relatively low DNA shedding has been proposed as a potential constraint on reptile detection (Nordstrom et al. 2022). Reptile recovery has also been sparse in other zoo-based airborne metabarcoding studies, with Lynggaard et al. (2022) detecting only one reptile species from an enclosure housing multiple reptile taxa. In our study, the contrast between qPCR and metabarcoding suggests that the absence of reptile metabarcoding detections may reflect low template abundance and marker-dependent amplification in community assays rather than an absence of reptile DNA from the sampled air.

Together, these results suggest that airborne eDNA recovery is strongly shaped by sampling strategy, effort, and environmental context. The low and variable detection consistency observed across replicate filters suggests that pooling multiple filters may improve recovery of low-abundance taxa by mitigating stochastic detection effects and increasing effective sampling effort. At broader monitoring scales, designs incorporating independent sites and repeated sampling would also enable occupancy modeling to estimate site-level occurrence while accounting for imperfect airborne eDNA detection.

4.5 | Background Signal and Filter Sterilization

Background signal in airborne eDNA was an expected feature of the workflow and varied predictably by marker and control type. Rather than attempting to eliminate this signal, we evaluated whether filter sterilization could reduce its impact and minimize expenditure of sequencing reads on ubiquitous non-target taxa. Filter sterilization reduced background taxon richness, particularly for the broad-range 12SV5 marker, but did not eliminate background signal, indicating that ambient airborne DNA deposition persists irrespective of filter treatment. Effects were largely confined to low-prevalence taxa, while common taxa were consistently detected across treatments, suggesting that sterilization reduces background complexity rather than signal presence. The magnitude of this effect is likely sterilization method-dependent; while we used autoclaving and UV irradiation, gamma irradiation has been shown to reduce amplifiable trace DNA (Deragon et al. 1990) and could be evaluated as a scalable batch-treatment approach for pre-assembled cartridges.

5 | Conclusion

Sampling design choices strongly shape airborne eDNA detection outcomes, and systematic evaluation of sampling parameters is needed to support broader adoption of this emerging monitoring approach. Using a simple passive platform, we tested the effects of sampling duration, filter surface area, and post-collection storage, providing practical guidance for scalable and reproducible passive airborne eDNA sampling. The modular design and ease of deployment of our device make it well suited to pilot studies, method development, and comparative testing across sampling parameters, environments, and taxa. By enabling replication at scale and rapid iteration of experimental design, such approaches facilitate hypothesis-driven investigation

of airborne eDNA behavior, including factors influencing signal recovery, transport, and persistence. More broadly, accessible and scalable sampling frameworks are likely to play a key role in translating airborne eDNA from proof-of-concept studies to routine biodiversity monitoring, particularly where traditional survey methods are limited by cost, logistics, or detectability.

Author Contributions

This study was conceived by E.E.H. E.E.H., R.L.T., and N.K. designed the airborne eDNA collection devices. The fieldwork was conducted by R.L.T., and lab work was conducted by R.L.T. and E.E.H. Bioinformatic analysis was conducted by R.L.T. and E.E.H. Figures and tables were prepared by E.E.H. All authors contributed to the writing and editing of the manuscript.

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Ethics Statement

All animal research conducted in this study complies with the Australian Code for the Care and Use of Animals for Scientific Purposes (2013) and protocols were approved by the CSIRO Wildlife and Large Animal Ethics Committee (AEC project number 2025-17).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Metabarcoding data supporting the findings of this study are openly available in the GBIF Metabarcoding Data Toolkit at <https://doi.org/10.15468/2ksb7e> for the Mam1/2 marker and <https://doi.org/10.15468/9v9sgr> for the 12SV5 marker. Quantitative PCR data are available as [Supporting Information](#).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1:** Supporting Methods. Construction of the filter surface area trial platform; filter preparation and device decontamination; DNA extraction from filter material; Species-specific quantitative PCR; metabarcoding PCR1 preparation; metabarcoding bioinformatic processing; metabarcoding statistical analysis; supporting methods references. **Data S1:** FAIRE-formatted raw qPCR Ct data associated with all experiments conducted in this study. **Data S2:** Per-sample sequencing and read-processing summary for all metabarcoding libraries, including raw read counts, filtering, denoising, merging, chimera removal, and final read retention across experiments, loci, and control types. **Data S3:** Taxon detection summaries across airborne eDNA experiments, including filter surface area, sampling duration, storage effect, and zoo deployments. Tables report taxa detected by metabarcoding, associated marker(s), taxonomic resolution, ecological category, and occurrence across sample and control types. **Table S1:** Full deployment conditions and replication for airborne eDNA experiments. Devices deployed indicates the number of sampling platforms operated concurrently, and filters collected indicates the number of filters processed separately. Weather conditions are summarized from Australian Bureau of Meteorology observations at the Canberra International Airport. **Table S2:** Zoo-held species list. Scientific and common names for all species housed at the National Zoo and Aquarium in Canberra, ACT, Australia, at the time of sampling. **Table S3:** Composition of vertebrate taxa detected by airborne eDNA metabarcoding across zoo sampling locations. Counts represent the

number of unique vertebrate taxa detected at each sampling location across two sampling events. Percentages indicate the proportion of detected taxa assigned to one of five categories within each location. Zoo-held = taxa housed at the zoo or aquarium, Ambiguous = zoo-held taxa also present in the surrounding area or provided as feed, Background wildlife = native or introduced free-living taxa present in the area, Domestic or feed = domestic taxa or taxa provided as feed. Total taxa represent per-location richness; outdoor locations share overlapping taxa. **Table S4:** Detection consistency across replicate filters by locus and taxonomic category from zoo deployments. Detection consistency was quantified for each taxon at each sampling location as the proportion of replicate filters out of eight (four filters $\times$ two deployments) in which the taxon was detected. *Taxon–location detections* indicates the number of taxon–location combinations with ≥ 1 detection. Median and interquartile range (IQR) of detection frequency across all taxon–location combinations is also provided within each locus and category group. **Table S5:** Relative read abundance of major signal categories within zoo samples, summarized by metabarcoding locus. Values represent the median, interquartile range (IQR), and minimum–maximum range of the proportion of reads assigned to each category within individual samples. Relative read abundance (RA) was calculated within each sample and locus following taxonomic filtering and aggregation.