



METHOD

Combining surface and soil environmental DNA with artificial cover objects to improve terrestrial reptile survey detection

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Abstract

Reptiles are increasingly of conservation concern due to their susceptibility to habitat loss, emerging disease, and harvest in the wildlife trade. However, reptile populations are often difficult to monitor given the frequency of cryptic in their life history. This difficulty has left uncertain the conservation status of many species and the efficacy of conservation actions unknown. Environmental DNA (eDNA) surveys consistently elevate the detection rate of species they are designed to monitor, and while their use is promising for terrestrial reptile conservation, successes in developing such surveys have been sparse. We tested the degree to which inclusion of surface and soil eDNA sampling into conventional artificial-cover methods elevates the detection probability of a small, cryptic terrestrial lizard, *Scincella lateralis*. The eDNA sampling of cover object surfaces with paint rollers elevated per sample detection probabilities for this species 4–16 times compared with visual surveys alone. We readily detected *S. lateralis* eDNA under cover objects up to 2 weeks after the last visual detection, and at some cover objects where no *S. lateralis* were visually observed in prior months. With sufficient sampling intensity, eDNA testing of soil under cover objects produced comparable per sample detection probabilities as roller surface methods. Our results suggest that combining eDNA and cover object methods can considerably increase the detection power of reptile monitoring programs, allowing more accurate estimates of population size, detection of temporal and spatial changes in habitat use, and tracking success of restoration efforts. Further research into the deposition and decay rates of reptile eDNA under cover objects, as well as tailored protocols for different species and habitats, is needed to bring the technique into widespread use.

KEYWORDS

detection probability, environmental monitoring, little brown skink, *Scincella lateralis*, surface eDNA

Resumen

El interés por la conservación de los reptiles es cada vez mayor debido a su susceptibilidad ante la pérdida del hábitat, enfermedades emergentes y la captura para el mercado de fauna. Sin embargo, las poblaciones de reptiles son difíciles de monitorear por lo frecuente que es la cripsis en sus historias de vida. Esta dificultad deja incierto el estado de conservación de muchas especies y desconocida la eficacia de las acciones de conservación. Los censos de ADN ambiental (DNAA) elevan sistemáticamente la tasa de detección de las especies que monitorean, y aunque su uso es prometedor para la conservación de los reptiles terrestres,

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han sido escasos los éxitos en el desarrollo de dichos censos. Analizamos el grado al que la inclusión del muestreo de DNAa superficial y del suelo a los métodos convencionales de cobertura artificial eleva la probabilidad de detección de una pequeña lagartija terrestre críptica: *Scincella lateralis*. El muestreo de DNAa de las superficies con cobertura de objetos con rodillos de pintura elevó las probabilidades de detección por muestra para esta especie 4–16 veces más que los censos visuales. Detectamos fácilmente el DNAa de *S. lateralis* bajo los objetos de cubierta hasta dos semanas después de la última detección visual y en algunos objetos de cubierta en donde no se había observado en los meses previos a *S. lateralis*. Con suficiente intensidad de muestreo, el análisis de DNAa del suelo bajo objetos de cubierta produjo probabilidades de detección por muestra comparables como métodos de rodillo superficial. Nuestros resultados sugieren que la combinación del DNAa y los métodos de objetos de cobertura puede incrementar considerablemente el poder de detección de los programas de monitoreo de reptiles, lo que permite estimaciones más precisas del tamaño poblacional, detección de los cambios espaciales y temporales en el uso de hábitat y el éxito de rastreo de los esfuerzos de restauración. Además, se necesita la investigación sobre las tasas de depósito y descomposición del DNAa de reptiles bajo objetos de cubierta, así como los protocolos hechos para diferentes especies y hábitats, para que la técnica entre al uso difundido.

PALABRAS CLAVE

DNAa superficial, monitoreo ambiental, probabilidad de detección, *Scincella lateralis*

INTRODUCTION

The sharp and recent global decline in abundance and diversity of herpetofauna is the result of habitat loss, introduction of novel predators and pathogens, and intentional hunting and trapping (Böhm et al., 2013; Stuart et al., 2014). Reptiles, in particular, have relatively large numbers of species categorized by the International Union for the Conservation of Nature (2021) as data deficient (14%), and it is generally accepted that many such species are threatened with extinction, but existing monitoring data are insufficient to support such a classification (Böhm et al., 2013). Thus, statistically robust population monitoring programs are urgently needed to resolve the status of data-poor species and to evaluate temporal and spatial changes in distributions of known-threatened and endangered species (Barata et al., 2017; Sewell et al., 2012). Visual counts under artificial cover objects (e.g., wood boards)—which attract individuals for use as protection or for thermo- and osmoregulation—are a standard survey method and provide a substantial boost to survey detection rates over conventional searching techniques for terrestrial reptiles (Hoare et al., 2009). Realized detection rates, however, are often still low enough that surveys do not provide adequate statistical power to accurately assess populations or habitat associations (Crawford et al., 2020; Matthias et al., 2021). Environmental DNA (eDNA) survey methods eliminate the need to directly observe the target organism, providing a larger window of time in which evidence of the species remains present and can be detected (Ficetola et al., 2019). We posit that incorporating an eDNA step in surveys of artificial cover objects can substantially increase reptile survey detection rates and add needed statistical power to evaluate population-level conservation status, response to anthropogenic stressors, and recovery after conservation invest-

ment. We designed and evaluated such a survey for little brown skink (*Scincella lateralis*).

Environmental DNA is DNA shed by organisms into their surroundings as they move, grow, breed, and decompose (Ruppert et al., 2019). Surveys based on collecting and detecting eDNA have revolutionized biodiversity monitoring in aquatic environments (Rees et al., 2014), providing robust and cost-effective sampling strategies, particularly for rare, cryptic, or endangered species, without having to directly observe or handle them (Yoccoz, 2014). Efforts to apply eDNA sampling for terrestrial species have recently advanced (e.g., Johnson et al., 2019; Kinoshita et al., 2019; Lyet et al., 2021; Thomsen & Sigsgaard, 2019; Williams et al., 2018) and include detection of terrestrial animal presence by sampling eDNA from vegetation and other surfaces (hereafter surface eDNA) (Valentin et al., 2020) and soil (Katz et al., 2020). Soil eDNA sampling for terrestrial reptiles is a growing field of research (Katz et al., 2020; Kucherenko et al., 2018), whereas sampling of surfaces for reptile eDNA appeared in the literature recently (Matthias et al., 2021). The “roller” method of Valentin et al. (2020), in particular, combines the strengths of both aquatic and terrestrial eDNA approaches by using dampened, commercially available paint rollers to recover eDNA across large surface areas and then bringing the eDNA into a solution where it can be easily concentrated via filtration. Surface and soil eDNA survey approaches for terrestrial reptiles, although promising, have yet to be fully vetted regarding their effectiveness in producing statistically robust spatial or temporal occupancy trends, which is the ultimate goal of improved monitoring schemes.

We integrated surface roller and soil eDNA methods with standard cover object sampling for *S. lateralis*, a small (8–15 cm long) lizard considered cryptic and elusive in the New Jersey Pine Barrens (DiLeo, 2016). Based on the litter-dwelling nature

of *S. lateralis* and their known use of artificial cover objects, we hypothesized that the undersides of these objects or the soil beneath them would concentrate skink eDNA allowing enhanced detection with molecular approaches. Through our case study on *S. lateralis*, we sought to encapsulate several common problems encountered in monitoring terrestrial reptiles more broadly (e.g., cryptic behavior, small activity range, and small body size), the solutions to which have implications for global efforts to assess conservation status comprehensively and accurately within this vulnerable group (Cruickshank et al., 2016).

METHODS

Proof-of-concept experiment and assay development

We completed a proof-of-concept experiment to establish how well the roller method could recover eDNA from commonly used cover object materials (metal and wood). This involved applying three different quantities of DNA-rich material—from a nonreptile species for which a reliable assay already existed and that did not naturally occur at the study site—to 15 plywood and 15 corrugated metal cover objects (Appendix S1). We evaluated the performance of roller sampling to detect this exogenous DNA based on the number of positive samples in each treatment group (Appendix S1). As a prerequisite for our primary study, we developed a species-specific qPCR assay for *S. lateralis* within the 12S mtDNA region. We then evaluated the sensitivity of this assay to detect trace amounts of DNA with standard lab-based techniques, estimated its limit of detection (LOD), and assessed specificity against closely related and co-occurring taxa with *in silico* methods (Appendix S2). We also obtained extracted DNA of 20 *S. lateralis* specimens used in Jackson and Austin (2010) to ensure the assay would amplify *S. lateralis* DNA across the extent of its native range (Appendix S2).

Evaluation of surface eDNA detection rates

We incorporated roller and soil eDNA sampling efforts into an existing herpetofauna cover object monitoring program in Wharton State Forest in the Pinelands National Reserve, New Jersey (United States). This landscape is an open-canopy, upland forest characterized by sandy, acidic soils, a predominantly pitch pine (*Pinus rigida*) overstory, and an understory of ericaceous shrubs (Collins & Anderson, 1994). We utilized an array of 82 sample sites spread along a 1163-m transect (Figure 1). Each site consisted of one metal and one 1-cm pressure-treated plywood cover object (0.6 × 0.6 m) (Figure 1) placed on each side of drift fencing (commercially available black plastic geotextile used for erosion control). Metal and wood cover objects alternated so that no two adjacent sites had the same cover material on the same side of the fence. The drift fence was buried ~10 cm into the ground and stood ~0.5 m tall, making it unlikely *S. lateralis* could move between paired objects. Because wood objects

provide a moister environment and metal objects are generally hotter and drier, this paired cover object design provided a broad array of microclimates throughout the year for use by reptiles. This array has been sampled since 2019, where from April through October each year, researchers conduct once daily visual checks. During each visual check, a cover object was lifted by a researcher, and all individual reptiles observed were counted and identified to species. The cover was then replaced in the same location.

From August 20, 2020 to October 22, 2020 (fall sample period) and from May 6, 2021 to June 24, 2021 (spring sample period), we performed weekly roller eDNA surface sampling of cover objects across a subset of the 82 sites in the array. On each eDNA sampling day, we targeted 10 sites where at least one of the two paired cover objects (wood or metal) had *S. lateralis* visually present within the prior 2 weeks. We sampled both cover objects at each of these 10 sites. As a result of this paired sampling scheme, our roller eDNA samples had wide variability with respect to the date at which the last skink was sighted, ranging from 0 days prior (eDNA roller sample taken immediately after visual skink sighting) to >100 days since visual sighting. This variability allowed us to document how long after a skink sighting we could detect skink eDNA. Our sampling design incorporated 81 eDNA surface samples from cover objects where no visual detections had occurred during that sampling period (spring or fall). Ultimately, our sampling scheme resulted in <50% of the 164 cover objects within the 82 array sites receiving roller eDNA sampling during each year (2020: $n = 144$ surface eDNA samples at 64 distinct cover objects; 2021: $n = 140$ surface eDNA samples at 76 distinct cover objects) (Figure 1).

Our roller eDNA surface sampling protocol followed Valentin et al. (2020) (Appendix S1). After lifting a cover object and completing visual counts, we used chlorine-sterilized commercial paint rollers mounted on a pole and dampened with deionized water to swab the entire ground-facing surface of the object. One roller was used for each cover object sampled. After use, it was placed in a sterile bag and in a cooler (~4°C) to preserve DNA during transport back to the lab. On each sampling day, we also included a field negative control (i.e., a check for in-field contamination of samples) by following the roller-handling protocol without performing the sampling. Within 2 h of initial collection, all roller samples were rinsed in the sterile bag with ~250 ml deionized water to bring collected eDNA into an aqueous solution. The water was then passed through 10- μ m polycarbonate track-etched filters with a peristaltic pump. The filters were stored in sterile 1.5 ml tubes and frozen at -20°C until further processing. Samples were thawed and DNA extracted using the DNeasy PowerSoil Pro extraction kit (Qiagen), which includes several PCR inhibitor removal and DNA purification steps. We considered this extraction process necessary given the amount of soil transferred from cover objects to rollers. Each filter extraction included a negative control to check for in-lab contamination of samples. We tested for the presence of *S. lateralis* with an eDNA TaqMan-based qPCR protocol we developed for this study (described in Appendix S2). There were three replicate reactions per sample, hereafter qPCR

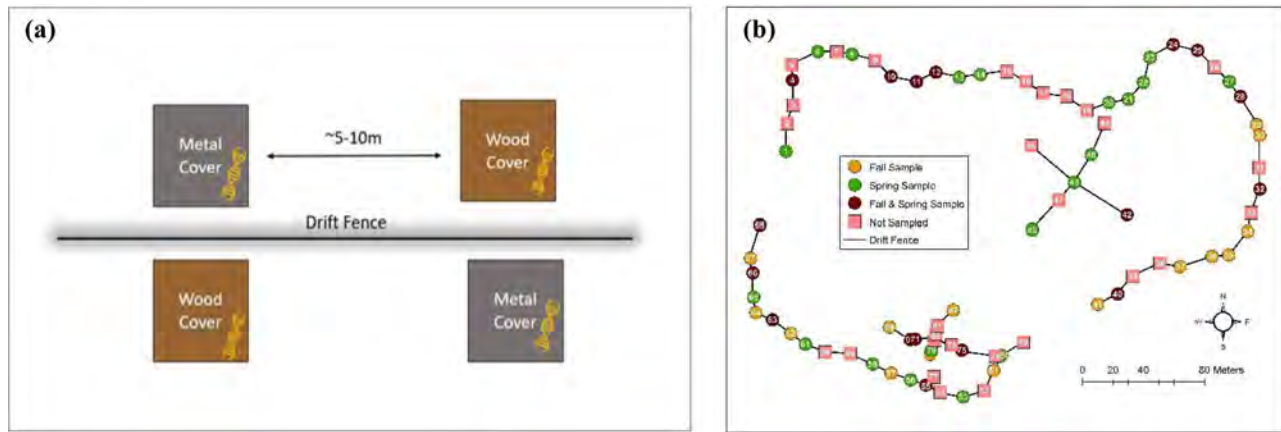


FIGURE 1 (a) Set up of two of the 82 sites included in the paired cover object and eDNA sampling design used to detect little brown skinks and (b) spatial design of the cover object sampling array (numbers: site number; drift fence length: 1163 m)

replicates. We considered a field sample positive for *S. lateralis* if at least one of three qPCR replicates amplified *S. lateralis* DNA.

Soil and surface eDNA comparison

To compare the performance of soil and surface (roller) eDNA methods in terms of detection probability, we collected soil samples under a nonrandom subset of 20 cover objects where roller samples had just been taken. We only collected soil eDNA samples under cover objects where *S. lateralis* was visually observed within the 2 weeks prior. This sampling design served to standardize the time since the last observation of skinks and the time since skink DNA was likely deposited, thereby allowing a more robust statistical comparison between methods (see below). We collected 25 soil samples under 20 cover objects (some objects had >1 sampling event in the fall); 15 samples were taken in fall 2020 and 10 in spring 2021 (fall 2020: three wood and seven metal; spring 2021: five wood and five metal). In fall 2020, we collected ~10 g of surface soil from 8 to 10 haphazardly chosen locations under each object. All soil was then placed in a single sterile 50 ml Falcon tube for transport to the lab. In spring 2021, we employed the same procedure but collected 40 g of soil from under each object to evaluate the extent to which this increase in sample volume and area covered would increase *S. lateralis* detection rates.

Soil samples were placed in a cooler for transport and stored at -20°C until DNA extraction. Soil samples were thawed at room temperature and extracted using the DNeasy PowerMax Soil Kit following manufacturer protocols (Qiagen). The 40 g of soil from spring sampling had to be separated into four 10-g extractions and was, therefore, run through qPCR independently. We considered positive returns from qPCR replicates from these four analyses as evidence of skink presence under a cover object. Steps to minimize field and in-lab contamination were identical to those used in roller sampling (see above and Appendix S1). Each sample was tested for the presence of *S. lateralis* eDNA with the qPCR protocol described above.

Occupancy modeling

To estimate and compare detection probability for the three methods (visual, visual plus roller eDNA, and visual plus soil eDNA), we fit a series of site occupancy models (MacKenzie et al., 2018) to the cover object survey data within a Bayesian framework. In all analyses, we defined occupied areas as those cover objects that were visited at some point by one or more *S. lateralis* individuals within a sample period. We defined visit-level detection probability as the joint probability of *S. lateralis*, or its eDNA, presence under an object during a given survey visit (i.e., availability or θ) (Nichols et al., 2008) and the visual or molecular determination of presence during a survey visit given availability, or $\theta \cap P(\text{detect} \mid \theta)$.

The visual-only model treated the repeated visual detection (1) and nondetection (0) data at cover objects during visits as the response variable, and covariates on both the occupancy and the detection submodels included sampling period (fall 2020 or spring 2021) and material (metal or wood). We only included detection information for visual survey visits that coincided with eDNA sampling to ensure statistical comparisons between the two methods were robust. To measure the increase in detection probability from also performing roller eDNA sampling on cover objects, we used the same model structure but treated either a visual or a molecular determination of presence (i.e., at least one qPCR replicate amplifying) as a successful detection.

In both models, we allowed sharing of information among the methods by setting the true occupancy state of skinks (z) at each object within a season to occupied at cover objects for which *S. lateralis* presence was confirmed by either method or if located visually at the cover object up to 30 days prior to eDNA sampling. We accomplished this by supplying the latent variable z as data in the model, with a value of 1 for known-occupied objects and not applicable (NA) otherwise (i.e., a blank value to be estimated). Finally, to evaluate whether our lab protocol of using three qPCR replicates per eDNA sample was adequate to confidently detect *S. lateralis* eDNA collected by rollers, we

fitted the multilevel occupancy model of Dorazio and Erickson (2018). This model allows estimation of the probability of detecting eDNA in a sample by an individual qPCR replicate. This probability was, in turn, used to estimate the cumulative power of detecting *S. lateralis* eDNA present in roller samples with varying numbers of qPCR replicates (Allen et al., 2021) (Appendix S3).

We compared the performance of soil versus roller eDNA sampling with multimethod occupancy models (Nichols et al., 2008). These models are multilevel and use shared detection information from multiple devices (in this case soil and roller eDNA samples) to inform availability, θ , and detection probability given availability, $P(\text{detect} \mid \theta)$. In this case, availability refers to the probability of *S. lateralis* eDNA presence under cover objects during a given visit. We incorporated visual survey information in the model as data for the latent variables z and a , where z is the true occupancy state of the cover object and a is the true availability state at cover objects during each visit (Kéry & Royle, 2015). We ran separate models per season due to the increase in soil sampling effort between fall and spring, as well as sample size limitations that precluded the use of model interaction terms. The dependent variable was the detection (1) or nondetection (0) of *S. lateralis* by each eDNA method by at least one qPCR replicate for each sampling event. We used the covariate device (soil vs. roller) on the detection submodel to estimate the method-specific probability of detecting *S. lateralis* given availability. We did not include cover material as a covariate due to sample size limitations.

All Bayesian models were fitted with noninformative priors (Kéry & Royle, 2015) in JAGS and jagsUI in R (Kellner, 2021; R Core Team, 2021). We ran three chains of 160,000 iterations each, including a burn-in period of 10,000; we kept every tenth draw. Model convergence was assessed by examining trace plots and Gelman–Rubin statistics ($\hat{r} < 1.1$). We compared estimates of visit-level detection probability for the various methods by examining and plotting posterior predictive distributions. The cumulative probability of detecting *S. lateralis* at least once given multiple samples (n) with a visit-level detection probability (p) was calculated using the formula $1 - (1 - p)^n$ (Allen et al., 2021).

RESULTS

Proof-of-concept experiment and assay development

Our proof-of-concept experiment confirmed that eDNA was readily recovered from cover objects with the roller surface eDNA aggregation method, and it revealed higher detection rates for metal objects (12 of 15 samples positive, or 80%) than for wood objects (4 of 15 samples positive, or 27%) (Appendix S1). Our *S. lateralis* qPCR assay targeted a 65 bp sequence in the 12S mtDNA region and was highly sensitive. The 95% LOD was 28.5 fg of genomic *S. lateralis* DNA per reaction, based on the assumption of three qPCR replicates (Appendix S2). In silico specificity testing revealed that no co-occurring reptile species would cross-amplify with the assay and that our assay

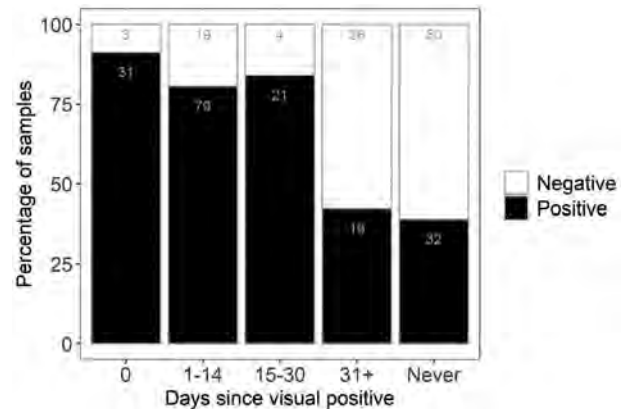


FIGURE 2 Number and proportion of eDNA samples in which *Scincella lateralis* (little brown skink) eDNA was amplified after collection from wood and metal cover objects as a function of time since the last *S. lateralis* visual sighting (positive: proportion of samples with eDNA; negative: proportion of samples without eDNA; x-axis: bins for number of days after visual detection until eDNA sampling occurred). *S. lateralis* sighting data extended prior to the onset of our sampling efforts (August 2020); thus, some cover objects never had visual detections prior to our eDNA sampling

successfully amplified *S. lateralis* DNA from across the species' range (Appendix S2).

Evaluation of surface eDNA detection rates

We found that 64% of the 284 total cover object samples had positive *S. lateralis* detections with the roller eDNA method (76% at metal and 51% at wood; Appendix S4), compared with only 11% for visual detections (15% at metal and 7% at wood). When we considered visual and eDNA as a combined survey method, the percentage of positive detections rose only slightly to 65% because only two samples had a visual detection but an eDNA nondetection. We found that, of the eDNA roller samples taken the same day *S. lateralis* was visually observed under a cover object, 91% returned positive detections (Figure 2). Of the remaining roller eDNA samples, we detected *S. lateralis* eDNA under 81% of the objects when the most recent visual detection at that object occurred 1–14 days prior. We detected eDNA under 57% of the cover objects when the most recent visual observation at that object was 15–127 days prior and under 39% of the objects when *S. lateralis* was never visually detected in a given season (Figure 2). All field and lab extraction negative control samples were negative for *S. lateralis* DNA, indicating no contamination.

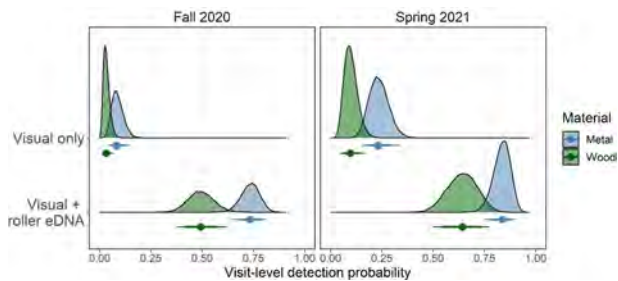
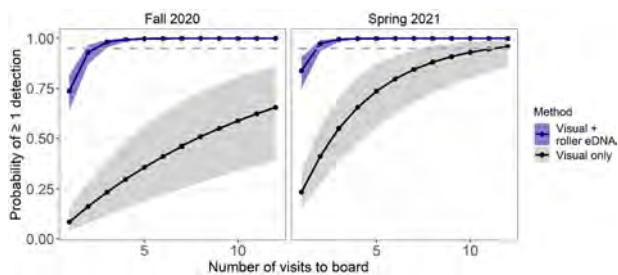
Occupancy modeling revealed that visit-level *S. lateralis* detection probability for visual surveys paired with roller eDNA sampling was 3.6–15.8 times higher than for visual surveys alone, depending on sampling period (fall and spring) and cover material (metal and wood) (Figure 3 & Table 1). The 95% credible intervals of detection probability did not overlap for the two methods (roller eDNA and visual) across either sample period or cover object material (Figure 3). The models also indicated that detection probability was higher in spring

TABLE 1 Detection probability of *Scincella lateralis* per visit to a cover object (metal or wood) based on visual sampling only and both visual and roller surface eDNA methods.^a

	Fall 2020		Spring 2021	
	metal	wood	metal	wood
Percent with visual only (95% credible interval)	9 (4–15)	3 (1–7)	23 (15–34)	10 (5–17)
Percent with visual + roller eDNA (95% credible interval)	74 (64–82)	49 (38–62)	84 (75–91)	64 (50–77)
Fold increase ^b	8.7	15.8	3.6	6.7

^aPercentages are posterior predictive medians.

^bImprovement in detection probability gained by adding eDNA sampling to traditional methods.

**FIGURE 3** Probability of detecting *Scincella lateralis* (little brown skink) per visit to a cover object with only visual surveys or with incorporation of the roller surface eDNA method (points: posterior median estimates; horizontal lines: 80% and 95% credible intervals)**FIGURE 4** Cumulative probability of detecting at least one *Scincella lateralis* (little brown skink) individual at a metal cover object based on visual detections alone (black curves) or paired visual and eDNA roller sampling efforts (purple curves) (shading: 95% credible interval; gray dashed horizontal line: cumulative 95% certainty of detecting *S. lateralis* at least once)

2021 than in fall 2020 and at metal compared with wood cover objects (slope parameter 95% credible intervals did not overlap 0) (Figure 3). Based on detection probability estimates, cumulative probability analyses revealed that 2–3 visits per cover object would have been required to detect *S. lateralis* with 95% confidence with the visual and roller eDNA methods concurrently, whereas 12–37 visits would have been required using visual methods alone (Figure 4). With our lab protocol of three qPCR replicates per roller sample, eDNA detection probability given *S. lateralis* eDNA presence in the sample was high (91–100%, mean = 97%) across both materials and sample periods. This result suggests that the number of qPCR replicates we performed did not hinder the overall detection probability (Appendix S3).

Soil and surface eDNA comparison

Multimethod occupancy models revealed that eDNA method-specific detection probability (i.e., detection based on eDNA method alone and not paired with visual) was 4.6 times higher with roller versus soil eDNA sampling in fall 2020, when only 10 g of soil was collected per object (60% vs. 13%, respectively), and 1.3 times higher in spring 2021, when 40 g of soil was collected per object (85% vs. 67%) (Figure 5). The 95% credible intervals for roller and soil eDNA detection probability estimates did not overlap in fall 2020, indicating a high likelihood that the roller method performed better. However, there was substantial overlap in spring 2021, suggesting that the two methods performed similarly well (for soil, 95% CI 37–89; for roller, 95% CI 59–97) (Figure 5).

DISCUSSION

The use of surface roller eDNA methods elevated survey detection rates up to 16 times higher than visual detections alone. Sampling soil eDNA under cover objects also boosted skink detection rates, provided that sufficient volumes of soil were collected. We showed that eDNA methods can detect skink presence when visual survey protocols failed to do so. Our results add to other recent eDNA applications for terrestrial reptiles, which collectively advance a promising avenue of research with the potential to reduce field time and support global monitoring efforts to gain accurate threat classification and recovery investments aimed at vulnerable terrestrial reptile species. Given that cover objects are regularly used in surveys of terrestrial amphibians (Marsh & Goicochea, 2003) and small terrestrial mammals (Lemm & Tobler, 2021), the benefits of adopting an eDNA-enabled cover object survey may extend to these taxa as well. However, like conventional surveys, any use of eDNA-enabled cover object surveys will require tailoring methods to match research questions, the local environment, and the natural history of the target species (Hampton, 2009; Hoare et al., 2009).

The ability to confirm the presence of a target species “sight unseen” is the main benefit to eDNA survey approaches (Jerde et al., 2011) and is particularly valuable when the target species is difficult to observe or very rare (Hunter et al., 2015). In

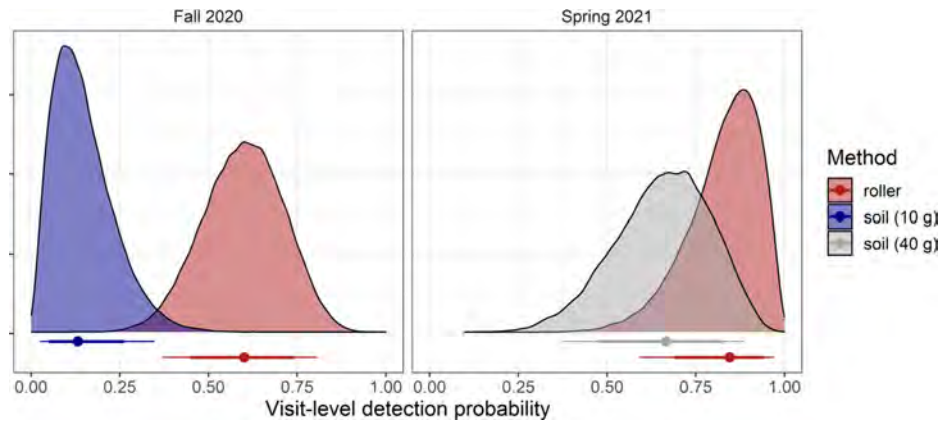


FIGURE 5 Relative performance of surface roller and soil samples under cover objects that were sampled using both protocols (fall 2020, $n = 15$; spring 2021, $n = 10$) to detect *Scincella lateralis* (little brown skink) eDNA (points: posterior medians; horizontal lines: 80% and 95% credible intervals). The posterior distributions from a multimethod occupancy model represent the predicted probability of detecting *S. lateralis* in one survey with each protocol given *S. lateralis* eDNA presence under the object

our study, only 11% of the 284 visual sampling events revealed *S. lateralis* sightings, compared with 65% of sampling events with the paired visual and roller eDNA approach. Similarly, low rates of visual detection are common for cover object surveys of terrestrial reptiles (e.g., Matthias et al., 2021). A low rate of skink visual detections under cover objects likely reflects the transient behavior of this species in which individuals make frequent movements among various forms of cover within their home range (DiLeo, 2016). Adding an eDNA step to artificial cover object sampling effectively extends the window of detection by revealing the eDNA trail left by target organisms even when they use a site only sporadically (Phoebus et al., 2020). Pairing visual cover object surveys with eDNA methods can thus logically only improve detection rates for skinks, and species like them, because eDNA allows recognition of skink presence under cover objects even if the object is only used briefly. The marginal benefits of adding eDNA to conventional surveys, however, are likely to vary by species depending on their rarity and behavior. Quantifying these marginal benefits for the world's terrestrial reptiles, as well as the feasibility of implementation due to costs and logistical factors, is a formidable research challenge, but it is one with important implications for improving monitoring schemes and documenting the response of terrestrial reptile populations to conservation investments and habitat management.

A key factor in evaluating any eDNA boost in detection rates is the length of time that eDNA persists in a state that remains available for detection with standard qPCR sample processing. We readily detected the presence of *S. lateralis* eDNA under cover objects at least 2 weeks, and up to 4 months (127 days), after the last confirmed visual observation. At a fraction of cover objects, we detected the presence of skinks despite no prior visual observations at that cover object in a season. This finding, in part, is certainly due to the sporadic use of cover objects by skinks and the nature of once-daily visual surveys. If a skink is not present at the moment a cover object is raised, it cannot be counted as present in visual surveys.

However, objects were checked daily increasing the likelihood that a visual survey captured skink presence, so some of the gaps between visual sightings and eDNA detection we observed likely also represented longer-term eDNA persistence under cover objects. The factors affecting the fluxes of eDNA into and out of an environment via deposition, degradation, and transport—the “ecology of eDNA”—have been studied in aquatic and aboveground terrestrial systems, but less so where deposited DNA is in contact with the soil surface, such as under cover objects (Barnes & Turner, 2016; Valentin et al., 2021). The environment under cover objects is shielded from UV light and most rainfall, which should favor eDNA persistence and subsequent detectability of target species. However, proximity to moist soil and its associated microbial community may accelerate DNA breakdown, eventually rendering any eDNA present on a cover object undetectable with qPCR-based assays. These questions cannot be answered with our study design, leaving an opportunity for more experimental evaluation of the rates of eDNA deposition and degradation at the soil surface (e.g., Kucherenko et al., 2018) and under cover objects specifically, including exploring how these rates vary by species and across a range of environmental conditions.

The roller method we used is one of a suite of new techniques that incorporates standard aquatic eDNA sampling frameworks to bring water to terrestrial surfaces in an attempt to capture, suspend, and concentrate DNA (Valentin et al., 2020). The fact that the entire surface of the cover object can be effectively sampled using a roller may explain our relatively high detection rates. For example, 91% of eDNA surface roller samples were positive at cover objects where *S. lateralis* was visually detected when that object was lifted. Matthias et al. (2021) found a 57% (13 of 23) positivity rate for eDNA of the snake *Contia tenuis* at visual positive cover objects in a comparable experiment with smaller swabs and covering a much smaller portion of the object. Matthias et al. (2021) also found roughly similar but somewhat lower recovery rates (45% or 9 of 20 samples) for soil sampling compared with swabbing cover objects.

Ultimately, the ability to sample where terrestrial reptiles dwell and deposit DNA (e.g., burrows and cover objects; Katz et al., 2020; Kucherenko et al., 2018), as well as the ability to collect and aggregate as much eDNA as possible, will determine the success of eDNA-based surveys. Such factors likely account for why some trialed eDNA survey approaches have been very successful in realizing improvements in reptile survey power and efficiency (Hunter et al., 2015), whereas others have been less successful (Baker et al., 2020; Halstead et al., 2017; Ratsch et al., 2020; Rose et al., 2019).

The material and size of a cover object, the microhabitat under cover objects, and other environmental conditions influence conventional survey detection rates (Hampton, 2009; Hesed, 2012), as well as eDNA recovery (Barnes & Turner, 2016; Valentin et al., 2021). In our study, the fold increase in detection under wood boards was a greater improvement on visual surveys than metal. However, metal cover objects outperformed wood cover objects in terms of *S. lateralis* occupancy and for visual and eDNA detection probability. The former finding demonstrates a potential preference of *S. lateralis* for metal over wood, which could be linked to the seasonality of our sample collection. We sampled primarily during spring and fall when conditions are generally cooler and skinks are more attracted to metal objects to aid in thermoregulation. The latter finding could result from high skink abundance and, therefore, greater residency time of skinks and eDNA concentrations under cover objects (Kéry & Royle, 2015). It could also relate to the generally drier conditions we observed under metal objects (J.F.B., personal observation), which may preserve eDNA by slowing microbial activity and degradation. Finally, it is possible that the chemicals used to treat wood cover objects inhibited PCR reactions and thus produced more false-negative eDNA results than metal objects. The results from any one study, such as ours, may not be consistent across all taxa, field methods, and locations; thus, there remains a need to optimize species-specific sampling using eDNA and cover objects (Hoare et al., 2009; Katz et al., 2020).

Our results suggest that merging surface eDNA with conventional cover object methods could become a critical tool in improving global reptile monitoring programs and thus greatly contribute to conservation, restoration, and management efforts (Ficetola et al., 2019; Halstead et al., 2017). These techniques provide an opportunity to overcome statistical noise in monitoring data, which in turn, will allow more robust estimates of changes in occupancy, site use, or habitat use (Lettink et al., 2011; Sewell et al., 2012). Our study provides proof that eDNA methods can provide much-needed statistical power boosts for terrestrial species' conservation monitoring. However, before this technique can be widely adopted, there is a need to assess the rates of eDNA deposition under cover objects, including exploring how these rates vary by species, material, sampling technique, and environmental conditions.

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