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Comparison of camera traps, eDNA, and visual encounter surveys for threatened species detection

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ABSTRACT

Accurate, cost-effective monitoring remains a major limitation to conservation efforts for many wildlife species. The USA federally threatened eastern indigo snake (*Drymarchon couperi*) exemplifies this sampling challenge. They occur at low densities, are cryptic, and highly mobile, which has historically made them challenging and expensive to monitor. However, emerging technologies and methodologies may provide new pathways to augment, complement, or replace conventional sampling to yield improved monitoring programs, both for eastern indigo snakes and for other imperiled and cryptic taxa. Here, we compare environmental DNA (eDNA) sampling, camera trapping, and visual encounter surveys as monitoring methods for the eastern indigo snake. We conducted an *in situ* study of eDNA decay rate in soil. Subsequently, we paired active searches across four sites in the southeast United States, eDNA sampling of gopher tortoise burrows and drift fences, and camera trapping of gopher tortoise burrows and drift fences and used these data to (1) assess eDNA detectability over time and (2) relative cost-effectiveness of the different survey methods. We found patterns of eDNA detectability that were concordant with previous experimental trials. Of 120 samples collected from locations with confirmed snake presence via visual encounter surveys, 66 amplified (55%) and eDNA was detectable up to six days after snake presence. Using multi-scale, multi-method occupancy modeling we estimated that the probability of eastern indigo snake presence in gopher tortoise burrows (0.29) was higher than at drift fences (0.24) and that cameras had a higher rate of detection (0.50) than eDNA sampling (0.38), although image processing time made camera trapping prohibitively expensive relative to other approaches. To reach a 95% likelihood of detection, the most cost-effective sampling method is visual encounter surveys augmented with eDNA sampling. Our results illustrate that visual encounter surveys remain an effective monitoring method, but supplementing with eDNA may decrease costs and increase detection probability.

1. Introduction

Basic information on species distribution is critical to conservation planning and understanding potential impacts of development, land conservation, and habitat restoration activities, but is often lacking. For example, approximately 20% of reptiles in the IUCN Sampled Red List Index are listed as "data deficient" (Bland and Böhm, 2016). This issue is particularly acute for cryptic, rare, and wide-spread species, for which gathering timely, accurate data may be cost-prohibitive (Jackson and Robertson, 2011; Martin et al., 2022). Such data deficiencies can hamper efforts to conserve rare species under legal frameworks (e.g., the U.S. Endangered Species Act) that are designed to protect species from

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extinction.

New tools and sampling designs are emerging to fill this gap and provide rapid, accurate, and cost-effective data, even for rare species: Acoustics (Wrege et al., 2017), environmental DNA (eDNA) sampling (Smart et al., 2015; Wilcox et al., 2018), non-invasive genetics (Mills et al., 2000), camera trapping (Burton et al., 2015; Sun et al., 2021), drones (Stephenson, 2020), and remote sensing (McDermid et al., 2009). Often, these tools are most powerful when used complementary to one another. For example, Sanderlin et al., (2019) used joint modeling with banding data (high cost) and point counts (lower cost) to generate greater precision and accuracy in population metrics than available from either data type alone. Pairing methods can also be more cost-effective; Magoun et al. (2011) used paired camera and hair traps to reduce monitoring costs for wolverine identification. However, leveraging multiple tools in this way effectively requires a strong understanding of their relative sensitivities and cost structures.

We assessed sensitivity and cost-efficacy of multiple potentially complementary sampling methods for monitoring of a rare, cryptic, and imperiled species. Eastern indigo snakes (*Drymarchon couperi*; hereafter EIS; Holbrook, 1842) were first listed by the U.S. Fish and Wildlife Service (USFWS) under the Endangered Species Act in 1978 as federally threatened (Federal Register 43:4026; IUCN RedList Status: Least Concern but Declining; Hammerson, 2007; NatureServe Rank: G3, Declining; NatureServe, 2024). They are highly mobile and occur in low-density populations, rendering them particularly challenging to monitor through much of their range (Bauder et al., 2020; Breininger et al., 2011; Hyslop et al., 2014; Landers and Speake, 1980). The primary threat to EIS populations is habitat loss, degradation, and fragmentation, but other threats include illegal collection, disease, introduced species, and human persecution (U.S. Fish and Wildlife Service, 2019a). Accurate and sensitive monitoring of the species is required as part of the USFWS recovery plan and as part of the regulatory process of the Endangered Species Act (1973) to track both wild and reintroduced populations and to survey possible development sites for species presence (U.S. Fish and Wildlife Service, 2019b). This is a species for which new, cost-effective sampling tools could have particularly important conservation impacts. This is also an extensively sampled species which acts as a useful model system for testing tools that may be transferable to other rare snake species.

Currently, visual encounter surveys (VES) are the primary EIS monitoring strategy (Stevenson et al., 2003). In the northern portion of their range (Georgia, Alabama, and northern Florida, USA), EIS rely on gopher tortoise (*Gopherus polyphemus*) burrows in xeric sandhills for thermal refugia during winter months (Diemer and Speake, 1983; Hyslop et al., 2009; Lawler, 1977; Stevenson et al., 2009). This allows for surveys of EIS in the northern portion of their range to focus on these well-defined landscape features to find snakes which are otherwise highly dispersed (Bauder et al., 2017). During these surveys, small teams of observers visually search sandhill sites, including all gopher tortoise burrows, for EIS during the winter months (Stevenson et al., 2009; Bauder et al., 2017). Visual encounter surveys often involve returning to a site three times during the survey season (late November – early March), although at reintroduction sites VES take place with much more frequency. While VES have low equipment costs and cover a large area during each survey, they incur personnel and travel costs. Moreover, due to time limitations and other logistical constraints, surveyors in current monitoring frameworks can only travel to a site a few times each season and only sample a portion of sites each season, which restricts the data to specific points in time and limits the spatial scalability (Bauder et al., 2017). Since EIS are a highly mobile species and often occur at low population densities, fewer site visits may lower the chances of species detection.

Camera trapping has recently emerged as an alternative EIS sampling tool. Camera traps have been successfully used to monitor a variety of terrestrial squamates, including rare species, in the southeastern United States (Adams et al., 2017, Neuharth et al., 2020, Ryberg et al., 2021). Pilot studies suggest that cameras may also be effective for detection of EIS (Erickson, 2023). While VES have the benefit of spatial scale, camera traps have the benefit of temporal scale. Camera traps can be placed at gopher tortoise burrows or drift fences and provide a large temporal sampling window (e.g., an entire sampling season) for a specific location. However, cameras have substantial up-front costs associated with equipment and installation, as well as potential image processing costs, which limits both how many cameras can be installed, and the number of sites at which cameras can be deployed (Welbourne et al., 2015).

Another emerging tool for sampling rare species, including EIS, is eDNA sampling. Environmental DNA sampling is the detection of a species' DNA in an environmental sample such as water or soil to infer species presence (Thomsen and Willerslev, 2015; Barnes and Turner, 2016). Environmental DNA sampling has the potential to provide information over moderate temporal scales (i.e., DNA often persists for hours to days; Allan et al., 2021; Collins et al., 2018; Thomsen et al., 2012) and large spatial scales, because field sample collection is often simple and inexpensive (Smart et al., 2015; Sternhagen et al., 2024; Wilcox et al., 2016). Costs of eDNA sampling are primarily associated with sample processing and lab technician costs. This ability to rapidly collect samples in the field makes eDNA well-suited to occupancy modeling frameworks. Species are also imperfectly detected by many survey methods including eDNA, as species are not directly observed during sampling and DNA may go uncollected when in fact, the species is present. Occupancy models have been increasingly used for eDNA studies and increase the effectiveness of the method (Tetzlaff et al., 2024; Kessler et al., 2020). Previous work has shown that EIS eDNA is detectable for as long as 10 days after snake exposure (Samuels et al., 2025; Santamaria et al., 2024).

Here, we sought to compare the sensitivity and cost-effectiveness of camera trapping, eDNA sampling, and VES for detection of EIS in overwintering habitats. We conducted a paired sampling design at four overwintering sites, sampling for EIS with all three survey techniques during a single winter season. We used these data to parameterize models that estimate detection probability for each method per unit effort. We then combined these detection parameter estimates with estimates of cost per unit effort to determine the overall cost associated with a 95% detection probability across methods to compare potential monitoring program options to leverage new tools for EIS monitoring.

2. Methods

2.1. Sampling

We conducted this field study at four locations across the northern portion of the EIS range in the United States: Conecuh National Forest in Andalusia, Alabama (hereafter CNF), Apalachicola Bluffs and Ravines Preserve in Bristol, Florida (hereafter ABRP), and two distinct sites on Fort Stewart near Hinesville, Georgia. We selected two distinct sandhill units on Fort Stewart where EIS are known to occur, and we refer to them as Fort Stewart East (hereafter FSE; 0.440 km^2) and Fort Stewart West (hereafter FSW; 0.735 km^2). These sites were chosen because they have relatively high-density populations and a history of EIS monitoring. At each site, we implemented five monitoring methods: (1) eDNA sampling directly from gopher tortoise burrows, (2) eDNA sampling from drift fence arrays, (3) camera trapping on individual gopher tortoise burrows, (4) camera trapping on drift fence arrays, and (5) VES.

2.1.1. Environmental DNA sampling

We parameterized two distinct models to estimate rate of detection using results from the eDNA sampling. First, eDNA samples were collected from locations where EIS were visually confirmed or there was evidence of EIS (i.e., the presence of snake tracks or sheds). Shed skins can be confirmed as EIS by their size and coloration, and we only sampled from snake tracks that we were confident were left by EIS because of their size, shape, or observations of nearby snakes. We

collected repeat samples from these locations three times over the course of the study to parameterize a model predicting eDNA persistence as a function of time since snake occurrence. We hereafter refer to these as "known-positive" locations. We returned up to three days later to resample these known-positive locations (Samuels et al., 2025). We used wire pin flags to mark sampling locations or locations for return samples to prevent resampling the exact same location, and therefore, likely decreasing detection rates. Second, eDNA samples were collected repeatedly (at the same location during each survey) from both gopher tortoise burrows and drift fences at Fort Stewart to parameterize occupancy models. Finally, we collected eDNA samples from rotating burrows and drift fences at all sites to estimate the overall rate of detection. Some of the known-positive locations also overlapped burrow and drift fence sampling locations for occupancy modeling.

Soil samples were collected with 50 mL conical tubes, skimming the surface to collect the top several millimeters of soil to catch eDNA until the tube was fully filled (~ 50 mL soil). All samples were collected while wearing sterile nitrile gloves that were changed between each sample. In the event of leaf litter or pine needles covering the soil substrate, one tube was filled to 25 mL with pine needles or leaf litter and another tube was filled with 25 mL of soil from underneath the pine needles and leaf litter.

We collected negative eDNA samples at each sampling visit by collecting soil from along the roadside away from all tortoise burrows and signs of EIS activity. We followed the same sample collection protocol for these samples.

2.1.2. Camera trapping and drift fences

Cameras were installed at Fort Stewart and had already been set up at CNF and ABRP as part of ongoing monitoring programs. We installed drift fences and cameras following similar methodologies to other studies (e.g., Brown et al., 2023; Erickson 2023; Martin et al., 2017). Briefly, we placed two sections of approximately 15 m of silt fencing in a straight line or in a "+" shape evenly spaced across the sandhill unit and within areas with higher density of gopher tortoise burrows. We mounted cameras in between sections of the drift fence by attaching the camera to the bottom of an inverted bucket placed on top of four rebar posts or wooden stakes. At each site, we also placed cameras pointed towards the mouth of five gopher tortoise burrows (Dziadzio and Smith, 2016). We chose camera locations to be spaced evenly across each site and in areas with higher burrow densities. We attached these cameras to wooden or metal posts driven into sandy soil in front of burrow entrances, to small trees near burrows, or set them directly on the ground facing burrow entrances. We used different models of cameras depending on the survey site. At Fort Stewart, all cameras were Reconyx HP2X. Fence cameras had a modified focal distance of approximately 40 cm, while burrow cameras were unmodified. We programmed the fence cameras at Fort Stewart to only acquire motion triggered photos, while cameras placed at burrows recorded both motion triggered photos and 30-second time lapse photos. At CNF, we used Browning Strike Force APEX trail cameras and set both fence and burrow cameras to only take photos when motion triggered. At ABRP, we used Browning Strike Force APEX trail cameras at drift fences, set to only take photos when motion triggered, each camera was modified with a reading glass lens (+3) affixed over the camera to allow them to focus on animals passing under the fence. At burrows at ABRP, we used Reconyx Hyper-Fire 2 mounted on T-posts ~ 1.5–2 m from burrow openings and set them to use time lapse and motion trigger. The timelapse for cameras at burrows at ABRP were set to either 30-second or 1-minute intervals, depending on the camera version. We processed all camera photos from this project manually.

2.1.3. Visual encounter surveys

For VES, small teams of 1–6 observers searched the entire sandhill unit and inspected all known gopher tortoise burrows for the presence of snakes or snake signs. We noted the presence of EIS shed skins and snake tracks and scoped burrows at FSE, FSW, and CNF to attempt to confirm the presence of a snake when snake signs were observed. We recorded the number of snakes encountered, along with the amount of survey effort (time * number of people). We completed visual surveys on three separate occasions across the entire winter survey season (surveys separated by at least two weeks).

2.1.4. Field sampling designs

Due to existing infrastructure associated with EIS monitoring programs, the sampling designs varied between the Fort Stewart sites and the other two sampling locations. The sampling methods and schedule at these two sites were identical. Five burrow camera traps and five drift fences were installed at each site at the beginning of the field season. Three full surveys were conducted at both FSE and FSW. Full surveys included a VES, eDNA sampling from the five burrows with camera traps, five drift fences with cameras, and five randomly selected burrows, as well as sampling and resampling when EIS were encountered (opportunistic known-presence locations). We also conducted additional eDNA-only surveys just from burrows with camera traps. Fort Stewart West surveys took place on 7 Dec 2023, 19 Jan 2024, and 21 Feb 2024, while FSE surveys took place on 6 Dec 2023, 11 Jan 2024, and 20 Feb 2024. Additional eDNA-only surveys from burrows with cameras for both locations took place on 18 Dec 2024, 01 Feb 2024, and 07 Mar 2024. Across the surveys, we collected a total of 156 eDNA samples at both FS sites.

Three surveys were conducted at CNF (0.928 km^2) on 21 Dec 2023, 06 Feb 2024, and 28 Feb 2024. Existing monitoring infrastructure on CNF included approximately 50 cameras associated with drift fences, along with camera traps placed at burrows. We randomly selected and rotated drift fences and burrows for collection of eDNA samples during survey visits. Across the three survey events, we collected 52 eDNA samples, 30 of which were taken from burrows (11 of which were associated with a camera trap).

Three surveys were also conducted at ABRP (0.931 km^2), as well as additional known-positive locations sampled over 18 additional days. Over the course of the study, 83 eDNA samples were taken. A total of 38 samples were taken at burrows, of which 19 had an associated camera. Surveys took place on 29 Nov 2023, 3 Jan 2024, and 24 Feb 2024, with the additional known-positive locations sampled between 29 Nov 2023 and 14 Mar 2024.

2.1.5. Environmental covariates

We recorded general weather conditions (air temperature, wind, cloud cover, and precipitation) while onsite at both Fort Stewart and CNF. All weather information was recorded at both the beginning and end of each survey event. We also recorded the number of tortoise burrows that were visually inspected and the number that were scoped with a tortoise burrow camera. At ABRP, we used the Florida Automated Weather Network (FAWN; fawn.ifas.ufl.edu) tower on site to record weather conditions on the day of each survey.

2.2. DNA extraction and qPCR

Prior to analysis we compared the two highest-yielding extraction methods from Samuels et al., (2025), the phosphate buffer (PB) method and the filter method. The PB method protocol uses a phosphate buffer (1.97 g of NaH_2PO_4 and 14.7 g of Na_2HPO_4 to 1 L diH_2O; 0.12 M, pH ~ 8) mixed at a 1:1 ratio with the soil sample for 15 – 30 min to displace extracellular DNA adhered to sediment. Then DNA is extracted from 700 μL of the buffer using the NucleoSpin Soil Kit (Macherey-Nagel), following the manufacturer's protocol, skipping the lysis step. The filter method utilizes filtration of solubilized DNA released from sediments using the method of Taberlet et al. (2012). Taking the remaining phosphate buffer (~50 mL) used in the PB method, the mixture was centrifuged for 5 min at 4430 × *g* and the supernatant filtered through 0.1 μm pore size, 47 mm diameter mixed cellulose ester membrane

filters using an electronic peristaltic pump. Filters were preserved in silica desiccant and frozen at −20 °C until DNA extraction using the modified DNeasy Blood and Tissue Kit (QIAGEN) protocol described in Franklin et al., (2019). We ran 21 field samples with both the PB and filter method. The seven same samples successfully amplified with both methods (mean of amplified samples Filter = 18.02 mtDNA copies/reaction, PB = 1.87 mtDNA copies/reaction). Although the filtration protocol yielded greater copy numbers on average, we used the PB method for extractions because performance on a detection/non-detection basis appeared similar across methods, but the filtration method is expensive and time-consuming for large numbers of samples.

To avoid contamination, all extractions were performed in pre-PCR laboratory spaces dedicated to low-quality DNA handling. Extractions were performed in a sterile hood and surfaces were cleaned with 20 % household bleach solution (~1.2 % sodium hypochlorite) and irradiated with ultraviolet (UV) light for 1 h between each set, in accordance with the Standard Operating Procedures of the NGC. Soil handling and disposal was in compliance with Animal & Plant Health Inspection Service (APHIS) Agreement # MT-SL-2024–01.

Using the EIS qPCR designed by Samuels et al. (2025), we analyzed all samples via targeted (i.e. species-specific) qPCR. This assay was extensively validated to ensure that there would be no cross-amplification with other, co-occurring snake species. Each qPCR reaction conisted of 7.5 μL of 2 × TaqMan Environmental Master Mix (ThermoFisher Scientific), 0.75 μL of 20 × assay mix (optimized primer concentrations), 0.95 μL of sterile water, 4 μL of template DNA, 1.5 μL of 10 × exogenous IPC assay, and 0.3 μL of 50 × exogenous IPC template (TaqMan Exogenous IPC Kit; ThermoFisher Scientific). Thermocycling conditions were 95 °C 10 min, (95 °C 15 sec, 60 °C 1 min) × 45. Each sample was analyzed in triplicate, and we determined any sample with a linear amplification phase in at least one replicate to be positive. We included an internal positive control (IPC) in each reaction to check for PCR inhibitors, with inhibited samples being characterized by a Ct shift of ≥ 1 relative to the negative control. Any inhibited samples were treated with an inhibitor removal column (n = 6; Zymo Inhibitor Removal Kit, Zymo Inhibitor Removal Kit; Zymo Research; Irvine, California, USA) and then re-analyzed. Each plate included a negative control, and a standard curve made with a gBlock gene fragment serially diluted fivefold, from 15,625 to 1 copy per reaction, with each dilution level in triplicate. Quantification was determined per sample by averaging across all replicates with positive amplification. All PCR preparations were conducted in a dedicated space, physically isolated from the post-PCR laboratory, and in a hood that was irradiated with UV light for 1 h between each set, in accordance with the Standard Operating Procedures of the National Genomics Center for Wildlife and Fish Conservation. We extracted samples in batches of 12 – 48 samples. The sample composition of each extraction batch was randomized to reduce the risk of bias due to batch effects.

2.3. Data analysis

2.3.1. DNA persistence in field

We used presence/absence data due to zero-inflation and low copy number (see Results) and a binomial generalized mixed-effects model with a logit link function to model EIS eDNA detectability from known-positive locations as in Samuels et al. (2025). We conducted model selection in two stages; first, random effect then fixed effect structure, as recommended by Zuur et al. (2011). We separately considered site and sample ID as random intercepts, with a fixed-effects structure that contained all possible covariates. Site refers to CNF, FSE, FSW, or ABRP (the four primary sampling areas), and sample ID gives locations that were resampled the same sample ID number (same known-positive location for specific repeat samples). We considered eight possible fixed effect model structures. All models included time since snake presence (soil collection time). We also considered sampling location (burrow vs non-burrow), mean temperature, and precipitation as potential fixed effects. We used the Akaike Information Criterion corrected for sample size (AICc) and Likelihood Ratio Tests (LRT) to test the impact of random effects and main effects (Burnham and Anderson, 2004). Models were fit with the *lme4* package (Bates et al., 2015) in R 4.3.1 (R Core Development Team, 2023) using the *glmer* function.

2.3.2. Occupancy modeling

We built multi-method occupancy models using camera trap and eDNA sample data from both Ft. Stewart sites. For these analyses, we only used eDNA samples that had been collected at paired camera trapping sites.

To incorporate these two data types into an occupancy model, we needed to discretize continuous camera sampling and time-integrative eDNA sampling into comparable timescales. We know that eDNA detection rates are low after 10 days post snake occurrence based on modeling of EIS eDNA persistence in Samuels et al., (2025) and results of this study (see *DNA* Persistence in Results). Based on this understanding, we considered each eDNA sampling session to represent an independent effort (minimum time between visits = 22 days), and we compared eDNA sampling results with camera trapping data from the prior 14 days. For both eDNA and camera trapping we had two different sampling location types: burrows or drift fences. Camera trapping and eDNA sampling occurred at shared local scale sampling locations (i.e., shared burrows and drift fences) within each larger site (i.e., FSE, FSW, CNF, ABRP). We modeled detection using a multi-scale, multi-method model which accounts for non-independence between sampling methods at shared, local scale sampling locations (Nichols et al., 2008). We fit these models using the *R* (R Core Team, 2023) package *RPresence* (MacKenzie and Hines, 2023), which incorporates code from *Presence* and *GENPRES* (Bailey et al., 2007) with model formulation and evaluation based on tutorials in R package *occupancyTuts* (Donovan et al., 2023). We used the Nichols et al. (2008) multi-scale model where we estimated method-specific detection parameters (p_{eDNA} and p_{camera}) and sampling location type availability parameters (θ_{burrow} and θ_{fence}). We fixed occupancy (Ψ) to 1.0, because snakes were known to occur across all four primary sampling locations. We compared three covariate structures that included variations of location of sample (drift fence and burrow) and method (eDNA and camera). We used AICc and AICc weights to compare models and a modified MacKenzie and Bailey (2004) goodness-of-fit test that used Fisher tests to account for small sample size.

2.3.3. Rate of detection

We calculated the rate of detection per site for a full survey season for each general method: (1) eDNA, (2) camera traps, including drift fences, and (3) VES. We first calculated rate of detection for a single survey, camera trap day, or eDNA sample, then adjusted values for average efforts for a single site across a single season. For eDNA, we estimated the rate of detection by dividing the number of positive eDNA samples by the total number of eDNA samples. We excluded known-positive samples and samples from burrows at Ft. Stewart that were repeatedly sampled, resulting in a total of 126 samples across sites (i.e., random samples with no indication of snake presence). We calculated the rate of detection of an average full-season, full-site eDNA sampling effort, assuming 20 samples per survey, and 3 total surveys. For camera traps, we estimated the rate of detection by dividing the number of total days across all cameras that EIS was detected by the total number of cameras multiplied by the average number of days in the survey season (120). We used our per day detection rate to calculate an average per season, per site detection rate for a single camera, using the 120-day average number of survey season days. For visual encounter surveys, we estimated detection rate by dividing the number of snakes found during a survey by the number of burrows searched in each survey. We calculated the average rate of detection per season by using an average of 90 burrows per survey, with three surveys per season.

2.3.4. Cost-benefit analysis

We considered costs associated with six different potential sampling approaches: (1) eDNA sampling at burrows, (2) eDNA sampling at drift fences, (3) camera trapping at burrows, (4) camera trapping at drift fences, (5) VES, and (6) VES *with* eDNA sampling. Building up cost structures for each sampling design means that we must estimate the costs associated with drift fence construction, operation of a camera trap for one season, collection and analysis of eDNA samples, VES, and travel to field sites. Cost estimates are difficult to make precisely due to situation-specific scenarios. For cost components with high variation (e.g., travel, photo processing time) we determined both 'low' and 'high' potential costs. Then for each sampling method we used all 'low' or 'high' estimates to build reasonable bounds around possible total costs and report the mean of these (Table 1).

In these systems, drift fences constructed from silt fencing are rapidly damaged, and so typically will require new construction or significant repair annually (Stegenga, *unpublished data*). Because of this, we considered drift fence construction to be a *per annum* expense. Based on fence construction for this study at the Fort Stewart sites, we estimate that the cost of fence construction (materials, travel, labor) is on average $538.75 per fence (low – high estimate = $393.50 – $684.00).

Camera trapping costs include camera purchase, camera equipment (SD cards and batteries), camera setup, maintenance, and removal (five travel events per season), and photo processing. Camera purchasing is a long-term capital expense. To account for this, we annualized camera costs by depreciating the value of the camera over the course of an eight-year average lifetime, with some budget for repair costs (Tavis Forrester, *personal communication*). For travel time, we assumed an average travel distance of 62.5 miles (based on average travel distance for annual EIS monitoring by The Orianne Society; Chandler, unpublished data), per mile cost using the standard U.S. government rates (67 cents a mile in 2024), and a field technician salary rate of $15.00 USD per hour (as in this study). For image processing, we assumed an average of 132,000 images per season for burrow cameras (range = 120,000 –144,000 images) and 1,800 images per season for drift fences (range = 1,200 – 2,400 images) and that a technician can screen approximately 4,300 images/hour with a salary rate of $15.00 USD per hour (based on time spent processing photos for this project).

Visual encounter survey costs included equipment, travel, and labor for one ('low' cost estimate) or two ('high' cost estimate) observers. Travel was calculated the same as for camera costs.

Environmental DNA field sampling costs were calculated similarly to VES costs, which included field time, travel time, and sample processing. Field time is shorter for eDNA sampling compared to visual surveys, but travel time remains the same. Cost of eDNA surveys were calculated by adding the average cost of field time to the number of samples multiplied by $95 to account for sample processing cost, including shipping. We assumed an average of 20 eDNA samples could be collected per visit. We used this cost per sample ($95) based on current rates for service laboratory analysis at the NGC, which are comparable to other federal, academic, and commercial laboratory rates. Environmental DNA laboratories require substantial capital investment and maintenance costs (e.g., equipment depreciation, laboratory staff salaries), which the per sample service price is designed to incorporate.

Table 1
Average costs for eDNA sampling, burrow cameras, drift fences and visual encounter surveys for EIS.

Type	Unit	Cost range	Average Cost
eDNA	1 eDNA survey (20 samples and travel)	$1,993.50 – $2,124	$2,058.75
Camera burrow	1 camera for 1 season, depreciated over 10 years	$1616.31 – $2373.98	$1995.15
Drift fence	1 fence for 1 season, depreciated over 10 years	$650.19 – $1265.87	$958.03
Visual encounter survey	1 survey (10–300 burrows)	$180.50 – $447	$313.75

We then calculated the level of sampling effort (cameras, samples, surveys) to reach a 95% probability of detection of EIS based on occupancy model parameter estimates from this study.

For an eDNA sample or camera trap, the probability of detection for a single sampling event is the probability that EIS is available for detection at the burrow or drift fence (θ) multiplied by the probability of detection (p). The probability of at least one detection across n sampling events is:

$$1 - (1 - \theta \times p)^n \quad (1)$$

In our occupancy models, we estimated p for cameras with a 14-day sampling window. To generalize our camera data, we calculated daily p (p_{daily}) with the understanding that p over a 14-day window ($p_{biweekly}$) is equivalent to the probability that at least one of 14 single-day sampling events results in a detection:

$$1 - \left(1 - p_{daily}\right)^{14} = p_{biweekly} \quad (2)$$

We then related daily p to season-long probability of detection, based on an average survey season length of 120 days (i.e., 120 sampling events for n in Eq. (1). Note that θ and p estimates differ depending on location (drift fence or burrow) and method (eDNA or camera).

For visual encounter surveys, we estimated detection rate by dividing the number of snakes found during a survey by the number of burrows searched during each survey for only Ft. Stewart sites, so it corresponded to occupancy parameters (mean = 0.014; range = 0.006 – 0.023 across 6 surveys). Detection probability is:

$$1 - (1 - p_{search})^n \quad (3)$$

Where n is the number of burrows and p_{search} was equal to 0.014. Sites annually monitored by The Orianne Society for EIS contain a range of 10 to 300 gopher tortoise burrows (but ~ 40, ~90, and ~ 150 are most common). We used 90 burrows for all average calculations, 40 burrows for a low estimate and 150 burrows for a high estimate.

Finally, we calculated a multi-method approach, combining VES and eDNA sampling. In this scenario, we assumed that technicians first conducted a survey, then, only if no snakes were observed do technicians collect eDNA samples and submit them for laboratory analysis. In this case, travel costs are shared between eDNA and VES and eDNA sampling costs are only incurred if VES does not detect snakes at the site. The detection probability for this sampling design is the sum of (1) the probability that snakes are detected during the VES stage and (2) the probability that snakes are not detected in the surveys, but are detected with eDNA sampling:

$$p_{search} + (1 - p_{search}) \times p_{eDNA} \quad (4)$$

3. Results

3.1. Field and lab work

3.1.1. Environmental DNA

In total, we collected 291 eDNA samples and 15 negative controls across four sampling sites. There was amplification in neither the negative control samples, nor the 15 no-template PCR controls, confirming that no field or lab contamination occurred. Standard curve efficiency ranged from 86–98% (mean = 90%, $r^2 > 0.98$). We had a total of 63 (21.6%) positive detections of EIS eDNA. Out of the known-positive samples (a snake was known or inferred to have occurred within three days of sample collection) 60/98 (61.2%) amplified. Across all four sites, 3/59 (5.1%) of drift fence samples amplified and 19/160 (11.9%) of burrow samples amplified.

We manually processed 2,230,345 photos from 72 cameras. Of these,

37 cameras resulted in at least one EIS detection over the course of field work (between late November 2023 and early March 2024; Fig. 1).

Visual encounter surveys resulted in 20 EIS detections across 12 searches, with 10/12 (83.3%) surveys resulting in at least one detection. Temperatures across all sites ranged from 4.51 °C – 25.50 °C and daily precipitation across all sites ranged from 0 – 0.762 mm daily (Supplemental Information Table S1).

3.2. DNA persistence

We collected 98 soil samples from known-positive EIS locations detected in the field. For our analysis of eDNA persistence, we also retroactively incorporated an additional 22 samples where we could confirm presence of EIS via camera trap within 14 days prior to soil sampling (total n = 120). This resulted in time from EIS occurrence to sample collection ranging from 0 (same day) to 11 days (median = 1 day; Fig. 2). Across these 120 samples, eDNA concentration ranged from 0 to 70 copies/reaction (median for amplified samples = 1 copy/reaction).

For random intercept, we included sample ID but not site. The LRT test found the inclusion of site was non-significant ($p = 1.000$), however the inclusion of sample ID was significant ($p = 0.006$), and the random effect variance of sample ID was 3.436. The model with sample ID also had a lower AICc than the model with site or without a random effect structure (ΔAICc = 7.508 and 5.291, respectively). Mean temperature and mean precipitation were non-significant, and did not improve model fit either individually or when both were added so they were excluded from the model. Sample location (non-burrow vs burrow) was included in the model; inclusion improved model fit (ΔAICc = 1.430). Our final model was amplification ~ soil collection time + sample location + (1|sample_id) and had moderately good fit (area under the curve [AUC] = 0.723; Table 2).

Likelihood of EIS eDNA detection was 80% on day one at a burrow and 50% when sampled at a drift fence. By day 7, detection was negligible (<5%) for drift fence sampling and by day 10 for burrow sampling (Fig. 3). Detection was > 50% for 3.5 days after snake presence for burrow sampling. As compared to the experimental eDNA decay curve for EIS (Samuels et al., 2025), detectability decreased more rapidly with time in the field samples than for captive enclosures (Fig. 3).

3.3. Occupancy modeling

In our dataset we included 22 sampling locations across FSE, FSW and CNF, each of which were sampled three times. Out of 66 eDNA samples, seven amplified (10.6%), and out of 66 2-week camera periods, 9 sampling periods detected an EIS (13.6%). These rates of detection correspond to constant camera operation across 2-week periods and singular environmental DNA samples that integrate signal across up to six days and possibly up to 10 days (see DNA Persistence Results). Therefore, these units of detection are not directly comparable and are instead used for a standardized cost analysis.

Our top model included sampling location (drift fence or burrow) as

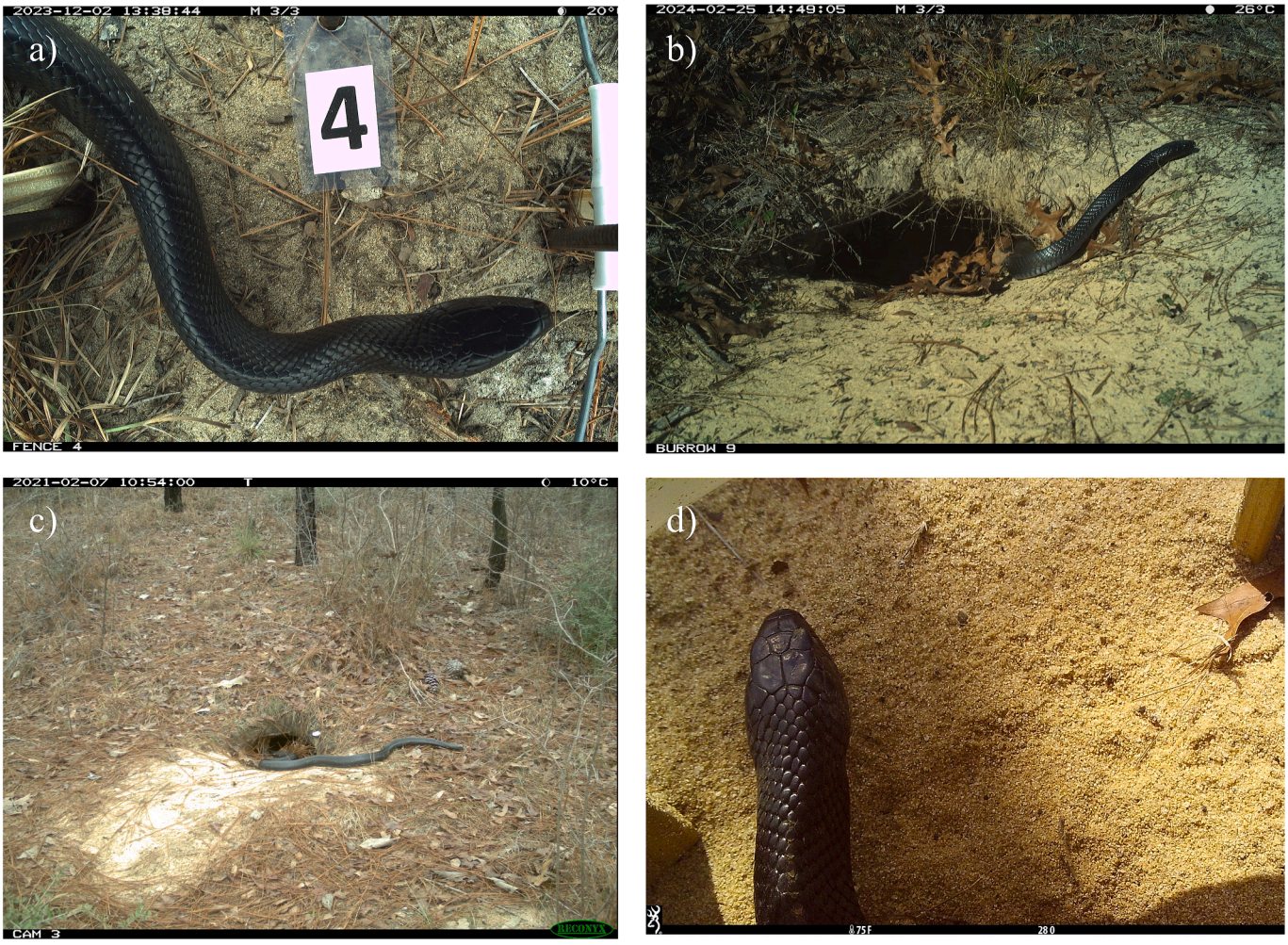

Fig. 1. Eastern indigo snakes caught via camera trap a) at a drift fence at Fort Stewart East, b) emerging from a burrow at Fort Stewart West, c) emerging from a burrow at Conecuh National Forest, and d) at a drift fence at Apalachicola Bluffs and Ravines Preserve.

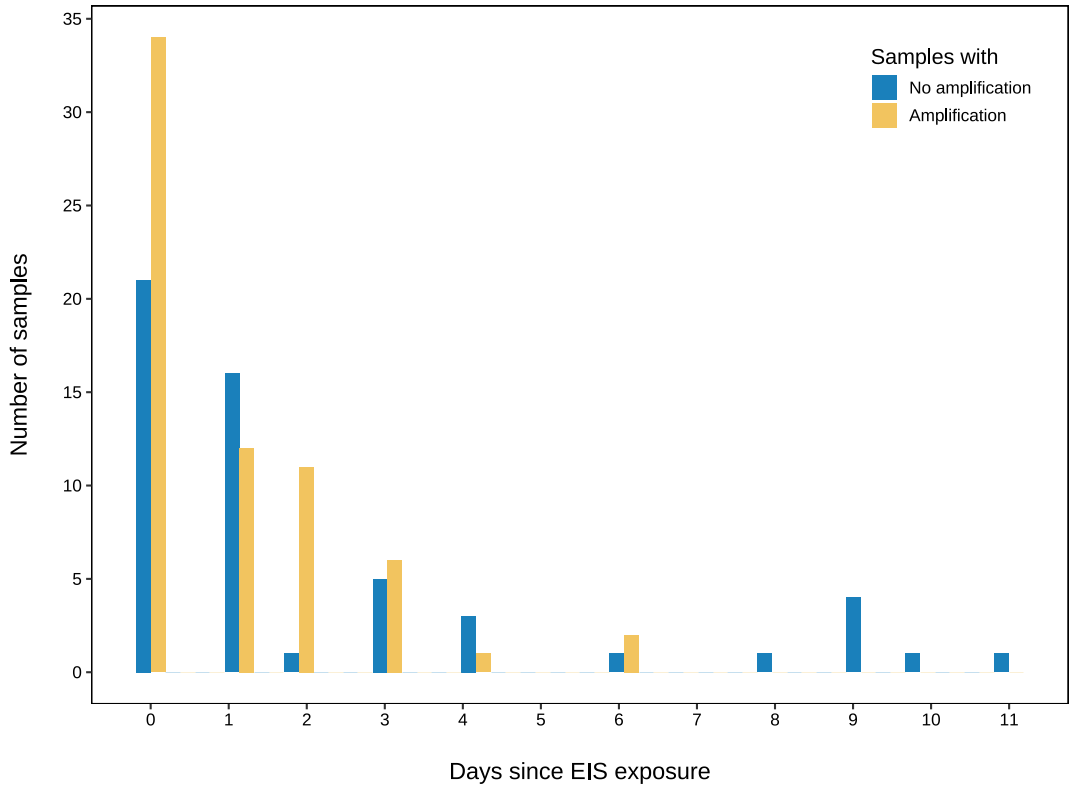

Fig. 2. Number of known-positive eastern indigo snake eDNA samples that amplified (yellow) and did not amplify (blue) by day they were collected. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Fixed effect estimates from models predicting EIS DNA amplification as a function of soil collection time and location of sample collection.

Parameter	Estimate	95 % CI	p-value
Intercept	1.414	0.312 – 12.937	0.026
Soil collection time	−0.017	−0.060 – −0.006	0.025
Sampling location	−1.397	−14.871 – −0.084	0.086

Note: The 95 % CI around each estimate were determined from 500 parametric bootstraps.

a covariate for availability (θ) and method (camera or eDNA) as the covariate for detection (p; Table 3) and showed good model fit (two-tailed $p = 0.24$). Further, all the parameter estimates were statistically significant. Camera traps had a higher predicted probability of detection per two-week period than eDNA sampling, and the burrow had higher predicted availability than drift fences (Fig. 4).

The sites we sampled in this study are higher quality sites with presumably higher abundance of EIS than is often observed in surveys of unknown habitats in the northern portion of the species' range. Our results are based on the local availability of EIS (θ) estimated from these relatively high-quality sites. Further, although we excluded known-occurrence samples from this analysis, randomized selection of burrows was not done formally and field sampling may have been biased towards habitats where field technicians expected to find snakes, which could upward bias our estimate of θ (see Discussion). To consider what overall detection probability might look like at low abundance, low burrow occupancy locations, we also calculated probability of detection across lower θ values typical of this landscape (Fig. 5).

3.4. Rate of detection

We compared the rate of detection for a full survey season at a single site of each of three methods: (1) eDNA, (2) camera traps, including drift fences, and (3) VES. Fort Stewart East had generally the highest rates of detection across methods and ABRP had the lowest (Fig. 6). The rate of detection values were similar to the estimated values from the occupancy models (Fig. 6). Fort Stewart West did not have any eDNA samples amplify that were included in the probability of detection analysis.

3.5. Cost-benefit analysis

To achieve 95% detection probability, burrow camera trapping has the highest associated cost. As a sole detection method, VES are the lowest cost method (Fig. 7). However, the combination of visual encounter surveys and conditional eDNA sampling has the overall lowest cost (Fig. 7).

4. Discussion

We found that eDNA and camera trapping, both at burrows and at drift fences, could detect wild EIS at sites with varying environmental conditions. As implemented in this study, both of these methods were expensive if used as an alternative to active searches, which are the primary existing survey approach for this species. However, in our modeling, we found that an even more cost-effective sampling design may be to use eDNA sampling and active searches in conjunction. If surveyors only collect eDNA samples after an active search has failed to reveal snakes, the expected cost for both eDNA sampling and repeated visits for additional searches can be reduced. This is because, consistent with previous experimental studies (Samuels et al., 2025), we found that eDNA was detectable for up to six days after snake occurrence, increasing the temporal window for detection relative to active searches alone. Ultimately, careful consideration is needed to identify survey methods that work towards meeting project goals for both short- and long-term monitoring programs.

Our modeling revealed that detection probability of EIS in eDNA samples collected at burrows was 80% when collected the same day as

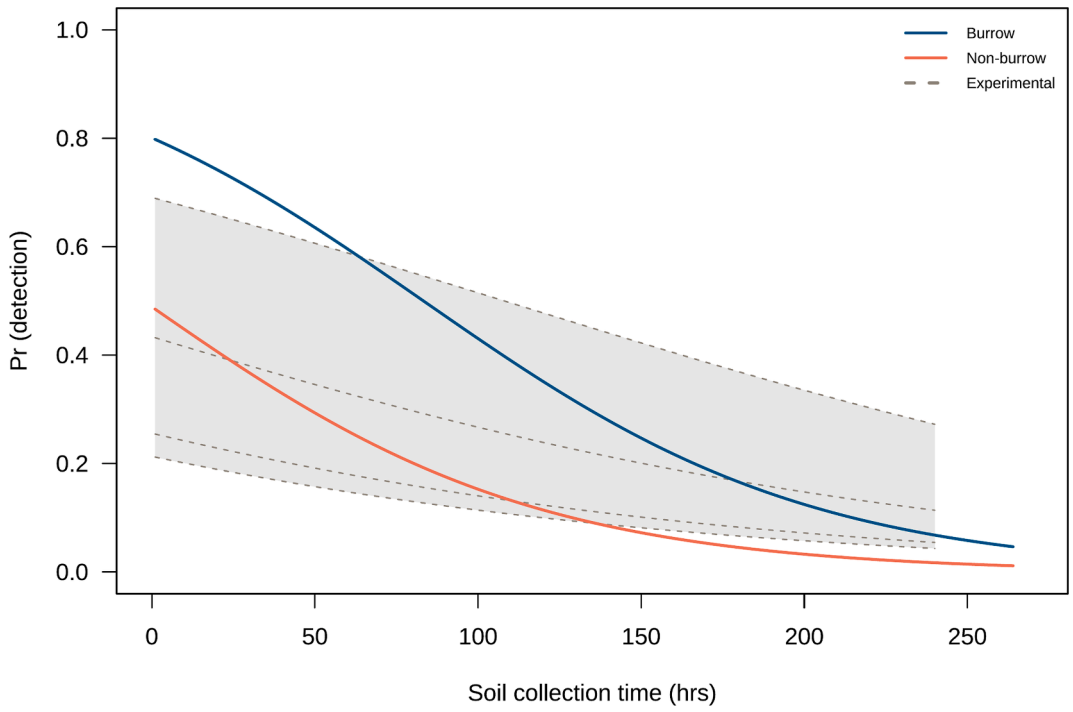

Fig. 3. Modeled probability of eastern indigo snake DNA detection as a function of soil collection time (hours) and sampling location. The blue and orange solid lines represent the detection probability of burrow and non-burrow, respectively. The gray shading indicates the range of eastern indigo snake DNA detection from experimental trials (Samuels et al., 2025), with the dotted experimental lines representing from top to bottom, 2 h of exposure of snake to substrate, 1 h, 15 min and 100 s. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Candidate models with covariates on both theta and p. ΔAICc is the difference in AICc value to the top model, *K* is the number of parameters estimated, AIC weight is the strength of the model given the other candidate models and model likelihood is the ratio of each model AIC weight over the model for the top ranked model.

Model	AICc	ΔAICc	AIC weight	Model likelihood	*K*
Ψ (1) θ (sample location) p (method)	94.82	0.00	0.83	1.00	5
Ψ (1) θ (1) p (method*sample location)	98.21	3.39	0.15	0.18	6
Ψ (1) θ (sample location) p (method* sample location)	103.08	8.26	0.01	0.02	7

snake presence could be confirmed and 27% after six days. The detection probabilities were much lower at our non-burrow sampling locations (drift fences and opportunistic sampling outside of burrows), with probabilities being 48% (same day) and 8% (6 days), respectively. This difference between microhabitats is consistent with literature showing that eDNA persistence tends to decline with UV exposure (e.g., Kessler et al., 2020; Pilliod et al., 2014) and also may reflect greater snake residence time in burrow habitats. However, our model predicts a more rapid decline in eDNA detectability over time than predicted from our previous experimental study looking at persistence of EIS eDNA in sand-filled enclosures (Samuels et al., 2025). These differences could be due to different experimental conditions. Our previous experiment was conducted in enclosures filled with sterilized sand, protected from local fauna. The substrate at the sites we sampled was a sandy soil, which has a higher organic carbon content than the sand used in our experimental study. Higher organic carbon content is associated with increased microbial activity and humic substances, which in turn increase DNA degradation and qPCR inhibition (Sirois and Buckley, 2019; Tsai and Olson, 1992). Along with substrate differences, it is important to note that we have a limited number of samples from our field study at longer times since exposure, reducing confidence in model estimates of detectability over many days (day 0–4: 107 samples; day 5–11: 10 samples). We used somewhat different extraction techniques between studies. To check that non-detections in samples from longer times after exposure was not due to differing extraction techniques, we re-extracted and analyzed all samples after day six using the highest yield method from Samuels et al. (2025). Even with the alternative, higher-yielding extraction protocol (the 'filter' method), none of the reprocessed samples amplified.

We conducted occupancy modeling using our results from eDNA sampling and camera trapping at both burrows and drift fences. Although model fit was adequate, due to the low number of sites and samples, our occupancy model parameters were estimated with a high level of uncertainty. This is a critical consideration when interpreting estimated detection probabilities from this study. Similarly, it is important to note that the sites we chose for the study were high-quality sites, with known presence of EIS and at (relatively) high densities. Therefore, it is likely our model estimates of overall detectability were greater than they would be at lower density, uncertain occupancy status sites where some EIS survey efforts are likely to occur. Additionally, the camera traps at burrows and drift fences were situated in higher use areas at FSE and FSW, possibly inflating occupancy parameter estimates even for the Fort Stewart sites in general. With those caveats in mind, we were able to use our occupancy model estimates and our rate of detection estimates for VES to calculate the cost for a 95% detection probability of EIS using each method. Among the three methods of monitoring EIS, VES is the most cost-effective stand-alone method. However, if eDNA sampling is deployed to augment VES, with samples being taken and processed only if no snake is found via VES, then the combination of eDNA and VES is overwhelmingly the most cost-effective method because it requires only a single site visit. It is worth noting that both eDNA and VES detection using this method relies on snakes being present and active at the site, and survey results could fail to detect snakes

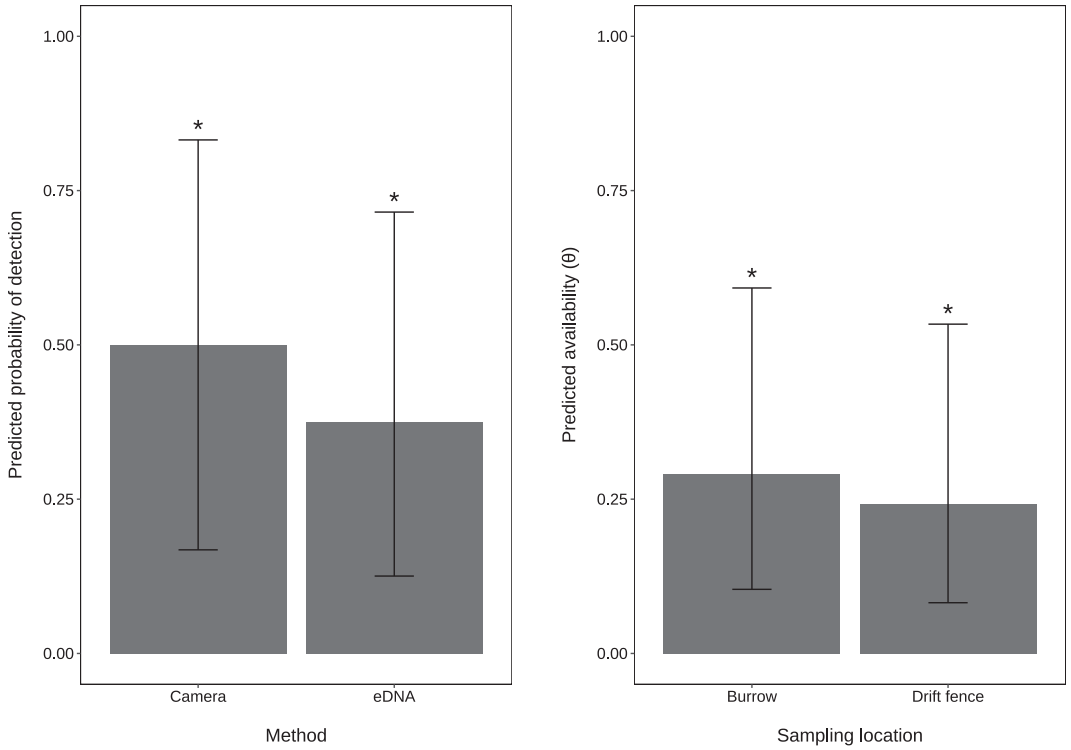

Fig. 4. Occupancy model parameter estimates for *p* (left) and θ (right) for EIS for camera and eDNA detection methods and burrow and drift fence sampling locations. Stars indicate if the parameter estimate was significant. Theta (θ) values indicate the probability that a snake visited any individual burrow within the last two weeks.

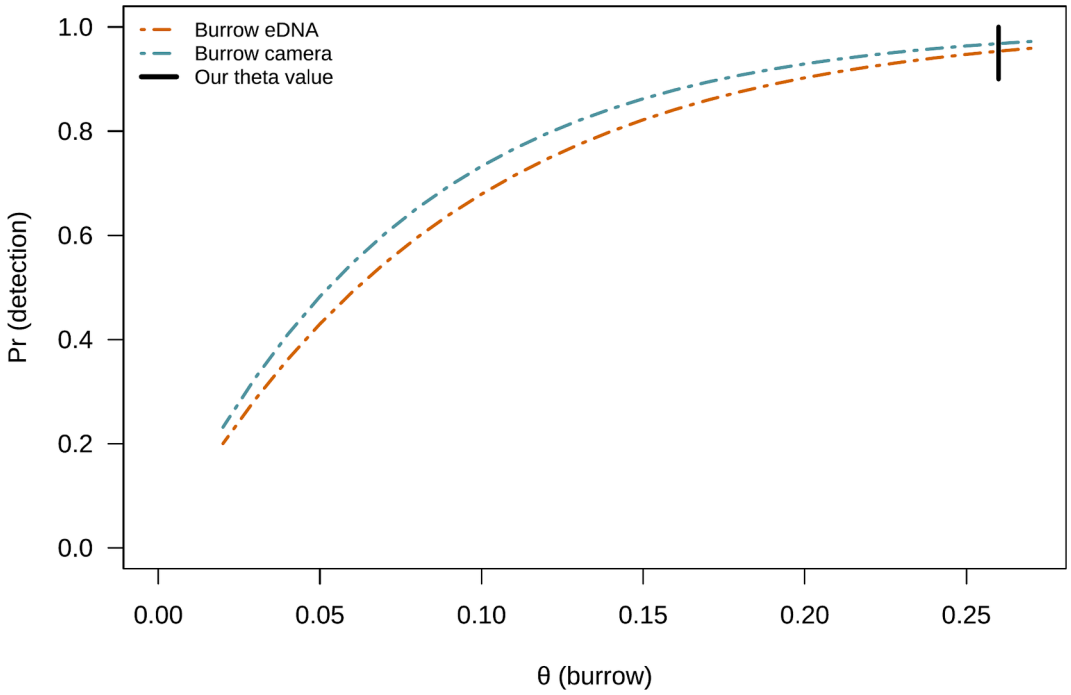

Fig. 5. Theoretical curve of detection probabilities of eastern indigo snakes as a function of θ (local occupancy or availability) when sampling gopher tortoise burrows. The orange and blue dotted lines indicate the curve for eDNA sampling and camera, respectively. The dark black line indicates the θ burrow value from our occupancy model, estimated at relatively high abundance, high occupancy study locations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

when climate conditions are unfavorable for EIS activity around tortoise burrows. Furthermore, repeat VES surveys offer additional benefits that passive survey approaches do not (e.g., marking individuals and disease sampling). Although camera traps have higher rates of detection than eDNA, the high costs associated with installation, maintenance, and photo processing make them a comparable method to eDNA when

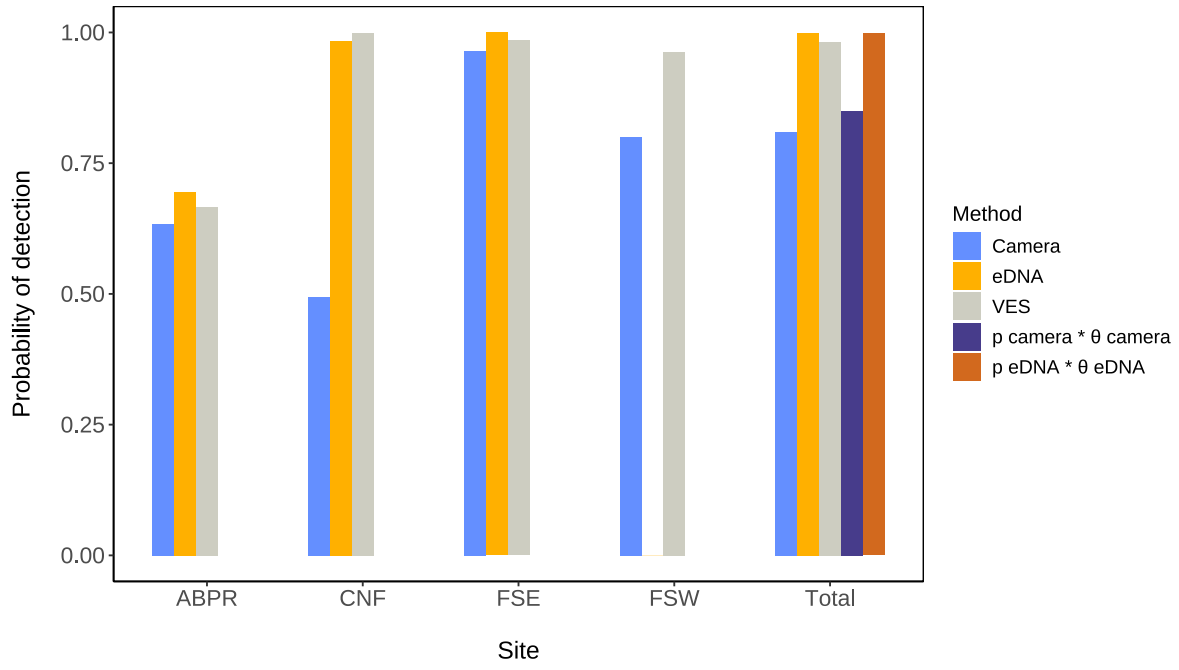

Fig. 6. Comparison of the detection probability for camera trapping (blue), eDNA sampling (yellow), and visual encounter surveys (VES; grey) across all four sites (ABPR = Apalachicola Bluffs Prairie Reserve, CNF = Conecuh National Forest, FSE = Fort Stewart East, FSW = Fort Stewart West, Total = all sites combined) for a full survey season, parameterized using site-specific per unit effort detection rates. This assumes that cameras are run continuously for a 120-day season, that 20 eDNA samples are taken per survey for three surveys and three visual encounter surveys are completed. Total rates of detection (all sites combined) are also compared to occupancy modeling parameters for cameras (purple) and eDNA (dark orange) sampling (burrow availability and per-sample detection probability; θ * p). Fort Stewart West did not have any eDNA samples amplify that were included in the probability of detection analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

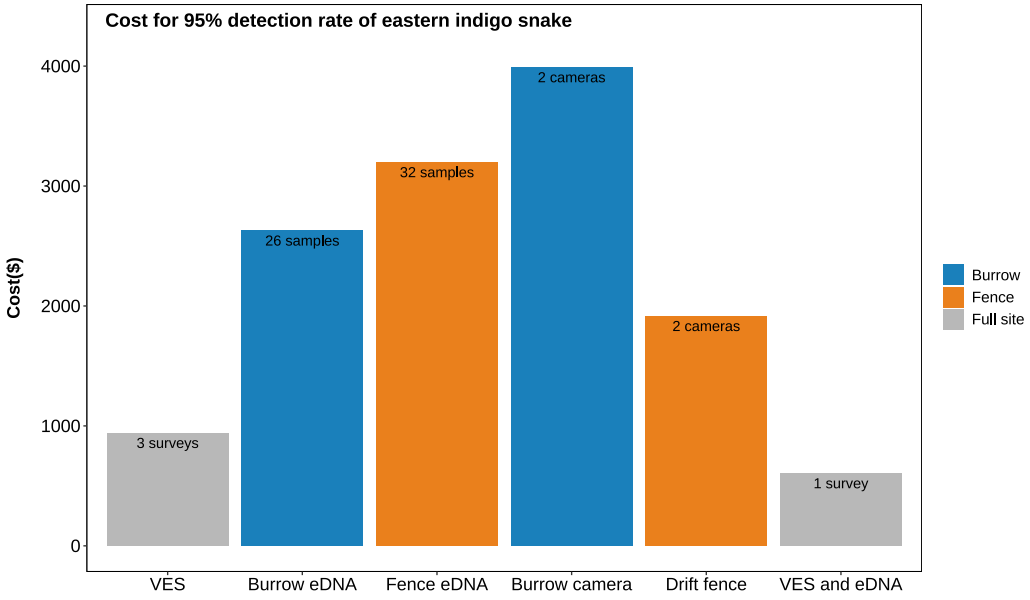

Fig. 7. The cost of detection methods (VES = visual surveys, eDNA sampling and cameras at burrows [blue] and drift fences [orange]) to reach a 95% detection probability of EIS using occupancy parameter estimates and visual encounter survey snake detection rates. The black text in the bar graphs indicate how many surveys, samples, or cameras are required to reach the 95% detection probability. Final column *VES and eDNA* indicates an adaptive, multi-method design: One survey of the search and eDNA multi-method approach indicates one visual search with 20 eDNA samples taken if EIS is not detected via the visual search. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

accounting for total project costs. Camera traps installed on burrows were the most expensive monitoring tool due to the high volume of photographs and the associated personnel time analyzing images. This was primarily due to these cameras being set to record time-lapse photos in addition to motion capture photos. It may be possible to decrease the volume of photographs (and the associated costs) by adjusting the positioning of cameras, only taking motion capture photos, and using image processing pipelines to identify photographs with snakes (Binta Islam et al., 2023). With current technology, training AI to detect wildlife in camera images can require large and diverse pre-processed

datasets to train algorithms (which were not available for our project), which would be difficult to acquire given the relative rarity of EIS and is particularly challenging for reptiles due to smaller body sizes and their ectothermy (Welbourne et al., 2017; Vélez et al., 2023). Artificial intelligence models also may have lower accuracy than humans, especially for small objects and time lapse images, so the use of image processing pipelines may be limited until further technological advances (Leorna and Brinkman, 2022).

Previous studies comparing eDNA sampling and camera trapping have shown that the optimal approaches can vary across species (e.g., Lyet et al., 2021, Holm et al., 2023). Here, our modeling revealed that relative technology performance can depend on the microhabitat sampled. Detection probabilities were lower for both eDNA and cameras at drift fences versus gopher tortoise burrows. However, costs for burrow cameras were greater than drift fences due to the higher volume of photographs taken, although this was primarily because these cameras were set to take time-lapse photos in addition to the motion trigger. At this time, it is more cost-effective to install drift fences than camera traps on burrows. Additionally, drift fence detection probabilities could be increased by modifying drift fence design, as the current set up may lead to snakes encountering the fence but not the associated camera. Our *in situ* eDNA persistence study as well as our occupancy parameters indicate that eDNA has higher detection probabilities at burrows. For these reasons, eDNA sampling efforts should likely be focused on burrows. However, in the southern portion of the EIS range, snakes do not rely on gopher tortoise burrows for thermal refugia. In these southern areas of the range where burrow sampling is less relevant, drift fences may offer a viable approach to concentrate snake movement through focal habitats. Drift fences and cameras, due to their high initial costs, are also likely most applicable to long-term monitoring at priority sites, as opposed to landscape scale surveys for detection of new snake populations.

Where discovery of new populations is the goal, our work suggests that the addition of eDNA sampling to single-visit VES surveys may substantially increase accuracy at a lower cost than implementing repeated visits within a single year. This could be especially important when temporal constraints (e.g., impending habitat modifications) limit the availability of surveyors to visit a site over months or even years. However, we caution that single-visit surveys may not be adequate for attempting to infer absence of species (which is not analogous to lack of detection) prior to habitat alterations. In such cases, extensive burrow scoping combined with other survey methods (VES and eDNA) likely presents the best methods for inferring absence.

Cost considerations are important, multifaceted components of ecological sampling designs and conservation plans (e.g., Wätzold and Schwerdtner, 2005). Environmental DNA is one of several tools that, in conjunction with current sampling approaches, may increase cost effectiveness and detection likelihood of species. Installation of concurrent sampling approaches may initially increase costs, however, could potentially decrease overall costs due to increased rates of detection and shorter time frames required to complete thorough surveys. Multiple visits may still be required with simultaneous monitoring methods installed, but overall, multiple monitoring methods increase confidence in detection of species presence or inference of a species' absence. Monitoring tools, and the assessment of their cost and sensitivity are an important part of species detection, especially for threatened, rare, or invasive species where rapid updates of distribution can greatly increase response time.

CRediT authorship contribution statement

Leah R.N. Samuels: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Taylor Wilcox:** Writing – review & editing, Supervision, Software, Resources, Methodology, Formal analysis, Conceptualization. **Michelle Hoffman:** Writing – review & editing, Validation, Investigation. **Michele Elmore:** Writing – review & editing, Methodology, Conceptualization. **Robert Aldredge:** Writing – review & editing, Methodology, Conceptualization. **Benjamin S. Stegenga:** Writing – review & editing, Validation, Investigation. **James E. Bogan Jr.:** Writing – review & editing, Resources, Methodology, Conceptualization. **Mark A. Davis:** Writing – review & editing, Methodology, Conceptualization. **Stephanie Hertz:** Writing – review & editing, Methodology, Conceptualization. **Michael K. Schwartz:** Writing – review & editing, Methodology, Conceptualization. **Houston C. Chandler:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnc.2025.126948.

Data availability

Data is available at github repository included in the manuscript document.

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