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Terrestrial environmental DNA survey substantially improves detection of satellite populations for an invasive insect

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Abstract

Early detection of satellite populations is essential for managing invasive species, but traditional methods often fall short when populations are sparse and widespread. We assessed the effectiveness of terrestrial environmental DNA (eDNA) surveys for detecting spotted lanternfly (*Lycorma delicatula*) along major highways in New York State, a region at the invasion front in North America. Over two years, we sampled 83 sites covering 1,650 km of high-traffic roadway using a roller-based, host-tree surface eDNA collection method and compared results with concurrent visual surveys and a government-run trap surveillance program ('circle traps'). We detected spotted lanternfly eDNA at 77% of sampled sites, while visual surveys indicated lanternfly presence at only 5% of these sites. Site occupancy models estimated detection probability at 0.77 per eDNA sample (95% CI: 0.66–0.86), compared with 0.25 per trap (95% CI: 0.21–0.31). Detection probability was lower in 2023 than in 2022 (eDNA: 0.68 vs. 0.89; traps: 0.13 vs. 0.32), likely due to our sampling in locations with lower population densities and distances farther from the invasion core. Extrapolation from hierarchical models indicated that three eDNA samples per occupied site would achieve a 95% cumulative detection probability, whereas circle traps would require 11–23 checks. Our surveys identified previously undocumented spotted lanternfly populations beyond the known range front at the time of the survey, including in high-risk agricultural and forested areas. These results show that terrestrial eDNA surveys can outperform traditional methods in detecting low-abundance invasive insects across large areas. Incorporating eDNA into early detection and rapid response efforts may improve management outcomes and reduce the risk of uncontrolled spread caused by human dispersal.

Keywords: environmental DNA, insects, early detection, detection probability, satellite populations, secondary spread, spotted lanternfly

Introduction

Non-native species become increasingly difficult to control or eradicate as they develop from small founding populations to widespread invasions (Simberloff et al. 2013). Early detection and rapid response (EDRR) programs are a frontline strategy for reducing costs and improving the effectiveness of invasive species management (Simberloff et al. 2013; Reaser et al. 2020). Quickly identifying newly established populations enables management actions that are more likely to eradicate and effectively control these species (Tobin et al. 2014). EDRR efforts are especially important for invasive species that exhibit a biphasic expansion pattern—beginning with a slow spread that shifts to exponential growth as a secondary human-assisted dispersal pathway (Siegert et al. 2014) emerges. The exponential phase of biphasic spread involves regular, long-distance dispersal events called 'jump dispersal,' which lead to the formation of satellite populations far ahead of the primary expansion front (Weston et al. 2019; Belouard et al. 2025). These satellite populations then grow locally and often serve as launch points for further jump dispersals, resulting in 'run-away' spread and higher control costs (Kovacs et al. 2011; Liebhold and Kean 2019). Therefore, finding and managing these satellite populations is critical. At the same time, locating small populations scattered across large areas can be difficult, akin to 'finding a needle in a haystack' (Leibhold et al. 2016; Larson et al. 2020). In this study, we evaluated the feasibility of conducting a terrestrial eDNA survey across a broad spatial area while maintaining the sensitivity required to detect low-abundance invasive insect populations. Our case study focuses on the secondary spread of the spotted lanternfly (SLF; *Lycorma delicatula*) across New York State (USA), aided by hitchhiking on vehicles.

Environmental DNA (eDNA) surveys have become vital components of EDRR programs for aquatic invasive species (Smart et al. 2015; Wittwer et al. 2018; Larson et al. 2020). Specifically, eDNA surveys have proven cost-effective for detecting emerging satellite populations that spread across freshwater and coastal waterways, where human-mediated jump dispersal is common (Sepulveda et al. 2022; Jarman et al. 2024). Recently, there have been significant advances in terrestrial eDNA techniques, which show similarly high potential, especially for detecting rare vertebrates and invasive insects (Allen et al. 2021; Kyle et al. 2022; Kirtane et al. 2022; Kelsey et al. 2025). However, terrestrial eDNA surveys have not yet been integrated into an EDRR framework, particularly for identifying satellite populations generated via human-assisted secondary pathways. We suggest that terrestrial eDNA methods hold considerable promise in this area because, like aquatic eDNA techniques, they rely on easy-to-deploy field methods, are highly sensitive, and can provide a relatively quick turnaround between sample collection, laboratory analysis, and obtaining actionable information (Allen et al. 2021; Kyle et al. 2022; Kelsey et al. 2025). Just as aquatic invasive species spread rapidly through human-assisted jump dispersal via watercraft, terrestrial invasive insects often disperse similarly via motor vehicles or trains (Leibhold et al. 2016). If eDNA survey methods prove effective at detecting these terrestrial satellite populations, they could become a powerful new tool within EDRR programs aimed at preventing and controlling the spread through secondary pathways.

The spotted lanternfly, a hemipteran insect native to China and Vietnam, was first discovered in Pennsylvania, USA, in 2014 (Barringer et al. 2015). Since then, it has spread quickly, causing problems for homeowners and threatening valuable crops such

as grapes and tree fruit (Dara et al. 2015). Once established, SLF can spread locally through self-powered dispersal, advancing about 1 km per year (Keller et al. 2020). In contrast, human-assisted jump dispersal—made more probable by the tendency of SLF to ‘hitch-hike’ on vehicles or trains and to withstand wind speeds of up to 100 km/h—can easily lead to transport distances of tens to hundreds of kilometers (Urban and Leach 2023; Elsensohn et al. 2024). These long-distance movements end when SLF are blown off or jump off as vehicles stop, forming new satellite populations ahead of the main infestation front (Ladin et al. 2023; Strombom et al. 2024). Over the last five years, jump dispersal events of SLF have nearly doubled in frequency, accelerating its spread across eastern North America (Belouard et al. 2025). A detailed understanding of secondary spread could help limit further range expansion by identifying and facilitating removal of roadside satellite populations while they are still small (Ladin et al. 2023; Belouard et al. 2025). However, the current invasion front for SLF spans hundreds of kilometers, and existing detection methods struggle to locate low-density satellite populations (Urban and Leach 2023).

Here, we used an existing terrestrial eDNA method, proven effective for detecting SLF at local scales (Valentin et al. 2020; Allen et al. 2021; Kelsey et al. 2025), and applied it across a broad area encompassing the northeastern North American invasion front in New York, USA. Our goal was to deploy the method at scale to evaluate its suitability for EDRR efforts. We assessed the performance of our eDNA surveys in four ways. First, we compared the locations of our positive eDNA detection sites with the overall understanding of SLF distribution based on traditional sources, including data collected by regulatory agencies. Second, we compared eDNA detection rates with

those from co-located timed visual searches (i.e., scouting; Urban and Leach 2023). We expected eDNA to outperform visual searches for SLF because scouting often performs poorly when SLF populations are at low levels (Urban and Leach 2023). However, this method remains widely used, especially for public engagement in surveillance (Urban and Leach 2023), so the comparison remains relevant to current practices. Third, we compared eDNA survey results with circle traps, which are the standard traps used by regulatory agencies to detect SLF (Nixon et al. 2020). Circle traps are cost-effective, easy to deploy, and have shown high capture rates across all mobile SLF life stages (Nixon et al. 2020; Urban and Leach 2023). We had no *a priori* assumptions about how eDNA sampling would compare with circle traps in terms of detection probability. Finally, we developed an optimized eDNA survey design by calculating the two components of eDNA detection probability (DNA capture within samples and detection given capture; Allen et al. 2021) and extrapolating to assess confidence in our overall power to detect SLF presence.

Methods

Roadside Survey Protocol

We examined all major highways within New York State, USA, and identified five routes (total distance: 1,650 km) that crossed or paralleled the known range edge of SLF as determined in August of each survey year (2022 and 2023; Fig. 1 Top). These roads are traveled by up to 130,000 vehicles daily (NYSDOT Traffic Viewer, 2019) and pass through a mosaic of temperate ecosystems, from fragmented urban forests near New

York City to the contiguous forests of Adirondack Park and extensive agricultural areas to the northwest near Lake Erie (Fig. 1 Top).

We conducted eDNA surveys (Fig. 1 Top) at sites roughly every 15-30 km along the routes, prioritizing rest areas with available parking. These roadside spots are at high risk of harboring satellite populations because they experience steady vehicle turnover, giving SLF individuals enough time to leave vehicles while they are stationary (Strombom et al. 2024). Also, these locations often have SLF host trees present, such as tree of heaven (*Ailanthus altissima*), maples (*Acer rubrum*, *A. platanoides, A. saccharum*), and black walnut (*Juglans nigra*), increasing the likelihood of SLF population establishment. When no formal highway rest areas were available, we picked sites near highway entry points and exit ramps, especially near commercial truck stops. These areas share a similar risk profile to roadside spots, characterized by high vehicle turnover and the presence of host trees. A smaller number of sites were in highway maintenance parking lots that included host trees and forested habitat, but were not open to the public. All survey activities followed New York State (NYS) Department of Transportation and New York Thruway roadside safety standards.

In 2022, we conducted surveys at 45 sites along 555 km of four interstate highways (I-495, I-87, I-81, I-86; Fig. 1 Top). We surveyed each site once between September 28 and October 12. In 2023, we mapped survey routes farther north and west, well beyond the known invasive range as of September 2023 (Fig. 1 Top). This decision was based on our 2022 results and other information suggesting that the SLF invasion front likely extended farther than previously thought, based solely on visual surveys and trap data. In 2023, we surveyed 46 sites along 1,150 km of four

interstates/highways (I-87, I-90, RT 20, and I-86). We visited each site twice — except those along I-90 and RT 20 — with each survey period lasting two weeks: once between September 12 and 22, and again between October 11 and 20. Of the 83 total survey sites, eight were visited in both years (2022 and 2023), all located on I-86 between the towns of Binghamton and Bath (Fig. 1 Top).

The timing of our eDNA surveys aligned with the maturation and breeding period of adult SLF, as they aggregate *en masse* to feed and mate on the trunks of a narrow range of host trees (e.g., *Ailanthus*; Leach & Leach 2020; Urban & Leach 2023). Most SLF eDNA found on tree trunks likely comes from the large amounts of excrement they produce, called honeydew (Valentin et al. 2021; Kelsey et al. 2025). Therefore, by planning our surveys to coincide with periods of adult SLF feeding and mating, we increased the likelihood of detecting SLF eDNA (Allen et al. 2021). Arthropod eDNA on plant surfaces degrades to undetectable levels within about 2 weeks due to UV exposure or microbial activity, and it often washes off during rain events (Valentin et al. 2021). As a result, we conducted all eDNA surveys at least 2 days after any significant rainfall was reported at our survey locations.

We worked in pairs, with one person collecting the samples (the surveyor) and the other processing them. At each site, we parked and staged all necessary equipment from the bed of a full-sized pickup truck. The surveyor then identified two sets of three host trees (‘sub-sites’) within approximately 60 meters of the truck (Fig. 2). We focused on collecting samples from *A. altissima*, *J. nigra,* and *Acer* spp. due to their prevalence as SLF hosts (Urban and Leach 2023). We sampled a total of 828 trees, 477 (58%) *Acer* spp., 144 (17%) *A. altissima*, 96 (12%) *J. nigra*, and 111 individuals of other

species (13%). The average diameter at breast height of these host trees across all sample sub-sites was 69 cm (SD: 68). The surveyor collected one eDNA sample from the three trees at each sub-site using a sterile, damp paint roller (6” woven mini roller with 1/4” nap, Home Depot “Best” Brand/ Linzer Products Corp.) attached to an extendable pole. To sample a tree, the surveyor moved the roller along its entire circumference, covering all surfaces from the ground to a height of 3 meters, applying gentle, consistent pressure to ensure contact between the roller's fibers and the tree surface (Valentin et al. 2020).

After sampling each sub-site, we processed the rollers at the truck bed workstation. We placed the roller in a sterile bag (304 x 114 mm Sterile Sampling Bags, Wards Science), added approximately 150 mL of deionized (DI) water to the bag, and massaged the roller for 30 seconds to suspend the collected material into aqueous solution. After removing the roller from the bag, we used a peristaltic pump (Alexis, Proactive Environmental Products, Inc.) to pass water through a self-desiccating filter assembly (Smith-Root, Inc.) equipped with a 47 mm diameter, 10 µm polycarbonate track-etched (PCTE) filter membrane. We continued pumping until the filter clogged or all the sample water was filtered. On average, we filtered 100 mL (SD: 41.12) of water per site sub-site (i.e., per roller). Each filter was then placed in a Smith-Root storage bag, sealed, and stored in a cardboard box at ambient temperature for transport back to the Rutgers eDNA lab for analysis. At three survey sites, no trees were available for roller sampling. Instead, we used the ‘spray aggregation’ eDNA sampling method (Valentin et al. 2020) on another preferred host species, wild grape (*Vitis sp.*). In this approach, our surveyors sprayed DI water over approximately 10 m^2 of vegetation,

collecting runoff from leaf surfaces into sterile bags (304 x 114 mm, Sterile Sampling Bags, Wards Science). We then filtered the collected drip water using the same filters and pump setup described above. We collected an average of 178 mL per spray sample (SD: 60).

We employed two field control measures to evaluate and prevent the introduction of external SLF DNA into our samples. First, upon arrival at each survey site, we created a field negative control sample by placing a pre-cleaned roller on the sampling pole, removing it with a sterile bag, adding about 150 mL of DI water to the bag, and processing it as described above. Since this negative control did not contact vegetation, any detection of SLF DNA would indicate contamination of sampling materials, suggesting that other survey results from that site might be similarly contaminated. Second, we wore clean nitrile gloves throughout all eDNA sampling steps and changed gloves between sample collection and processing. This helped reduce the risk of transferring SLF DNA from our hands into the samples.

After eDNA sampling, the surveyor conducted a timed 2.5-min visual survey for SLF at each sub-site. During that period, all bark and other vegetation surfaces within a 3 m radius of each sampled tree were carefully examined for SLF presence, and all individuals sighted were recorded. In total, the eDNA effort, visual surveys, and processing took 30–45 min per site. Considering travel time between sites, we could complete about one site per hour, or 8–10 sites (spanning ~130–160 km) in a typical workday.

Lab Processing

All eDNA filters collected in the field were returned to the Rutgers eDNA lab within three days of collection and stored at -20 °C until processing. We conducted all DNA extractions and qPCR setup in a clean lab that is free of PCR products. Additionally, we performed all qPCR setup in a secondary, positive-pressure laminar flow hood equipped with a UV sterilization unit. We handled any PCR products in a separate laboratory space with its own dedicated equipment. All laboratory benches were cleaned with 10% bleach and DI water before sample handling, and all lab surfaces were regularly disinfected with 10% bleach to maintain a low-DNA environment. We wore nitrile gloves whenever handling sample tubes and changed them at regular intervals between tasks. All sample tubes and pipette tips were purchased sterile and kept clean before use.

We removed filters from the self-desiccating housings on a bleach-cleaned lab bench using bleach- and flame-sterilized forceps. We extracted DNA from the filters using the DNeasy Blood and Tissue Extraction Kit (Qiagen, Inc.) according to the manufacturer's protocol. We tested each extracted sample for the target species' DNA using a previously developed SLF qPCR assay targeting a 63-bp region of the ITS1 genetic region (Valentin et al. 2020). We followed the published protocol, except that we ran each qPCR for 50 rather than 45 cycles. Species specificity of this assay has been validated against local specimens and through *in silico* testing using NCBI's PRIMER-BLAST (Valentin et al. 2020). We performed in-lab dilution experiments using synthetic DNA (gBlocks Gene Fragments, Integrated DNA Technologies, Inc.) to determine that the assay's limit of detection is 2-3 copies, based on three technical qPCR replicates (95% Limit of Detection; see Supplementary Information). All qPCR assay reagents

were prepared in a HEPA-filtered, positive-pressure PCR hood (AirClean 600 PCR Workstation, AirClean Systems, Inc.). We used a StepOne Plus™ Real-Time PCR System (Applied Biosystems, Inc.) to amplify target DNA from environmental samples and considered amplification that crossed the default fluorescence threshold as positive SLF detections. Thresholds were set by the StepOnePlus™ software based on a standard curve consisting of a six-level, ten-fold dilution series of synthetic SLF DNA (gBlocks Gene Fragments, Integrated DNA Technologies, Inc.) with known concentrations ranging from approximately 1.5 million to 15 copies. We tested for potential PCR inhibition on a random subset of our samples (n = 24) to ensure that environmental organic compounds did not disrupt PCR amplification of the target DNA. This process involved spiking the extracted DNA from field samples with a known amount of synthetic SLF DNA, then analyzing for shifts in Ct values using the same qPCR protocol as above. No evidence of inhibition was observed in the subset tested, so no further inhibitor treatment was performed.

We prepared laboratory negative control samples at each stage of processing: during filter pulling and DNA extraction, and again during qPCR processing (Supplementary Information). These controls were analyzed alongside our field samples to assess potential contamination. To verify that the DNA detected in our qPCR analyses was truly from SLF, we performed bidirectional Sanger sequencing on a subset of 20 of the qPCR products from field samples that tested positive for SLF DNA, including samples at each end and the midpoint of all routes sampled. If the sequence matched our 63-bp SLF target, we considered the SLF detection confirmed (Supplementary Information).

Statistical Analysis

We first qualitatively compared the spatial pattern of our detection results with the known distribution of SLF based on regulatory agency data. We then used site occupancy models within a Bayesian framework to estimate and analyze detection probabilities. We performed three analyses: (1) comparing eDNA detection probabilities with visual survey detection probabilities, (2) comparing eDNA detection probability with that of circle traps, and (3) extrapolation of DNA capture and detection probabilities to inform lab and field survey design considerations (e.g., qPCR technical replicates, number of samples). All analyses were conducted in R version 4.40 (R Core Team 2024). All models were run in JAGS via the 'jagsUI' R package, with three chains of 30,000 iterations each, of which 5,000 were discarded as burn-in, yielding 75,000 total posterior samples. All parameters successfully converged with R-hat values below 1.1.

eDNA Mapping — Here, we compared the spatial alignment of our SLF eDNA detections with visual and circle trap detections reported by regulatory agencies. First, we evaluated the locations of our SLF survey results against known (breeding) populations and visual data from regulatory agencies as of May 2025. In this comparison, we aimed to determine whether our eDNA detections fell outside the 'infested' or 'sighting' zones shown on state-level maps (Fig. 1 Top). Second, we assessed agreement between eDNA and circle-trap surveys on the presence or absence of SLF. This comparison can yield only four outcomes: both eDNA and trap detection, eDNA detection without trap detection, no eDNA detection but trap detection, or neither method resulting in detection. Our goal was to understand how often our results aligned with those from conventional survey approaches, how often they

differed, and whether discrepancies occurred within or outside the core SLF-occupied area.

Probability of Detection: eDNA vs. Visual — For this comparison, we considered a survey site to be 'occupied' by SLF if at least one of the three qPCR technical replicates amplified SLF DNA within either of the two site sub-sites, or if ≥ 1 individual was observed during our timed visual surveys. Due to the limited number of visual positives, we were unable to effectively compare visual and eDNA detection probabilities using site-occupancy models. Instead, we estimated detection probability as the proportion of positive detections for each method in the subset of sites with confirmed SLF presence (i.e., confirmed by visual inspection or eDNA). This is consistent with defining detection probability as the probability of detection conditional on presence. For this analysis, we used a logistic regression model with the form:

$$y_i \sim Bernoulli\ (p_i)$$

$$logit(p_i) = \alpha + \beta * method_i$$

where y_i is the binary detection and non-detection outcome for SLF for each occupied site, p_i is the probability of SLF detection given presence, and β is the effect size of 'method', a binary variable set to 1 for eDNA and 0 for visual.

Probability of Detection: eDNA vs. Traps — To compare the performance of eDNA versus circle traps, we treated eDNA samples from each sub-site as an 'occasion' (i.e., repeat visit) within each site in a site occupancy model. We considered an eDNA sub-site positive for SLF if at least one qPCR technical replicate amplified SLF

DNA. This step converted our three qPCR technical replicates into a single binary presence-absence outcome (0 = absent, 1 = present), with two replicate survey results recorded per site visit.

Circle traps detect the presence of SLF by capitalizing on their tree-climbing behavior to enclose the insects, and then capture them in a collection cup (Nixon et al. 2020). To compare detection rates with eDNA sampling, we used data from the US Department of Agriculture trapping effort in New York during 2022-2023 (G. Para, *pers. comm.*). During each trap check (mean interval: 11.8 d ± 7.5 SD), agency staff recorded the number of SLF captured before emptying the trap. We converted the circle-trap data into presence-absence records, where capturing one or more individuals during a trap check was considered a presence, and capturing none was considered an absence.

New York State had a total of 569 and 691 actively checked circle traps deployed in 2022 and 2023, respectively. Of these, we retained 91 traps for analysis located within 5 km of our eDNA survey sites and checked at least once during the date intervals covered by our eDNA sampling efforts (i.e., any checks in September or October). We excluded two traps with unusually high (~weekly) check frequencies. The final dataset included 204 checks across 64 traps at 13 eDNA sites in 2022, and 119 checks across 27 traps at 14 eDNA sites in 2023. These individual trap checks were treated as sampling occasions, alongside the eDNA sub-site, to inform detection probability in the occupancy model.

To compare the detection probability of circle traps versus eDNA sampling, we fit a two-level occupancy model to the combined eDNA and circle trap data for each year separately with the following structure:

$$z_i \sim Bernoulli\ (\Psi_i)$$

$$y_{ij} |\ z_i \sim Bernoulli\ (z_i * p_{ij})$$

where z_i is the occupancy status of a given site *i* by SLF, which is determined by a Bernoulli process with probability Ψ_i; y_{ij} is the observed detection or non-detection data per sample *j* (i.e., each eDNA sample or trap check interval), with detection probability p_{ij} given that a site is occupied. We estimated Ψ_i using linear models with a logit link function and no covariates, while p_{ij} was similarly estimated, but with a single 'method' covariate (1 = eDNA sub-site, 0 = trap check). Therefore, our model directly compared the probability of detecting SLF at an occupied site between individual eDNA sub-site and the mean (~12 d) program trap-check intervals.

Survey Design Optimization – Presence and detection with eDNA sampling methods can be viewed as the product of three distinct component probabilities, each nested within hierarchical levels of sampling (Schmidt et al. 2013). First is the probability that SLF are present at a site within the landscape (Ψ) and have therefore deposited DNA somewhere within the site. Second is the probability that SLF DNA present at a site is captured within a given field sample (Θ). Lastly, there is the probability that SLF DNA captured within a sample is amplified in a qPCR reaction (*p;* Allen et al. 2021; Keller et al. 2022). The overall probability ($p_{overall}$) of detecting SLF DNA that is present at the site in any given sample can therefore be defined as the joint probability of DNA capture (Θ) and molecular detection (*p*). To estimate these probabilities and explore the sampling design implications, we fit a model for each sampling year, using 86 eDNA samples across 43 sites in 2022 and 158 eDNA samples across 45 sites in 2023.

We created a 3-level hierarchical model with the following structure:

$$z_i \sim Bernoulli\ (\Psi_i)$$

$$a_{ij} |\ z_i \sim Bernoulli\ (z_i\ \cdot\ \Theta_{ij})$$

$$y_{ijk} |\ a_{ij} \sim Bernoulli\ (a_{ij}\ \cdot\ p_{ijk})$$

where z_i is the occupancy status of a specific site *i* by SLF with occupancy probability Ψ; a_{ij} indicates the presence or absence of SLF DNA within sample *j* with capture probability Θ_{ij}; y_{ijk} is the observed data, a positive or negative qPCR result for each technical replicate *k*; and p_{ijk} is the probability of DNA amplification in a technical replicate given its presence in the sample. We included a binary 'year' covariate (2022 or 2023) in logistic models of Ψ, Θ, and *p* to account for interannual differences in survey locations.

We used cumulative probability formulas based on these detection probabilities to inform tradeoffs in survey protocols for SLF eDNA sampling. We determined the minimum number of samples needed at a site to achieve a 95% chance of successfully capturing SLF DNA within a sample using the following equation:

$$p_{samples} = 1 - (1 - \theta_{ij})^s$$

Where θ_{ij} is the probability of DNA capture for site *i*, sample *j*, and *s* is the total number of samples. For a 95% probability, we set $p_{samples}$ to 0.95 and solved for *s*.

Similarly, the minimum number of qPCR technical replicates needed to achieve a 95% probability of SLF eDNA detection within a sample that contains SLF DNA was estimated using:

$$p_{replicates} = 1 - (1 - p_{ijk})^n$$

where p_{ijk} is defined as above, and *n* is the total number of technical replicates. We set the $p_{replicates}$ to 0.95 and solved for *n* to determine the minimum number of replicates

needed to achieve a 95% probability that at least one replicate is positive when SLF DNA is truly present in a sample.

We used the same approach to analyze the sampling design implications of detection rates estimated in the model comparing eDNA and circle traps. We estimated how the cumulative detection probability of detecting SLF in at least one sub-site or trap check, given presence at the site, increased with sampling effort using the following formula:

$$p_{cumulative} = 1 - (1 - p_{ij})^n$$

where p_{ij} is the detection probability of a method at site *i*, sample *j,* and *n* is the total number of sampling occasions, defined as eDNA samples or trap check intervals.

Results

We covered over 1,600 km of highway during six weeks of eDNA field surveys. We processed and tested all eDNA samples over an eight-week period, resulting in a total effort of 14 weeks to collect samples and return associated detection data. Except for a single field negative control that was dropped during lab processing and tested positive for SLF DNA, all 126 other field negative controls and all 59 lab negative controls showed no detections, indicating no field or lab contamination. The eDNA samples from the site associated with the dropped field negative were excluded from our analyses as a precaution. Overall, our eDNA-positive detections far exceeded the known SLF range in New York as mapped by regulatory agencies during the survey years (Fig. 1, Bottom).

Detections of SLF using eDNA were far more common than with the timed visual surveys. We detected SLF at 64 of the 83 sites surveyed with eDNA, but only at four sites with visual surveys. All four sites with visual detections also had eDNA detections, while 61 of the sites with eDNA detections had no visual detections. When considering only the 65 sites with confirmed occupancy, logistic regression estimated visual detection probability to be 0.04 (95% CI: 0.02, 0.09). We did not attempt to fit this model for eDNA, as SLF DNA was detected at 100% of confirmed-positive locations.

eDNA also revealed more positive SLF detections than circle traps. Of the 27 eDNA sites with spatially comparable data, six were positive based on both trap and eDNA methods, 17 were positive based on eDNA only, and six had no detections with either method (Fig. 3). None of the sites had positive detections from circle traps alone. Overall detection probability was also higher for eDNA (0.77; 95% CI: 0.66, 0.86) than for circle traps (0.25; 95% CI: 0.21, 0.31). Detection probability was lower in 2023 than in 2022 for both eDNA (2022: 0.89, 95% CI: 0.73, 0.97; 2023: 0.68, 95% CI: 0.53, 0.81) and circle traps (2022: 0.32, 95% CI: 0.25, 0.38; 2023: 0.13, 95% CI: 0.07, 0.21).

eDNA capture and detection probabilities were both lower at the 2023 survey points than at the 2022 points, likely due to lower SLF densities at the 2023 points, which were farther north and east relative to the invasion front. We estimated an overall detection probability of 0.85 (95% CI: 0.75, 0.92) and 0.43 (95% CI: 0.30, 0.56) for 2022 and 2023, respectively. Combining data from both years produced an overall joint probability of 0.63 (95% CI: 0.55, 0.71; Fig. 5). SLF DNA capture probability was 0.85 (95% CI: 0.75, 0.93) in 2022 and 0.55 (95% CI: 0.37, 0.74) in 2023. The qPCR technical

replicate detection probability was 0.80 (95% CI: 0.74-0.85) in 2022 and 0.42 (95% CI: 0.31-0.52) in 2023.

Extrapolation from these detection probabilities indicated that, at an occupied site, a minimum of 2 and a maximum of 7 samples would be required to capture SLF DNA 95% of the time, depending on the year. Upon successful eDNA capture, a minimum of two and a maximum of ~ 6 qPCR technical replicates were needed to be 95% confident of detecting SLF DNA in a sample (Fig. 4). Using our methods (i.e., 2 samples per site and 3 technical replicates), 2 to 9 samples are required to both capture and detect SLF eDNA when present at a site 95% of the time, depending on the year.

Using detection probabilities from both 2022 and 2023 efforts at the 27 trap-associated sites, the median number of samples needed to reach a 95% chance of detection when SLF are present at a site was three roller samples for eDNA or 11 trap checks for circle traps (Fig. 5). The lower detection probabilities in 2023 increased the number of trap checks required to 23 for circle traps, while eDNA only required three roller samples to achieve 95% confidence in SLF presence.

Discussion

Early detection of emerging invasive populations is vital for reducing their negative impacts and facilitating eradication (Simberloff et al. 2013). This task becomes especially challenging when species have effective human-assisted secondary dispersal methods (Leibhold and Kean 2019). We found that an emerging terrestrial eDNA survey technique improves early detection of satellite populations of an invasive insect across a wide spatial area compared with existing methods. Our eDNA survey

reliably detected SLF at sites where no individuals were observed, and we show that eDNA samples were more than three times as likely to identify satellite SLF populations as commonly used circle traps. Our survey resulted in the first known detections of SLF in areas at high risk of significant impacts, including important wine-growing regions and protected forests. Our easy-to-deploy approach allowed efficient sampling along over 1,600 km of highway across diverse terrain, from wilderness and farmland to major urban areas. Ultimately, our findings reinforce existing evidence that heavily traveled roads often serve as secondary pathways for SLF spread, leading to regular jump dispersal events (Strombom et al. 2023; Belouard et al. 2025).

A key aspect of EDRR is target analysis, which involves evaluating survey methods based on their ability to detect invasive species when they are rare, their ease of deployment across different logistical settings, and their cost-effectiveness over large temporal and spatial scales (Reaser et al. 2020). We show that eDNA surveys for SLF are effective in all these areas. Specifically, eDNA samples can detect adult SLF at a site with an overall probability of 43-85%, depending on population rarity (the lower the abundance, the lower the probability). This range of estimates closely matches that of Kelsey et al. (2025) for similar eDNA protocols used in Ohio forests (US). Together, these findings strongly indicate that terrestrial eDNA surveys can reliably support EDRR efforts for SLF as they spread across North America and could also be useful in other invasion scenarios, such as biosecurity checks at ports of entry outside North America.

Although we did not conduct a cost analysis comparing survey methods (eDNA, circle traps, visual scouting), we estimate that eDNA surveys can detect the presence of SLF at an occupied location with 95% confidence with just three to six samples. In

comparison, we estimate that 8-22 circle-trap sampling checks (roughly 12-day trap-check intervals) are required to achieve the same confidence level. The number of circle trap sampling events can be increased by deploying more traps, increasing the frequency of checks on existing traps, or both. However, no matter how many additional circle trap sampling events are conducted — doubling or even quadrupling them to match the detection certainty of eDNA, along with the associated costs of revisiting trap sites — this approach may be less cost-effective than eDNA. Our visual scouting surveys detected very few SLF individuals; however, our protocol involved only a brief search period (2.5 min per location), which likely limited the number of visual detections (Urban and Leach 2023). Kelsey et al. (2025) carried out a more thorough comparison between the roller eDNA methods used here and visual scouting, finding that the detection probability was twice as high with eDNA. Based on our results and those of Kelsey et al. (2025), we recommend that eDNA methods are a comparatively cost-effective and reliable approach for surveying satellite populations of SLF. Substituting eDNA surveys for at least some of the effort (person-hours) spent on visual searches within EDRR frameworks for SLF, perhaps as a supplement to existing trap surveys, appears to be justified based on our data and that of Kelsey et al. (2025).

The methods and results we present here demonstrate a new application of eDNA surveys in managing invasive terrestrial insects. Therefore, it is crucial to consider how our findings apply to other invasive insects, especially those spreading through human-related secondary pathways (e.g., moving firewood, stowaways in produce or other goods; Liebhold et al. 2016). The success of our eDNA surveys for SLF relies on two key factors. First, adult SLF feed heavily on the phloem of host trees

for energy needed for mating and egg-laying before they die later in the fall (Dara et al. 2015; Leach & Leach 2020). Phloem-feeding insects like SLF produce large quantities of ‘honeydew’ as waste. These honeydew deposits on tree surfaces are often not visible to observers but can be easily collected using the roller or spray methods we employ here (see also Kelsey et al. 2025). Using qPCR assays allows detection of small amounts of DNA from these deposits, confirming the presence of SLF at the site. However, not all insect species generate large volumes of excrement or leave their DNA on accessible surfaces. In those scenarios, the detection methods will need to be modified, such as using drones or other tools to reach difficult locations, such as tree canopies or the tissues of host species, to improve eDNA capture. Second, as SLF mature into adults, they narrow their host range (Urban and Leach 2023), allowing us to target a few key plant species for sampling. The degree to which other invasive insects specialize on one or a few hosts will similarly influence the success of eDNA surveys within EDRR programs. We anticipate that insects with high host specialization will yield much better eDNA detection results.

Since eDNA surveys often indicate a species’ presence even when it cannot be visually confirmed, there is concern that the target species might not actually be present at a location (Jerde 2019; Sepulveda et al. 2023). For example, positive detections from eDNA surveys could be caused by the target species' DNA being transferred to a site through wind or other mechanisms such as predation, by the survey crew accidentally transferring DNA to a new site on their clothes or equipment, or by contamination of samples with the target species' DNA during lab processing (Jerde 2019; Sepulveda et al. 2020; Darling et al. 2021). These complications are relevant in all applications of

eDNA surveys (Jerde 2019), but they are especially critical when eDNA results directly inform eradication and control efforts (Sepulveda et al. 2023), as could be the case with SLF. Given these concerns, we note that our field and lab controls (n = 185 samples) did not contain SLF DNA, except for one clear case where the field control was dropped in the lab. We also confirmed, for a subset of samples, that the field-obtained DNA matched SLF by complete amplicon sequencing. Therefore, we are confident that the positive detections are not due to amplification of non-target DNA. However, these controls cannot determine the origin of the detected DNA (Jerde 2019; Mathieu et al. 2020). Still, we have confidence in our SLF detections, including the first detections of new satellite populations, because of the strong agreement between our results and data from the large-scale deployment of circle traps by regulatory agencies. Our eDNA results were often verified by subsequent or concurrent detections in circle traps within the region (e.g., county). Additionally, we consistently detected SLF in areas where circle traps successfully captured them.

The uncertainty about how to interpret initial eDNA detections remains significant, even given the confidence level achieved here (see also Kelsey et al. 2025). For aquatic invasive species, an established decision framework now guides subsequent actions following an initial eDNA detection of a target species (Sepulveda et al. 2020, 2022). The numerous emerging DNA-based survey tools in terrestrial environments, including for SLF (this study; McHale et al. 2025; Lin et al. 2025), would benefit from being viewed within a similar framework. In particular, understanding the spatial scale over which terrestrial DNA-based detection methods operate is crucial. Such information is vital for guiding managers on where and how to implement follow-up

surveillance and for assessing risks to sensitive ecosystems and to assets with high economic value (e.g., vineyards, orchards, urban areas).

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Declarations

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References

Allen, M. C., Nielsen, A. L., Peterson, D. L., & Lockwood, J. L. (2021). Terrestrial eDNA survey outperforms conventional approach for detecting an invasive pest insect within an agricultural ecosystem. *Environmental DNA*, *3*(6), 1102–1112. https://doi.org/10.1002/edn3.231

Barringer, L. E., Donovall, L. R., Spichiger, S. E., Lynch, D., & Henry, D. (2015). The first new world record of *Lycorma delicatula* (Insecta: Hemiptera: Fulgoridae). *Entomological news*, *125*(1), 20-23.

Belouard N, De Bona S, Helmus MR, Smith IG, Behm JE (2025). A method to quantify jump dispersal of invasive species from occurrence data: the case of the spotted lanternfly, *Lycorma delicatula*. *NeoBiota* 98: 319-334.

Beric, B., & MacIsaac, H. J. (2015). Determinants of rapid response success for alien invasive species in aquatic ecosystems. *Biological Invasions*, *17*(11), 3327–3335. https://doi.org/10.1007/s10530-015-0959-3

Bradley, B. A., Laginhas, B. B., Whitlock, R., Allen, J. M., Bates, A. E., Bernatchez, G., Diez, J. M., Early, R., Lenoir, J., Vilà, M., & Sorte, C. J. B. (2019). Disentangling the abundance–impact relationship for invasive species. *Proceedings of the National Academy of Sciences*, *116*(20), 9919–9924. https://doi.org/10.1073/pnas.1818081116

Bradshaw, C. J. A., Leroy, B., Bellard, C., Roiz, D., Albert, C., Fournier, A., Barbet-Massin, M., Salles, J.-M., Simard, F., & Courchamp, F. (2016). Massive yet grossly underestimated global costs of invasive insects. *Nature Communications*, *7*(1), 12986. https://doi.org/10.1038/ncomms12986

Cooperband, M. F., & Murman, K. (2022). Responses of adult spotted lanternflies to artificial aggregations composed of all males or females. *Frontiers in Insect Science*, 2, 981832. https://doi.org/10.3389/finsc.2022.981832

Cuthbert, R. N., Diagne, C., Hudgins, E. J., Turbelin, A., Ahmed, D. A., Albert, C., Bodey, T. W., Briski, E., Essl, F., Haubrock, P. J., Gozlan, R. E., Kirichenko, N., Kourantidou, M., Kramer, A. M., & Courchamp, F. (2022). Biological invasion costs reveal insufficient proactive management worldwide. *Science of The Total Environment*, 819, 153404. https://doi.org/10.1016/j.scitotenv.2022.153404

Dara, S. K., Barringer, L., & Arthurs, S. P. (2015). *Lycorma delicatula* (Hemiptera: Fulgoridae): A New Invasive Pest in the United States. *Journal of Integrated Pest Management*, *6*(1), 20. https://doi.org/10.1093/jipm/pmv021

Darling, J. A., Jerde, C. L., & Sepulveda, A. J. (2021). What do you mean by false positive? *Environmental DNA*, *3*(5), 879–883. https://doi.org/10.1002/edn3.194

De Brauwer, M., Clarke, L. J., Chariton, A., Cooper, M. K., de Bruyn, M., Furlan, E., MacDonald, A. J., Rourke, M. L., Sherman, C. D. H., Suter, L., Villacorta-Rath, C., Zaiko, A., & Trujillo-González, A. (2023). Best practice guidelines for environmental DNA biomonitoring in Australia and New Zealand. *Environmental DNA*, *5*(3), 417–423. https://doi.org/10.1002/edn3.395

eDNA Task Team (2024). National Aquatic Environmental DNA Strategy. National Science & Technology Council. https://www.whitehouse.gov/wp-content/uploads/2024/06/NSTC_National-Aquatic-eDNA-Strategy.pdf

Elsensohn, J. E., Wolford, S., Tabb, A., & Leskey, T. (2024). Experimental evidence supports the ability of spotted lanternfly to hitchhike on vehicle exteriors as a

mechanism for anthropogenic dispersal. *Royal Society Open Science*, *11*(7), 240493. https://doi.org/10.1098/rsos.240493

Essler, J. L., Kane, S. A., Collins, A., Ryder, K., DeAngelo, A., Kaynaroglu, P., & Otto, C. M. (2021). Egg masses as training aids for spotted lanternfly Lycorma delicatula detection dogs. *PLOS ONE*, *16*(5), e0250945. https://doi.org/10.1371/journal.pone.0250945

Fediajevaite, J., Priestley, V., Arnold, R., & Savolainen, V. (2021). Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. *Ecology and Evolution*, *11*(9), 4803–4815. https://doi.org/10.1002/ece3.7382

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., Spear, S. F., McKee, A., Oyler-McCance, S. J., Cornman, R. S., Laramie, M. B., Mahon, A. R., Lance, R. F., Pilliod, D. S., Strickler, K. M., Waits, L. P., Fremier, A. K., Takahara, T., Herder, J. E., & Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, *7*(11), 1299–1307. https://doi.org/10.1111/2041-210X.12595

Hajibabaei, M. (2022). Demystifying eDNA validation. *Trends in Ecology & Evolution*, *37*(10), 826–828. https://doi.org/10.1016/j.tree.2022.06.015

Highway Data Services Bureau. (2019). Traffic Data Viewer [TDV GDB Shapefile AADT 2019.zip]. New York State Department of Transportation. https://www.dot.ny.gov/tdv

Jarman, S., Ackerman, F., Marnane, M., Berry, O., Bunce, M., Dawkins, K., Furlan, E., Lukehurst, S, McDonald, J., Pochon, X., Wilkinson, S., Zaiko, A., and Harvey, E.

(2024). Research horizons for invasive marine species detection with eDNA/eRNA. *Biological Invasions*, 26, 3715-3731.

Jerde, C. L. (2021). Can we manage fisheries with the inherent uncertainty from eDNA? *Journal of Fish Biology*, *98*(2), 341–353. https://doi.org/10.1111/jfb.14218

Jones, C., Skrip, M. M., Seliger, B. J., Jones, S., Wakie, T., Takeuchi, Y., Petras, V., Petrasova, A., & Meentemeyer, R. K. (2022). Spotted lanternfly predicted to establish in California by 2033 without preventative management. *Communications Biology*, *5*(1), 558. https://doi.org/10.1038/s42003-022-03447-0

Keller, A. G., Grason, E. W., McDonald, P. S., Ramón-Laca, A., & Kelly, R. P. (2022). Tracking an invasion front with environmental DNA. *Ecological Applications*, *32*(4), e2561. https://doi.org/10.1002/eap.2561

Keller, J. A., Johnson, A. E., Uyi, O., Wurzbacher, S., Long, D., & Hoover, K. (2020). Dispersal of *Lycorma delicatula* (Hemiptera: Fulgoridae) Nymphs Through Contiguous, Deciduous Forest. *Environmental Entomology*, *49*(5), 1012–1018. https://doi.org/10.1093/ee/nvaa089

Kelly, R. P., Shelton, A. O., & Gallego, R. (2019). Understanding PCR Processes to Draw Meaningful Conclusions from Environmental DNA Studies. *Scientific Reports*, *9*(1), 12133. https://doi.org/10.1038/s41598-019-48546-x

Kellner, K. (2024). jagsUI: A Wrapper Around 'rjags' to Streamline 'JAGS' Analyses_. R package version 1.6.2, https://CRAN.R-project.org/package=jagsUI

Kelsey, D., Lee-Rodriquez, J., Michel, A., Ranger, C.M., Canas, L., and Leach, A. (2025). eDNA sampling detects early colonization of spotted laternfly *Lycorma*

delicatula better than in-person scouting in an urban landscape. *Environmental DNA*, e70123

Kery, M., & Royle, J. (2020). *Applied hierarchical modeling in ecology: Analysis of distribution, abundance and species richness in r and bugs: volume 2: dynamic and advanced models* (1st ed.). Elsevier.

Kirtane, A., Dietschler, N.J., Bittner, T.D., Lefebvre, M.B., Celis, S., O'Connor, K., Havill, N., and Whitmore M.C. (2022). Sensitive environmental DNA (eDNA) methods to detect hemlock woolly adelgid and its biological control predators *Leucotaraxis* silver flies and a *Larodobius* beetle. *Environmental DNA*, 4, 1136-1149.

Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., Jackson, C. A., Lance, R. F., Mangan, A. M., Monroe, E. M., Piaggio, A. J., Stokdyk, J. P., Wilson, C. C., & Richter, C. A. (2020). Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA*, *2*(3), 271–282. https://doi.org/10.1002/edn3.29

Kotani, K., Kakinaka, M., & Matsuda, H. (2009). Dynamic economic analysis on invasive species management: Some policy implications of catchability. *Mathematical Biosciences*, *220*(1), 1–14. https://doi.org/10.1016/j.mbs.2009.03.009

Kovacs, K., Haight, R.G., McCullough, D.G., Mercader, R.J., Siegert, N.W., and Liebhold, A.M. (2010). Cost of potential emerald ash borer damage in the U.S. communities, 2009-2019. *Ecological Economics*, 69, 569-578.

Kyle, K. E., Allen, M. C., Dragon, J., Bunnell, J. F., Reinert, H. K., Zappalorti, R., Jaffe, B. D., Angle, J. C., & Lockwood, J. L. (2022). Combining surface and soil

environmental DNA with artificial cover objects to improve terrestrial reptile survey detection. *Conservation Biology*, 36(6), e13939. https://doi.org/10.1111/cobi.13939

Ladin, Z. S., Eggen, D. A., Trammell, T. L. E., & D'Amico, V. (2023). Human-mediated dispersal drives the spread of the spotted lanternfly (*Lycorma delicatula*). *Scientific Reports*, *13*(1), 1098. https://doi.org/10.1038/s41598-022-25989-3

Langlois, V. S., Allison, M. J., Bergman, L. C., To, T. A., & Helbing, C. C. (2021). The need for robust qPCR-based eDNA detection assays in environmental monitoring and species inventories. *Environmental DNA*, *3*(3), 519–527. https://doi.org/10.1002/edn3.164

Larson, E. R., Graham, B. M., Achury, R., Coon, J. J., Daniels, M. K., Gambrell, D. K., Jonasen, K. L., King, G. D., LaRacuente, N., Perrin-Stowe, T. I., Reed, E. M., Rice, C. J., Ruzi, S. A., Thairu, M. W., Wilson, J. C., & Suarez, A. V. (2020). From EDNA to citizen science: Emerging tools for the early detection of invasive species. *Frontiers in Ecology and the Environment*, *18*(4), 194–202. https://doi.org/10.1002/fee.2162

Leach, H., & Leach, A. (2020). Seasonal phenology and activity of spotted lanternfly (Lycorma delicatula) in eastern US vineyards. *Journal of Pest Science*, *93*(4), 1215–1224. https://doi.org/10.1007/s10340-020-01233-7

Leibhold, A.M., Berec, L., Brockerhoff, E.G., Epanchin-Niell, R.S., Hastings, A., Herms, D.A., Kean, J.M., McCullough, D.G., Suckling, D.M., Tobin, P.C., and Yamanaka, T. (2016). Eradication of invading insect populations: from concepts to applications. *Annual Review of Entomology*, 61, 335-352.

Leibhold, A.M., and Kean, J.M. (2019). Eradication and containment of non-native forest insects: successes and failures. *Journal of Pest Science*, 92, 83-91.

Lin W-J, Liu F-LC, Cho L, Yang C-CS (2025). Let ants find them: Using ants as eDNA samplers for detecting the invasive spotted lanternfly. *NeoBiota*, 98: 261-267.

Locke, A., Mandrak, N. E., Therriault, T. W., & Department of Fisheries and Oceans, Ottawa, ON(Canada); Canadian Science Advisory Secretariat, Ottawa, ON(Canada). (2011). A Canadian rapid response framework for aquatic invasive species. Canadian Science Advisory Secretariat= Secrétariat canadien de consultation scientifique.

Marshall Meyers, N., Reaser, J. K., & Hoff, M. H. (2020). Instituting a national early detection and rapid response program: Needs for building federal risk screening capacity. *Biological Invasions*, 22(1), 53–65. https://doi.org/10.1007/s10530-019-02144-0

Martinez, B., Reaser, J. K., Dehgan, A., Zamft, B., Baisch, D., McCormick, C., Giordano, A. J., Aicher, R., & Selbe, S. (2020). Technology innovation: Advancing capacities for the early detection of and rapid response to invasive species. *Biological Invasions*, *22*(1), 75–100. https://doi.org/10.1007/s10530-019-02146-y

Mathieu, C., Hermans, S. M., Lear, G., Buckley, T. R., Lee, K. C., & Buckley, H. L. (2020). A Systematic Review of Sources of Variability and Uncertainty in eDNA Data for Environmental Monitoring. *Frontiers in Ecology and Evolution*, 8, 135. https://doi.org/10.3389/fevo.2020.00135

McCarthy, M. A., Moore, J. L., Morris, W. K., Parris, K. M., Garrard, G. E., Vesk, P. A., Rumpff, L., Giljohann, K. M., Camac, J. S., Bau, S. S., Friend, T., Harrison, B., &

Yue, B. (2013). The influence of abundance on detectability. *Oikos*, *122*(5), 717–726. https://doi.org/10.1111/j.1600-0706.2012.20781.x

McHale, E., Kwait, R., Kerwin, K., Kyle, K., Crosby, C., & Maslo, B. (2025). Detection of Spotted Lanternfly (*Lycorma delicatula*) by Bats: A qPCR Approach to Forest Pest Surveillance. *Forests*, *16*(3), 443. https://doi.org/10.3390/f16030443

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M., & Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. *Royal Society Open Science*, *2*(7), 150088. https://doi.org/10.1098/rsos.150088

Mosher, B. A., Bernard, R. F., Lorch, J. M., Miller, D. A., Richgels, K. L., White, C. L., & Campbell Grant, E. H. (2020). Successful molecular detection studies require clear communication among diverse research partners. *Frontiers in Ecology and the Environment*, *18*(1), 43–51. https://doi.org/10.1002/fee.2141

Nixon, L. J., Leach, H., Barnes, C., Urban, J., Kirkpatrick, D. M., Ludwick, D. C., Short, B., Pfeiffer, D. G., & Leskey, T. C. (2020). Development of Behaviorally Based Monitoring and Biosurveillance Tools for the Invasive Spotted Lanternfly (Hemiptera: Fulgoridae). *Environmental Entomology*, *49*(5), 1117–1126. https://doi.org/10.1093/ee/nvaa084

Pennsylvania Invasive Species Council (2014). Rapid Response Plan and Procedures for Responding to Aquatic Invasive Species in Pennsylvania. Pennsylvania Invasive Species Council.

R Core Team (2024). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Reaser, J. K., Burgiel, S. W., Kirkey, J., Brantley, K. A., Veatch, S. D., & Burgos-Rodríguez, J. (2020). The early detection of and rapid response (EDRR) to invasive species: A conceptual framework and federal capacities assessment. *Biological Invasions*, *22*(1), 1–19. https://doi.org/10.1007/s10530-019-02156-w

Schenekar, T. (2023). The current state of eDNA research in freshwater ecosystems: Are we shifting from the developmental phase to standard application in biomonitoring? *Hydrobiologia*, *850*(6), 1263–1282. https://doi.org/10.1007/s10750-022-04891-z

Schmidt, B. R., Kéry, M., Ursenbacher, S., Hyman, O. J., & Collins, J. P. (2013). Site occupancy models in the analysis of environmental DNA presence/absence surveys: A case study of an emerging amphibian pathogen. *Methods in Ecology and Evolution*, *4*(7), 646–653. https://doi.org/10.1111/2041-210X.12052

Sepulveda, A. J., Dumoulin, C. E., Blanchette, D. L., McPhedran, J., Holme, C., Whalen, N., Hunter, M. E., Merkes, C. M., Richter, C. A., Neilson, M. E., Daniel, W. M., Jones, D. N., & Smith, D. R. (2023). When are environmental DNA early detections of invasive species actionable? *Journal of Environmental Management*, *343*, 118216. https://doi.org/10.1016/j.jenvman.2023.118216

Sepulveda, A. J., Nelson, N. M., Jerde, C. L., & Luikart, G. (2020). Are Environmental DNA Methods Ready for Aquatic Invasive Species Management? *Trends in Ecology & Evolution*, *35*(8), 668–678. https://doi.org/10.1016/j.tree.2020.03.011

Sepulveda, A., Smith, D., O'Donnell, K., Owens, N., White, B., Richter, C., Merkes, C., Wolf, S., Rau, M., Neilson, M., Daniel, W., Dumoulin, C., & Hunter, M. (2022). Using structured decision making to evaluate potential management responses to detection of dreissenid mussel (Dreissena spp.) environmental DNA. *Management of Biological Invasions*, *13*(2), 344–368. https://doi.org/10.3391/mbi.2022.13.2.06

Smart, A. S., Tingley, R., Weeks, A. R., Van Rooyen, A. R., & McCarthy, M. A. (2015). Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. *Ecological Applications*, *25*(7), 1944–1952. https://doi.org/10.1890/14-1751.1

Siegert, N.W., McCullouth, D.G., Liebhold, A.M., and Telewski, F.W. (2014). Dendrochonological reconstruction of the epicenter and early spread of emerald ash borer in North America. *Diversity and Distributions*, 20, 847-858.

Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E., & Vilà, M. (2013). Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution*, 28(1), 58–66. https://doi.org/10.1016/j.tree.2012.07.013

Strömbom, D., & Pandey, S. (2021). Modeling the life cycle of the spotted lanternfly (Lycorma delicatula) with management implications. *Mathematical Biosciences*, 340, 108670. https://doi.org/10.1016/j.mbs.2021.108670

Strömbom, D., Sands, A., Graham, J. M., Crocker, A., Cloud, C., Tulevech, G., & Ward, K. (2024). Modeling human activity-related spread of the spotted lanternfly

(*Lycorma delicatula*) in the US. *PLOS ONE*, *19*(8), e0307754. https://doi.org/10.1371/journal.pone.0307754

Thomas, A. C., Tank, S., Nguyen, P. L., Ponce, J., Sinnesael, M., & Goldberg, C. S. (2020). A system for rapid eDNA detection of aquatic invasive species. *Environmental DNA*, 2(3), 261–270. https://doi.org/10.1002/edn3.25

Tobin, P. C., Kean, J. M., Suckling, D. M., McCullough, D. G., Herms, D. A., & Stringer, L. D. (2014). Determinants of successful arthropod eradication programs. *Biological Invasions*, *16*(2), 401–414. https://doi.org/10.1007/s10530-013-0529-5

Urban, J.M., and Leach. H. (2023). Biology and management of the spotted lanternfly *Lycorma delicatula* (Hemiptera: Fulgoridae), in the United States. *Annual Review of Entomology*, 68, 151-167.

Valentin, R. E., Fonseca, D. M., Gable, S., Kyle, K. E., Hamilton, G. C., Nielsen, A. L., & Lockwood, J. L. (2020). Moving eDNA surveys onto land: Strategies for active eDNA aggregation to detect invasive forest insects. *Molecular Ecology Resources*, 20(3), 746–755. https://doi.org/10.1111/1755-0998.13151

Valentin, R. E., Kyle, K. E., Allen, M. C., Welbourne, D. J., & Lockwood, J. L. (2021). The state, transport, and fate of aboveground terrestrial arthropod eDNA. *Environmental DNA*, *3*(6), 1081–1092. https://doi.org/10.1002/edn3.229

Weston, J.D., McClaran, M.P., Whittle, R.K., Black, C.W. and Fehmi, J.S. (2019). Satellite patches, patch expansion, and doubling time as decision metrics for invasion control: *Pennisetum ciliare* expansion in southwestern Arizona. *Invasive Plant Science and Management*, 12:36-42

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, 4(43), 1686. https://doi.org/10.21105/joss.01686

Wilke, C. (2024). cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'. R package version 1.1.3, https://CRAN.R-project.org/package=cowplot

Wittwer, C., Stoll, S., Strand, D., Vrålstad, T., Nowak, C., & Thines, M. (2018). eDNA-based crayfish plague monitoring is superior to conventional trap-based assessments in year-round detection probability. *Hydrobiologia*, 807(1), 87–97. https://doi.org/10.1007/s10750-017-3408-8

Wolfin, M. S., Myrick, A. J., & Baker, T. C. (2020). Flight Duration Capabilities of Dispersing Adult Spotted Lanternflies, Lycorma delicatula. *Journal of Insect Behavior*, 33(2–4), 125–137. https://doi.org/10.1007/s10905-020-09754-w

Xiao, N. (2024). ggsci: Scientific Journal and Sci-Fi Themed Color Palettes for 'ggplot2'. R package version 3.0.3, https://CRAN.R-project.org/package=ggsci

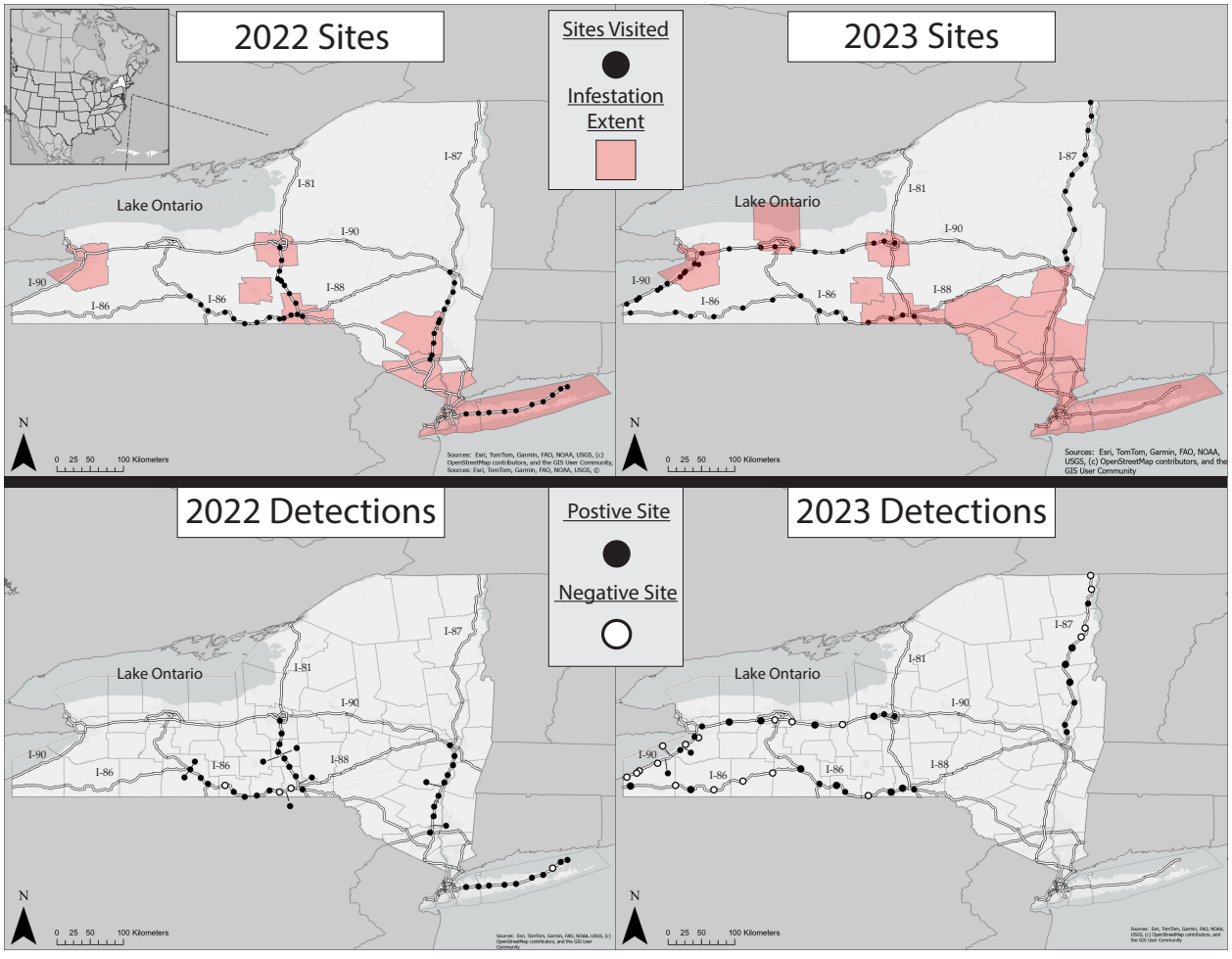


Figure 1. Map of our survey sites along major highways in New York State, USA. (Top) We selected survey locations at the edges or outside areas where spotted lanternfly were known to be established at the time of our surveys (September – November 2022, 2023). Known occupied areas are shown at the county level, and the spotted lanternfly distribution is based on the Cornell University Integrated Pest Management Spotted Lanternfly Distribution Map (cals.cornell.edu). (Bottom) Map of sites where spotted lanternfly (*Lycorma delicatula*) eDNA was detected (black points) or not detected (white points) during a roadside eDNA survey in New York State, USA. Across 83 sites, we detected lanternfly eDNA at least once at 64 sites (77%) during our two-year surveys (2022-2023).

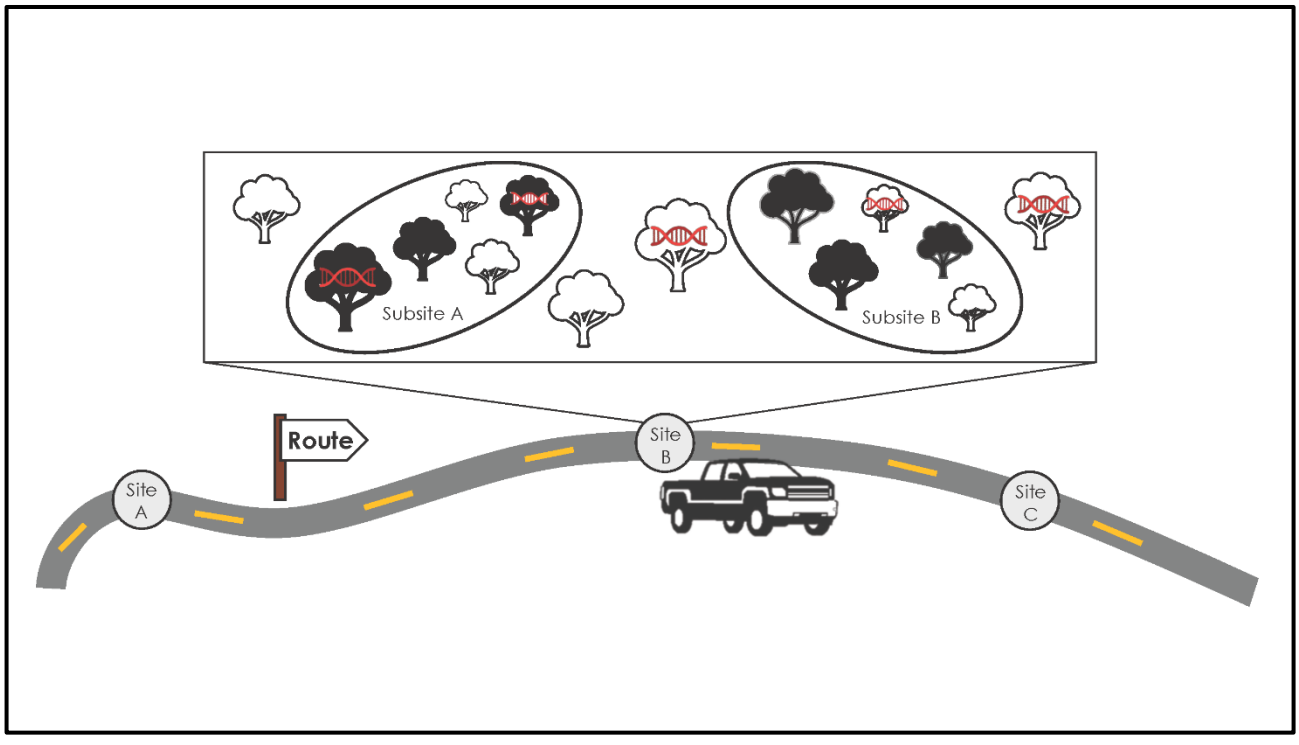


Figure 2. Conceptual diagram showing the roadside eDNA study design for spotted lanternfly (*Lycorma delicatula*) in New York State, USA. Sites were rest stops or similar way stations along major highways. At each site, we sampled two sub-sites. At each sub-site, three trees were sampled with a single paint roller. Timed (2.5-minute) visual surveys for lanternflies were also conducted at each sub-site.

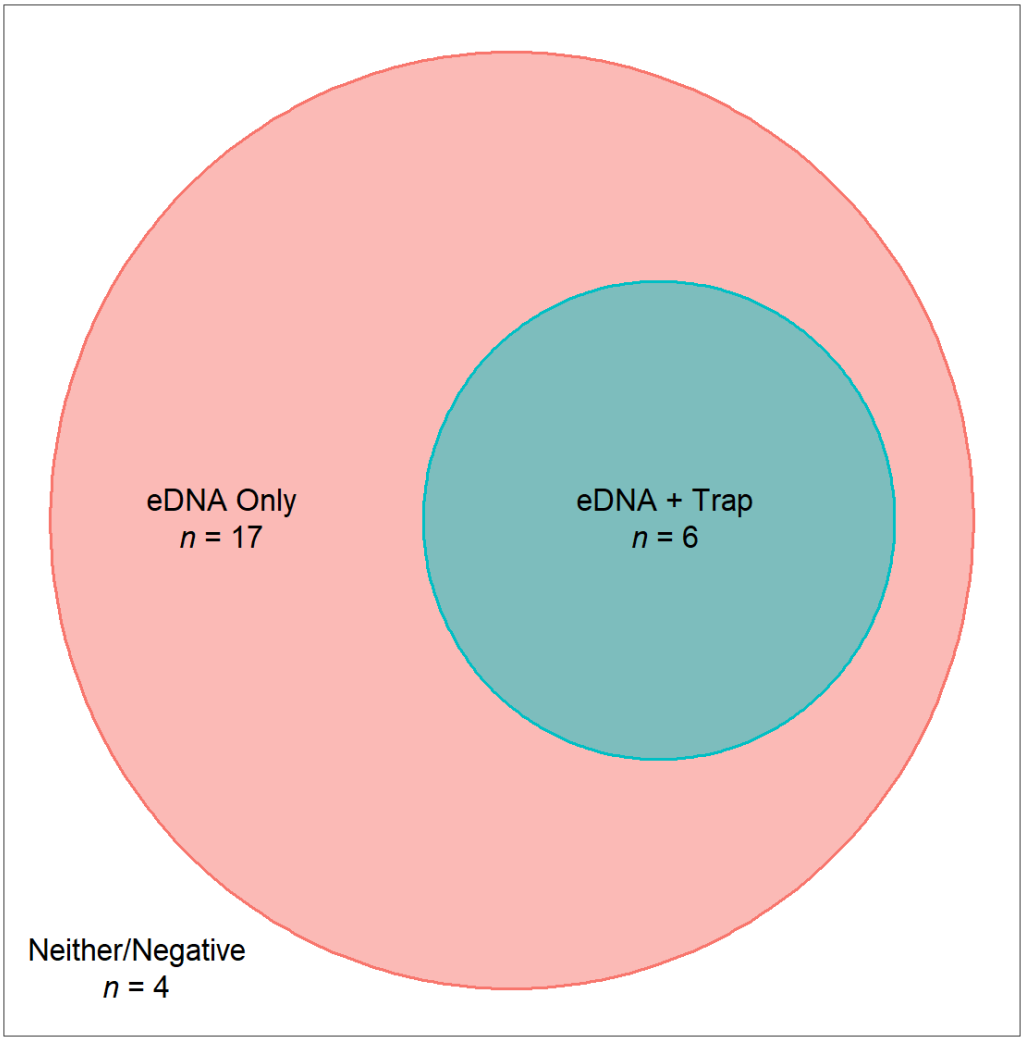


Figure 3. Venn diagram showing the overlap between positive detections resulting exclusively from eDNA (red) and those from both eDNA and traps (blue) at all 27 sites where both methods were used in 2022 and 2023. Only traps within 5 km of an eDNA site and limited to trap visits during the months when eDNA surveys were conducted (September and October) are included. Detections at 17 sites were unique to eDNA, while none were unique to traps. Four sites had no positive detections. There were no sites where circle traps detected SLF and eDNA did not.

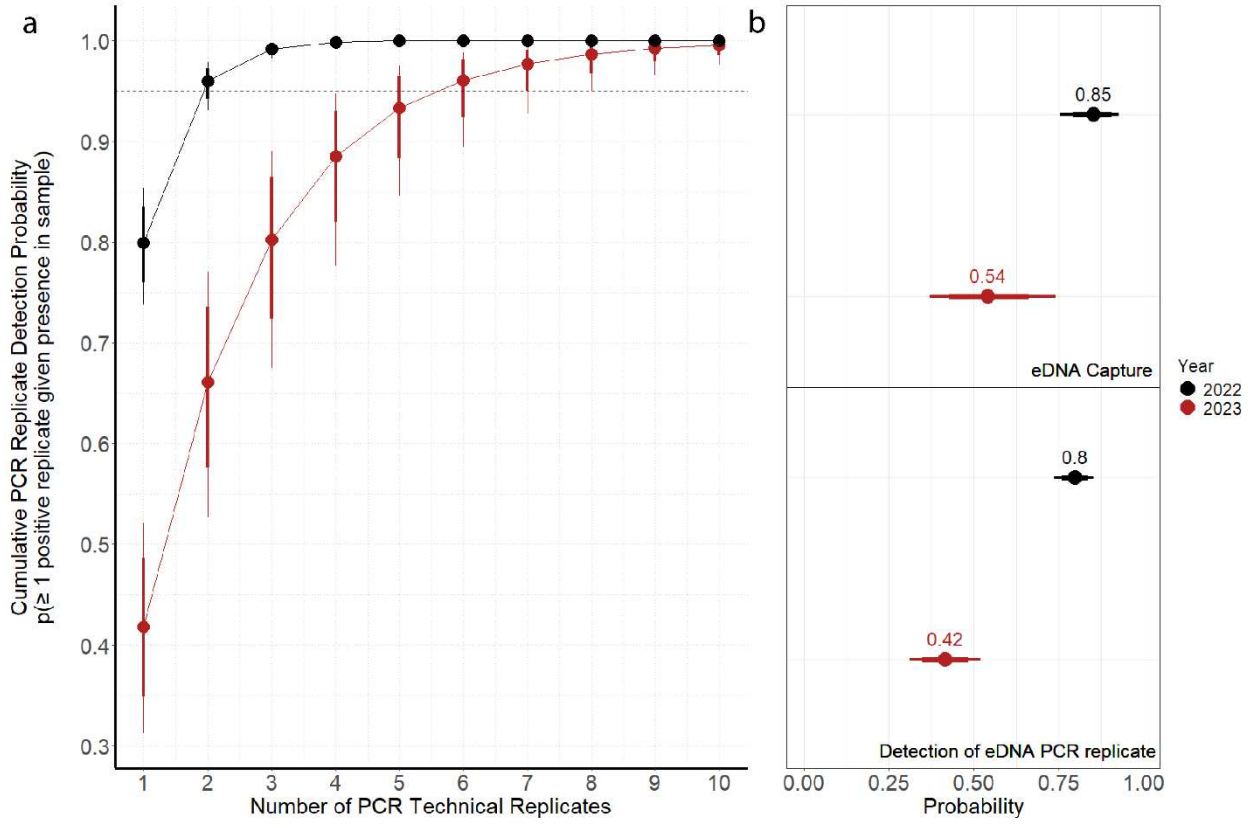


Figure 4. (a) Cumulative detection probability for eDNA in 2022 (black) and 2023 (red) across 10 theoretical qPCR technical replicates, assuming the sample contains SLF DNA. Dots show median posterior estimates, while thick and thin error bars indicate 80% and 95% credible intervals, respectively. A dotted horizontal line marks a 95% cumulative detection probability. (b) Probability of capturing SLF DNA using the roller method at a site occupied by SLF (top panel), and the detection probability of a single qPCR technical replicate from a sample containing SLF DNA (black bottom panel) by year, with medians (dots and text), as well as 80% (bold line) and 95% (thinner line) credible intervals.

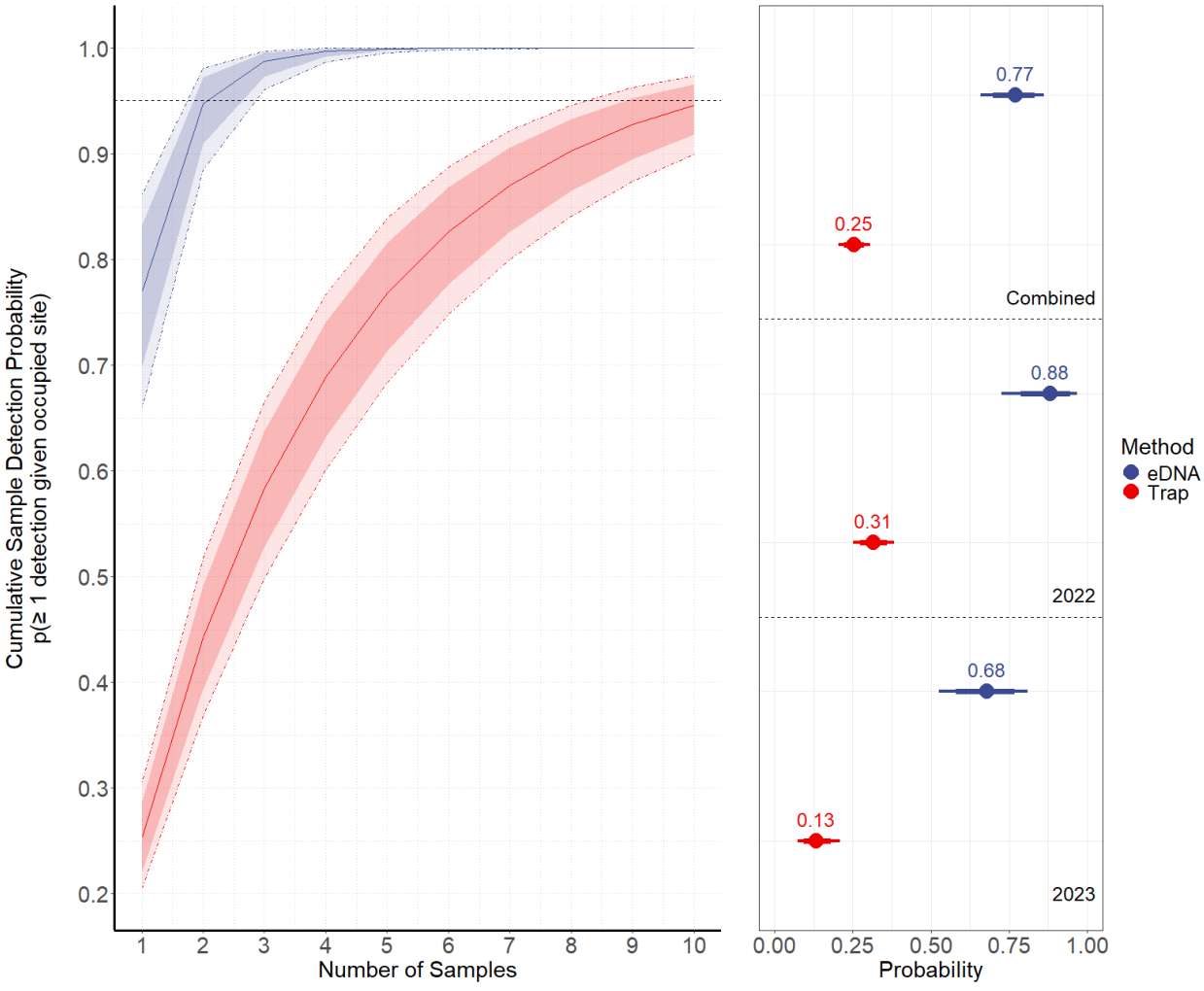


Figure 5. (a) Cumulative detection probabilities for eDNA and circle trap methods over 10 theoretical sampling occasions, assuming sampled sites are occupied by SLF (2022 and 2023 data combined for this model run). Only traps within 5 km of an eDNA site, and including only trap-visit data collected in September and October each year, were used in this analysis. Solid lines, dark-shaded areas, and light-shaded areas represent median values, 80% credible intervals, and 95% credible intervals, respectively. A dotted horizontal line indicates the desired 95% cumulative detection probability. (b) Median detection probabilities (shown as dots and text), along with 80% (bold line) and

95% (line) credible intervals for eDNA (blue) and circle trap sampling (red) methods for detecting SLF across combined years, 2022 only, and 2023 only sampling efforts.

Supplemental Information

Lab Protocols to Ensure eDNA Surveys Detected SLF

To further confirm SLF eDNA detections, we Sanger sequenced qPCR products from a subset of samples. To do so, we added extension sequences (bold font) to our initial 63 bp amplicon primers, and our qPCR product was further amplified using a standard PCR reaction (SLF_EXT_F2: **ATGATACGGCGACCACCGAGATCgACAACACTCTTagCCTACACGACGCTCTTCC GATCT**AGCGTTTGACAGCTGACTCTTG; SLF_EXT_R2: **CAAGCAGAAGACGGCATACGAGATGaGACTGGAGTTCAGACGaGTGCTCTTCCG ATCT**CGCCGAAGCGCAAAAA; DNA Oligo, Integrated DNA Technologies Inc.). Extension sequences are modified (lowercase and underlined) adapter primers used for Illumina sequencing (Miya et al. 2015). Each reaction consisted of 2 µl of Applied Biosystems™ 10x Buffer II, 0.3 µl of dNTPs (10 nM each), 0.4 µl of each extension primer at 10 µM, 2 µl of 25 mM $MgCl_2$, 12.7 µl of nuclease-free water, 0.2 µl of AmpliTaq Gold™ DNA polymerase at 5 U/µl, and 2 µl of qPCR product. The extension PCR was conducted with an initial denaturation step at 96 °C for 10 min followed by 8 cycles of: 96 °C for 30 sec, 55 °C for 30 sec, and 72 °C for 1 min, with a final extension step at 72 °C for 5 min and a 4 °C hold. The extension PCR products were cleaned using ExoSAP-IT and prepared in separate forward and reverse reactions, consisting of ~10 ng of product and 2.5 µL of sequencing primer (SLF_Sanger_ FWD: ATGATACGGCGACCACC; SLF_Sanger_REV: CAAGCAGAAGACGGCAT; DNA Oligo, Integrated DNA Technologies Inc.) at 10 µM and sent for Sanger sequencing at an

external lab. Subsequent sequence data were trimmed and aligned using Geneious Prime software (Dotmatics) and aligned to the target SLF sequence for species confirmation.

To differentiate between any potential contamination that could occur during qPCR setup, we designed our synthetic SLF DNA to match the target amplicon sequence with sacrificial base pairs (lower case, italic font) at each end, a reverse primer binding site (bold font), and a target sequence inversion (lower case font) between the forward primer (bold and underlined) and the probe (underlined) binding sites (5'-*atgcatgatctacgtgcgtcacatgcagtac***AGCGTTTGACAGCTGACTCTTG**CatgccacGCCGCGGGACCGGTAGC**TTTTTGCGCTTCGGCG***cactagctcagattcagtagaccgctgttgc*-3'; gBlocks Gene Fragments, Integrated DNA Technologies, Inc.).

Limit of Detection (LOD) Analysis

To conduct the Limit of detection (LOD) analysis, we prepared a 10-fold serial dilution (ranging from ~100,000 copies to ~0.1 copies) of the target amplicon using synthetic SLF DNA (gBlocks Gene Fragments, Integrated DNA Technologies, Inc.). The dilution series was analyzed using the SLF qPCR assay, consisting of 12 replicates per dilution level. The results were fit to a modeled curve (after Klymus et al. 2020), and we determined that the 95% LOD for the assay is ~3 copies of target DNA per reaction when run in triplicate.