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RESEARCH

No longer a pipe dream: monitoring a cryptic, endangered skink population (*Oligosoma otagense*) using passive eDNA detection devices

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Abstract: Using a sequential adaptive experimental design, we successfully demonstrate that a passive eDNA detection field tool, consisting of pear-baited alkathene pipes equipped with Dacron filters for eDNA capture, can effectively monitor occupancy by a cryptic Otago skink (*Oligosoma otagense*) population. Detection rates using our eDNA tool were approximately 30–50% higher than those recorded by field teams historically in the Awa Nohoaka Conservation Area. Furthermore, our findings show that the Awa Nohoaka Otago skink population likely occupies less habitat in 2024 than it did between 2005–2007, equivalent to a range retraction of 38% (95% CI: 14–70%). This decline aligns with expectations given that 50 animals were removed from the wider site for the establishment of a captive population between 2009 and 2014. Additionally, the site has been exposed to ongoing predation by unmanaged invasive predators since 2017 and was only subject to limited predator control before this time. Our findings suggest that our eDNA field technique could be useful for periodic long-term monitoring of other cryptic or sparse lizard populations, particularly those inhabiting rock outcrops due to the ease of inferring probable movement routes. While eDNA methodologies remain expensive, they offer advantages in terms of minimising observer heterogeneity, increasing detectability, and improving field team safety and field scheduling certainty. To become cost-competitive with single species visual occupancy monitoring, the 2024 costs of eDNA field tools and laboratory analyses would need to decrease by c. 25%. However, given that eDNA assays can detect thousands of species simultaneously, the technique is likely to be cost-effective for occupancy scenarios requiring simultaneous monitoring of two or more hard-to-detect species, or where non-economic factors are prioritised.

Keywords: conservation, environmental DNA, lizard, occupancy, reptile, threatened, variable detection

Introduction

Lizards in Aotearoa | New Zealand (hereafter referred to as NZ) are in a precarious ecological state, with more than 115 of 119 described and putative lizard taxa classified as nationally "At Risk" or "Threatened" (Hitchmough et al. 2021). Invasive mammalian predators and habitat modification are key drivers of lizard population declines (e.g. Reardon et al. 2012; Harris et al. 2014; Monks et al. 2024) and emerging crises, such as climate change, are predicted to compound these agents of decline (Jarvie et al. 2022). Without careful management of threats, many of NZ's lizard species are predicted to suffer further range contractions (Jarvie et al. 2022) and localised extinctions (Hoare et al. 2007).

In NZ there is a large impetus for top-down control of invasive mammalian predators, as evidenced by the Predator Free New Zealand movement (King 2023). Concurrently, a global sense of urgency in conservation has inadvertently led to the widespread rollout of initiatives without attention to whether they are supported by robust statistical or causal inference (Coetzee & Gaston 2021). This has manifested in NZ with the creation and expansion of predator trapping programmes without careful examination of whether such trapping is achieving the species protection it is tacitly assumed to provide (King 2023; Monks et al. 2024). Simultaneously, there is a push for evidence-based decision making coming from the scientific community in which wildlife managers are expected to demonstrate the effectiveness of their management via outcome monitoring (Gitzen & Millspaugh 2012; Walsh et al. 2019). To further complicate the challenges for wildlife managers, evidence-based management requires a monitoring system that is both sensitive enough to detect changes and affordable enough to implement (Gitzen & Millspaugh 2012). At the same time there is often a professional reluctance to redirect financial resources from core management activities, such as predator trapping, to species monitoring (Kapos et al. 2008).

Most wildlife managers in NZ face a quandary: they must manage and monitor a portfolio of species, including native lizards, within the confines of a small budget. Many of

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NZ's most critically threatened lizard species exhibit cryptic behaviour (Lettink & Monks 2016; Purdie 2022) and have low population densities (Hoare et al. 2007; Bell & Patterson 2008). Effective monitoring strategies such as pitfall trapping, artificial covers and retreats (e.g. Bell 2009; Lettink & Monks 2016), and photo-resight methodologies (Reardon et al. 2012) are context dependent and can be unfeasible for many of NZ's cryptic or sparsely distributed lizard species. While population trend data for many NZ lizards remains limited (Hitchmough et al. 2016), it is important to recognise that this gap is more likely driven by broader social and cultural influences on conservation funding (Towns et al. 2016) than by monitoring challenges alone.

In recent decades, a major advancement in wildlife monitoring has been the widespread adoption and normalisation of estimator approaches, which allow the direct estimation of abundance, density, and occupancy across diverse taxa (White 2005). Estimator approaches are advantageous over traditional indices (typically based on counts) because the former account for uncertainty in animal detection, thereby reducing the risk of biased estimation and erroneous conclusions (Mazerolle et al. 2007; Wiewel et al. 2007; Kellner & Swihart 2014). Occupancy methods, which do not require the identification of individuals, just evidence of their presence as a species, are likely to be useful when an understanding of colonisation and extinction is needed (e.g. Mazerolle et al. 2007; Budy et al. 2015) and when expenses associated with abundance monitoring are prohibitive (Keane et al. 2012; Noon et al. 2012; MacKenzie et al. 2018).

Occupancy is usually defined as the probability that an area is occupied by a species of interest during a specified period (MacKenzie et al. 2018). As a method it has the advantage that it can be scaled at a comparatively low cost across extensive areas, as it assesses units of habitat which are entirely user defined (Bailey et al. 2014). Although occupancy has been used in ecological studies of lizards in NZ (e.g. Roughton & Seddon 2006; Gebauer et al. 2013; Harris et al. 2014) it is seldom used for outcome monitoring. Despite their practical advantages, many wildlife managers in NZ are reluctant to adopt occupancy methods, because the resulting estimates are usually perceived as being less tangible than count indices or abundance estimates (NW, pers. obs.). Conversely, Goldstein et al. (2024) have noted that in other contexts occupancy methods are sometimes adopted under the mistaken assumption that site occupancy probability directly reflects population characteristics such as density or abundance. These contrasting perspectives reflect two sides of the same issue: (1) some practitioners misinterpret occupancy metrics as proxies for density or abundance, leading to inappropriate application; while (2) others, aware of the methodological distinction, are hesitant to use occupancy because they remain unsure of its practical value.

The Otago skink (*Oligosoma otagense*) is one of NZ's largest endemic skink species (≤ 142 mm snout-vent length; Bogisch et al. 2016) and is currently classified as Nationally Endangered (Hitchmough et al. 2021). Otago skinks are viviparous, diurnal, and strongly heliothermic (van Winkel et al. 2018; Purdie 2022). They inhabit schist rock outcrops in the Otago region where their populations have been decimated by exotic mammalian predators and habitat modification (Reardon et al. 2012). The Otago skink is believed to now occupy less than 8% of its former distribution (Whitaker & Loh 1995).

Wild Otago skinks are currently managed as two genetically distinct populations (Chapple et al. 2012): (1) a large, eastern population with a stronghold at Macraes Flat; and (2) a small, western population comprising a series of remnant subpopulations in the mountains around Lindis valley and a recently translocated population at Mokomoko Dryland Sanctuary, near Alexandra (Fig. 1). Between 2005 and 2008, a well-financed experimental management programme for Otago skinks was undertaken by the Department of Conservation (DOC) at Macraes Flat (Reardon et al. 2012) (Fig. 1). This programme demonstrated that Otago skink populations can rebound over a three-year period when they are supported by intensive predator-trapping or predator exclusion (Reardon et al. 2012). The findings of Reardon et al. (2012) were acquired using a labour-intensive photo-resight monitoring programme that yielded precise abundance estimates (Reardon et al. 2012). However, the same monitoring programme failed to obtain precise abundance estimates for an Otago skink population inhabiting the Awa Nohoaka Conservation Area in the Lindis valley (Fig. 1) (Hutcheon et al. 2011). Between 2006 and 2010, the mean abundance of Otago skinks within the study site declined from 38 individuals (95% CI: 31–62) to 22 individuals (95% CI: 19–52) (Hutcheon et al. 2011). However, low and variable resight probabilities, which were believed to be in part a symptom of the complex and precipitous topography, led to high uncertainty. As of 2023, the status of the Awa Nohoaka Otago skink population was unknown.

In recent years there have been rapid advances in environmental DNA (eDNA) research which allows the detection of species from residual traces of DNA (Beng & Corlett 2020). We hypothesised that if Otago skinks were willing to pass through pipes containing a brush-like filter (to collect eDNA) placed on and around inhabited rock outcrops, then an eDNA based occupancy approach could form the basis for population monitoring. Potentially, this technique could have utility for cryptic or sparsely distributed threatened lizard species in NZ and elsewhere. In order to explore this possibility, we investigated the validity of eDNA detection for Otago skinks under a sequential research programme beginning with (1) a proof-of-concept on captive animals, (2) a field trial with a high abundance, wild, intensely managed population,

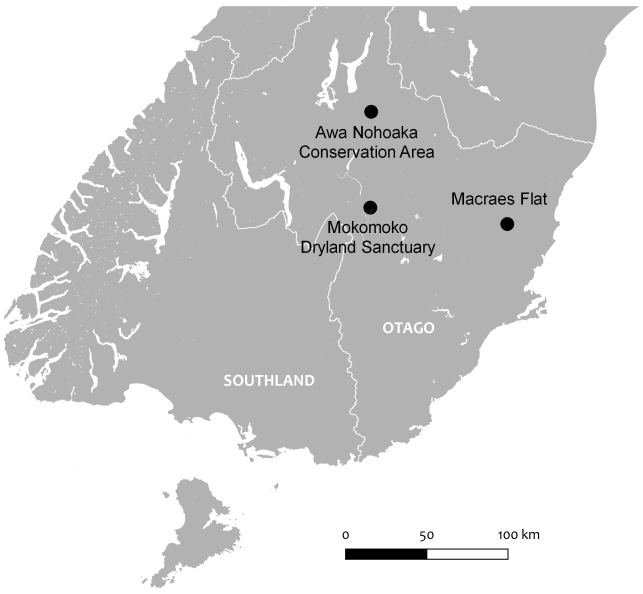

Figure 1. Field locations mentioned in this study.

(3) a comparison between baited and unbaited devices in a high abundance, wild, intensely managed population, and (4) a full-scale occupancy trial at Awa Nohoaka Conservation Area: a scenario with a sparse, unmanaged population.

Methods

In each trial, sterile eDNA pipe kits supplied by Wilderlab Ltd. were used (Fig. 2). Each pipe consisted of a 200 × 50 mm diameter alkathene pipe with a Dacron (polyethylene terephthalate) filter attached to the inside with double-sided adhesive mounting tape. These filters were designed to act like a brush and capture residual DNA whenever an animal moved through the pipe. To prevent cross-contamination, each kit also contained two sets of gloves, a pair of tweezers, sample containers, and a syringe containing a proprietary preservative (DNA/RNA shield, Zymo Research). To minimise the risk of environmental contamination, new sets of sterile gloves were worn whenever an eDNA pipe was being deployed. All samples were individually stored in the preservative and shipped to Wilderlab for processing.

Trial 1: Captive trial

To understand whether eDNA pipes could be a viable tool for occupancy monitoring, we conducted an exploratory proof-of-concept study with captive lizards at Kiwi Park Queenstown (Otago, NZ) on 8 January 2023. The purpose of the study was to identify whether sufficient Otago skink eDNA could be captured on Dacron filters to enable accurate species identification when another species was present. The study design was intentionally simple and had the sole objective of determining whether the development of a more rigorous trial was justified. We considered two scenarios: (1) a two-species scenario in which each lizard species crossed the filter once, and (2) a two-species scenario in which each lizard species crossed the filter three times. The choice of a trial involving both geckos and skinks was based on a suspicion that differences in scale morphology (i.e. granular non-overlapping scales in geckos and overlapping scales in skinks; van Winkel et al. 2018) and behaviour could lead to differences in eDNA shedding and the potential swamping of an Otago skink eDNA signal by gecko eDNA in the wild. Each scenario was replicated three times at room temperature. The lizard species used were an Otago skink and a gecko (either a Duvaucel's gecko, *Hoplodactylus duvaucelii*; or a forest gecko, *Mokopirirakau granulatus*). To minimise stress on any one lizard, multiple individuals were used for the captive trial. As few captive geckos were available, we used a mix of two different gecko species.

Our experimental configuration consisted of two eight-litre plastic containers (each 275 × 235 × 125 mm) that were connected by an eDNA pipe. One container was empty, while the other contained a selection of live insects (e.g. yellow mealworms, *Tenebrio molitor*; woodlice, *Porcellio scaber*; migratory locusts, *Locusta migratoria*; black soldierfly larvae, *Hermetia illucens*) and canned pear to serve as attractants. For each trial, we gently placed a lizard into the empty container and allowed it to move freely into the other container via the eDNA pipe. If after five minutes the focal animal had not moved, it was coaxed to move with a feather. A new eDNA pipe was used for each replicate. Our threshold for further investigation was a minimum of four positive detections of Otago skinks from six trials (based on an assumption that detection did not have to be 100% accurate but would have to be > 50% accurate to be viable in the field).

Figure 2. The eDNA pipe in the field.

Trial 2: In situ detection trial

To understand whether eDNA pipes could be used to monitor a wild Otago skink population, we installed 36 unbaited eDNA pipes at Mokomoko Dryland Sanctuary on 5 April 2023 for a three-week period. We deployed the pipes in Otago skink habitat, characterised by large schist outcrops. Otago skinks were known to inhabit four of the six rock outcrops surveyed, as they had been observed in these locations previously (Grant Norbury, Central Otago Ecological Trust, pers. comm.). We deployed two sets of three eDNA pipes on each outcrop. We positioned the pipes in areas that we suspected would be frequented by Otago skinks (e.g. rock crevices, slabs, vegetative cover, and large crenulations). Where necessary, we secured the eDNA pipes with small rocks so they would not be dislodged by wind. The three pipes that made up each set were placed within 2 m of each other. Each week, one filter from each set of three pipes was selected for removal (via a random number table). In total, we acquired 12 samples for each deployment period (i.e. week 1, 2, and 3).

To determine the optimal pipe deployment period, we analysed possible differences in naïve detection probability (i.e. without explicitly incorporating the influence of occupancy probability) between the three different deployment periods using logistic regression. We compared two models using Akaike's Information Criterion (with a small sample correction; AICc) (sensu Burnham & Anderson 2002): (1) an intercept only null model (in which the naïve detection probability was

constant), and a (2) time varying model (in which the naïve detection probability varied through time).

Trial 3: Efficacy of bait type

To identify whether baiting pipes with canned pear (sensu Whitaker 1967) enhanced Otago skink eDNA detection, we installed 24 eDNA pipes at Mokomoko Dryland Sanctuary on 21 November 2023. In this trial we deployed the pipes across the four rock outcrops that were known to be occupied by Otago skinks, with six pipes on each outcrop. We randomly selected half of the pipes ($n = 12$) to be baited with canned pear (the remaining twelve pipes did not have bait). The canned pear was picked out with sterile gloves and stored in small plastic containers for field use. In each of the baited pipes a small amount of canned pear was spooned into the centre of the Dacron filter (as per our standard protocol, gloves were replaced with new, sterile sets between pipe deployments). After two weeks, on 5 December 2023, we removed the eDNA pipes from the field and sent the filters to Wilderlab for analysis.

We analysed the data from this trial using logistic regression, in which four models were compared via AICc: (1) an intercept only (null) model in which there were no naïve detection differences between bait types, (2) a model in which there were naïve detection differences between bait types, (3) a model in which naïve detection differed only by outcrop, and (4) a model in which naïve detection differed by outcrop and bait type.

Trial 4: Awa Nohoaka Conservation Area field trial

To understand whether eDNA pipes could be used to monitor a wild Otago skink population in a complex, difficult-to-survey environment using an occupancy methodology, we conducted four monitoring sessions at Awa Nohoaka Conservation Area from 14 March–30 April 2024. The trial took place across ten discrete rock outcrops that were historically monitored by DOC. We positioned eDNA pipes in areas that we suspected would be frequented by Otago skinks and baited all pipes with canned pear (following the outcome of Trial 3). At Awa Nohoaka Conservation Area, the Otago skink habitat is mostly composed of large, schist towers with deep fractures and many interstitial spaces. Therefore, we assumed that skinks would be more difficult to detect at this location (and would thus have much lower detection probabilities) than at Mokomoko Dryland Sanctuary. To maximise Otago skink detection, and to have some within-outcrop redundancy (i.e. to reduce the likelihood of failing to detect Otago skinks when they were present) we deployed three eDNA pipes on each rock outcrop across a total of ten different rock outcrops. When combined with the four monitoring sessions, this resulted in 120 eDNA pipes being deployed over the course of the trial.

For the first three monitoring sessions, we left eDNA pipes in the field for 12 days. After this period, we removed the pipes and replaced them following the protocol of Trial 3. However, due to forecast inclement weather, we shortened the final monitoring session by one day, resulting in an 11-day monitoring duration. Due to the suspected intra-outcrop redundancy, any positive detection within a set of three eDNA pipes was considered a positive detection for the entire rock outcrop (which was considered a proxy unit of territory). In addition to analysing our eDNA survey data, we analysed our data in relation to historic occupancy records for the same rock outcrops sampled by DOC over a three-year period from 2005 to 2007. The historic DOC monitoring method involved 4–6 experienced field crew, working in teams of two, surveying each rock outcrop using binoculars for a maximum period of 10 minutes, and recording whether an Otago skink was sighted. These surveys were historically repeated 5–9 times over a period of several weeks (in November or December).

We undertook three separate modelling approaches to understand occupancy rates (ψ) in Trial 4. Firstly, we modelled the eDNA survey data with a suite of three candidate models to determine whether the eDNA pipe method was sufficiently sensitive to identify within-survey changes in detection rate (p) via model selection (including a model which expected that detection probability would decline progressively throughout the autumn months). Next, we modelled each year of the historic data and the eDNA data as independent single season analyses to determine baseline differences in detection probability between the methods (without the added complications of Markovian extinction dynamics). Finally, we analysed the historic data jointly with the eDNA data to determine the probable extinction rate (ε) in our sample of rock outcrops. Preliminary modelling indicated that extinction rates were not calculable when visual occupancy methods were treated as discrete primary periods, so we aggregated the three visual occupancy seasons (2005–2007) and modelled the potential extinction dynamic between the historic survey period and the eDNA survey via four candidate models. As all rock outcrops were occupied in 2005, the colonisation parameter (γ) was set to zero in the candidate models. We then used model averaging to estimate extinction probability from all the resultant models. The extinction probability was then converted into an annual rate to reveal changes in habitat occupancy at Awa Nohoaka Conservation Area over the intervening c. 16.4 years. All model selection undertaken in Trial 4 used AIC rather than AICc, as the latter requires an explicit sample size, which is not defined in occupancy modelling (MacKenzie et al. 2018).

Cost effectiveness

Finally, we assessed the cost effectiveness of our eDNA tool by comparing the relative costs associated with the naïve detection rate of the 2005–2007 visual occupancy surveys and the 2024 eDNA survey (on the assumption of equal transport costs and 2024 labour costs with realised field staffing; see https://osf.io/z6ubq/ for data and working). For the 2024 eDNA survey, we considered two scenarios: (1) where the detection probability was best represented by the mean, and (2) where the detection probability was that of the first session (based on the assumption that detection probability would decline progressively throughout the autumn months and therefore the detection probability of the first session would be indicative of expected detection probabilities during a typical summer field season). Historic time data were used to estimate the labour hours associated with the 2005–2007 visual occupancy surveys. To account for unrecorded surveys abandoned due to changing weather, the number of field days required by the visual occupancy method was estimated as 20% more than what was recorded. We treated the cost effectiveness of each monitoring season as total cost per percent of realised detection probability.

eDNA analysis

On receipt of the samples at the Wilderlab laboratory, each preserved Dacron filter was removed from its specimen jar using sterile tweezers and placed in the barrel of a 60 ml syringe containing an internal 100 micron nylon mesh prefilter. The

plunger was inserted and the preservative squeezed into a low-bind (Eppendorf) tube, with the internal prefilter excluding any particles > 100 µm.

DNA extraction, Next-Generation Sequencing library preparation, sequencing and bioinformatics followed methods outlined in Wilkinson et al. (2024). For DNA extraction and purification, 200 µl of each sample lysate were loaded into a Genolution GD141 cartridge and run on the Nextractor NX-48S system (Genolution, Korea) using the standard extraction settings. DNA quality/quantity analysis, adapter-fusion, indexing, and amplification were carried out in a single step Polymerase Chain Reaction (PCR: Mullis et al. 1986) on an Applied Biosystems ProFlex PCR instrument. DNA extracts were PCR-amplified in duplicate using fusion-tag mitochondrial assays for the detection of target DNAs (see Appendix S1 in Supplementary Material for primer sequences and associated taxon targets). Fusion tag primers included Illumina P5 and P7 adapter sequences, Illumina TruSeq™ sequencing primer bind site (forward primer only), unique 8 or 9 bp index sequences, and locus specific primers, respectively. All indexes differed from each other by at least 3 bp. Each PCR reaction contained 3 µl MyTaq 2× Red Mix (Bioline) with 2 mg ml^{-1} BSA (Sigma Aldrich), 0.5 µl forward primer (10 µM), 0.5 µl reverse primer (10 µM) and 1.5 µl template DNA. PCR cycling conditions included an initial denaturation step of 3 min at 95°C, followed by 38 cycles of 5 s at 95°C, 10 s at the assay-specific annealing temperature and 15 s at 72°C. At least one negative control reaction containing 1.5 µl of DNase-free water (IDT) in place of the template DNA was included with each sequencing run. Sequencing libraries were pooled, cleaned, and double-end size selected using AMPure XP magnetic beads (0.9 × and 1.2 × for lower and upper size bounds, respectively). The final pooled library concentration was determined using a Qubit 4 Fluorometer (ThermoFisher Scientific) and the concentration adjusted to 50 pM in sterile DNAse/RNAse free water (IDT). Each library was loaded onto an iSeq i1 V2 reagent cartridge with 5% Phi X and run for 200 cycles in a single direction on an Illumina iSeq 100 instrument.

The FASTQ files (text-based format for representing sequences) were separated using the "insect" package in R (Wilkinson 2018; R Core Team 2022), and the trimmed sequences were filtered to generate a table of exact amplicon sequence variants (ASVs) with the "dada2" package (Callahan et al. 2016). Amplicon sequence variants were identified to the lowest possible taxonomic rank using a global reference sequence database primarily compiled of trimmed reference sequences downloaded from GenBank (Benson et al. 2010). Any ASV matching with 100% identity and 100% coverage to at least one reference sequence was assigned at the lowest common ancestor level (i.e. assigned to genus level if matched to more than one species, or to family level if matched to more than one genus). Unmatched sequences > 50 bp in length were queried against the same reference database using the SINTAX classification algorithm (Edgar 2016) with a conservative assignment threshold of > 0.99 and taxon assignment restricted to genus level or above. Under this protocol Otago skinks (*O. otagense*) could not be differentiated from scree skinks (*O. waimatense*), a result that was not unexpected given Chapple et al. (2012) had inferred historic introgression took place between these species in the northern Otago-southern Canterbury region, nor concerning as the two species are not sympatric (Chapple et. al. 2012).

Data analysis

Data from the field trials were analysed using program R version 4.2.1 (R Core Team 2022) with additional functionality from the packages "AICcmodavg" (Mazerolle 2020), "arm" (Gelman & Su 2021), "DHARMa" (Hartig 2022), "lubridate" (Grolemund & Wickham 2011), "oddsratio" (Schratz 2017) and "tidyverse" (Wickham et al. 2019). Model diagnostics were conducted with program R for all analyses involving generalised linear models using the package "DHARMa" but no issues were encountered. The R package "RPresence" (MacKenzie & Hines 2024) was used as an external interface for running the program PRESENCE (Hines 2006). Occupancy analyses were conducted following the protocols of MacKenzie et al. (2018).

Results

Trial 1: Captive trial

eDNA from the Otago skinks was correctly detected in five of the six trials. eDNA from the Otago skinks and geckos was always correctly identified when multiple (three) passes had been conducted. Detection failure was only recorded when single passes had been conducted, which occurred in two different replicates, affecting one Otago skink and one gecko replicate. This suggests that the probability of false absences is c. 0.17 (i.e. 1/6). However, as our minimum detection threshold for further investigation was exceeded (i.e. ≥ 4 Otago skink detections in six trials) the investigation was continued.

Trial 2: In situ occupancy trial

Model selection suggested that naïve detection probabilities of Otago skinks at Mokomoko Dryland Sanctuary did not vary with deployment time (Table 1), with the null model (a time invariant model) receiving 88% of the model support. The time varying model held some support (12%) with detection peaking after two weeks of deployment (Fig. 3). Based on these findings, and operational compatibility, a deployment period of two weeks was targeted for all subsequent eDNA pipe trials.

Trial 3: Efficacy of bait trial

The top-ranked model (with 78% model weight) supported differences between bait treatments, with pear-baited pipes returning a higher detection rate than unbaited pipes (Table 2). Predicted naïve detection probabilities for unbaited pipes and baited pipes were 0.08 (95% CI: 0.01–0.41) and 0.50 (95% CI: 0.24–0.76) respectively. The odds of baited pipes detecting an Otago skink were 11 × greater than the odds for unbaited pipes (Appendix S2l) although there was substantial uncertainty (95% CI: 1.4–235.1). Based on these findings, we decided to bait the eDNA pipes in Trial 4 with canned pear.

Trial 4: Awa Nohoaka Conservation Area field trial

Although Otago skinks were not sighted by the field team during the process of deploying or retrieving the eDNA pipes, positive eDNA detections indicated that a spatially dispersed Otago skink population remained (Fig. 4). These recorded presences were notable for their spatial consistency (Fig. 4). The top-ranked occupancy model was $\sim\psi(.)p(\text{trend})$ with 68% model support (Table 3). The resultant trend from the top-ranked model suggested that detection probability was declining as autumn deepened from $p = 0.85$ (95% CI: 0.48–0.97) at the

Table 1. Model selection table: prediction of naïve Otago skink detection rates in Trial 2 using logistic regression. Models ranked by AICc (AIC with a small sample correction). Key: K = number of parameters; Δ AICc = difference between the AICc of the current model and that of the top ranked model; Model weight = the relative likelihood of the model; Log likelihood = maximised value of the log-likelihood function.

Model name	K	AICc	Δ AICc	Model weight	Log likelihood
Null model	1	51.02	0.00	0.88	−24.45
Time varying	2	54.96	3.94	0.12	−24.11

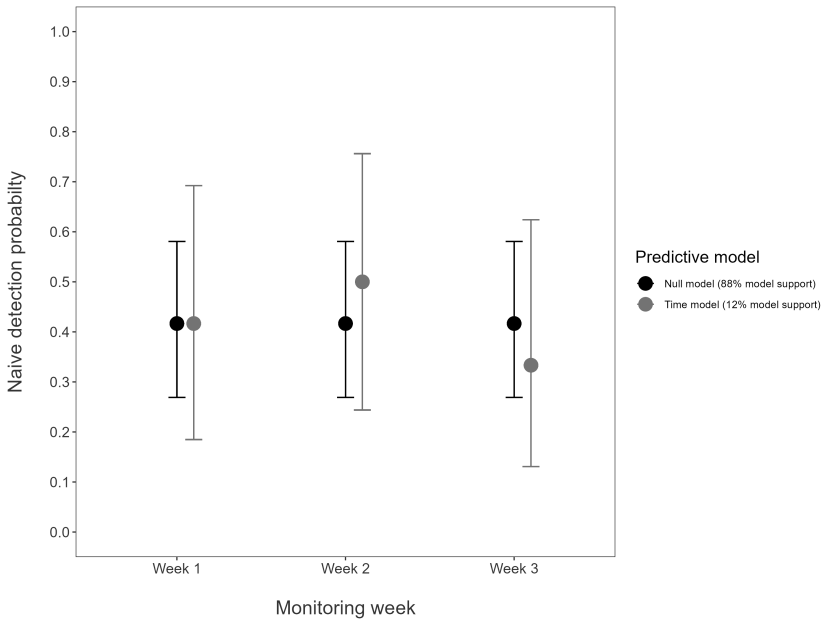

Figure 3. The variation in the estimated probability of detection for eDNA pipes in Trial 2 given the different deployment regimes.

Table 2. Model selection table: naïve detection rates and the influence of pear bait in Trial 3 using logistic regression. Models ranked by AICc (AIC with a small sample correction). Key: K = number of parameters; Δ AICc = difference between the AICc of the current model and that of the top-ranked model; Model weight = the relative likelihood of the model; Log likelihood = maximised value of the log-likelihood function. Model parameters: Null model = detection rate modelled as a simple mean; Bait = detection rates vary by bait type; Outcrop = detection rates differ across outcrops; Outcrop + bait = detection rates vary by both outcrop and bait type.

Model name	K	AICc	Δ AICc	Model weight	Log likelihood
Bait	2	28.09	0.00	0.78	−11.76
Null model	1	31.16	3.07	0.17	−14.49
Outcrop + bait	5	33.95	5.86	0.04	−10.31
Outcrop	4	36.87	8.78	0.01	−13.38

start to p = 0.27 (95% CI: 0.08–0.62) by the end of the trial (Fig. 5). The top-ranked model estimated the occupancy rate within the rock outcrops as 0.61 (95% CI: 0.30–0.85).

Differences in detection rates between the current and historic sampling seasons (on the assumption of a constant detection rate, ignoring the declining seasonal trend just identified) suggest that the eDNA methodology may be superior to visual occupancy sampling, with an expected mean detection rate of 0.56 using the eDNA occupancy method compared to 0.37–0.43 for the visual occupancy method (Fig. 6), an improvement of c. 31–52%.

Multi-season occupancy analysis (with a focus on determining the extinction rate) identified the top ranked model as ~ψ(.)γ(0)ε(.)p(method + trend) with 63% model support (Table 4). Model averaging estimated the extinction rate (ε, i.e. the probability of occupied habitat becoming unoccupied) to be 0.38 (95% CI: 0.14–0.70) which, over an interval of c. 16.4 years, is equivalent to a mean annual extinction rate of c. 2.9%.

Cost effectiveness

We assessed that the mean cost per percent of detection probability was NZ$248 for visual occupancy surveys (range: NZ$132–409) compared to NZ$491 and $324 for our eDNA method at mean (56%) and first session (85%) detection probabilities respectively. On this basis, to be cost comparable to a visual occupancy method, the cost of the eDNA consumables and analysis would need to reduce by c. 54% if the mean eDNA detection rate we recorded was generally representative of the

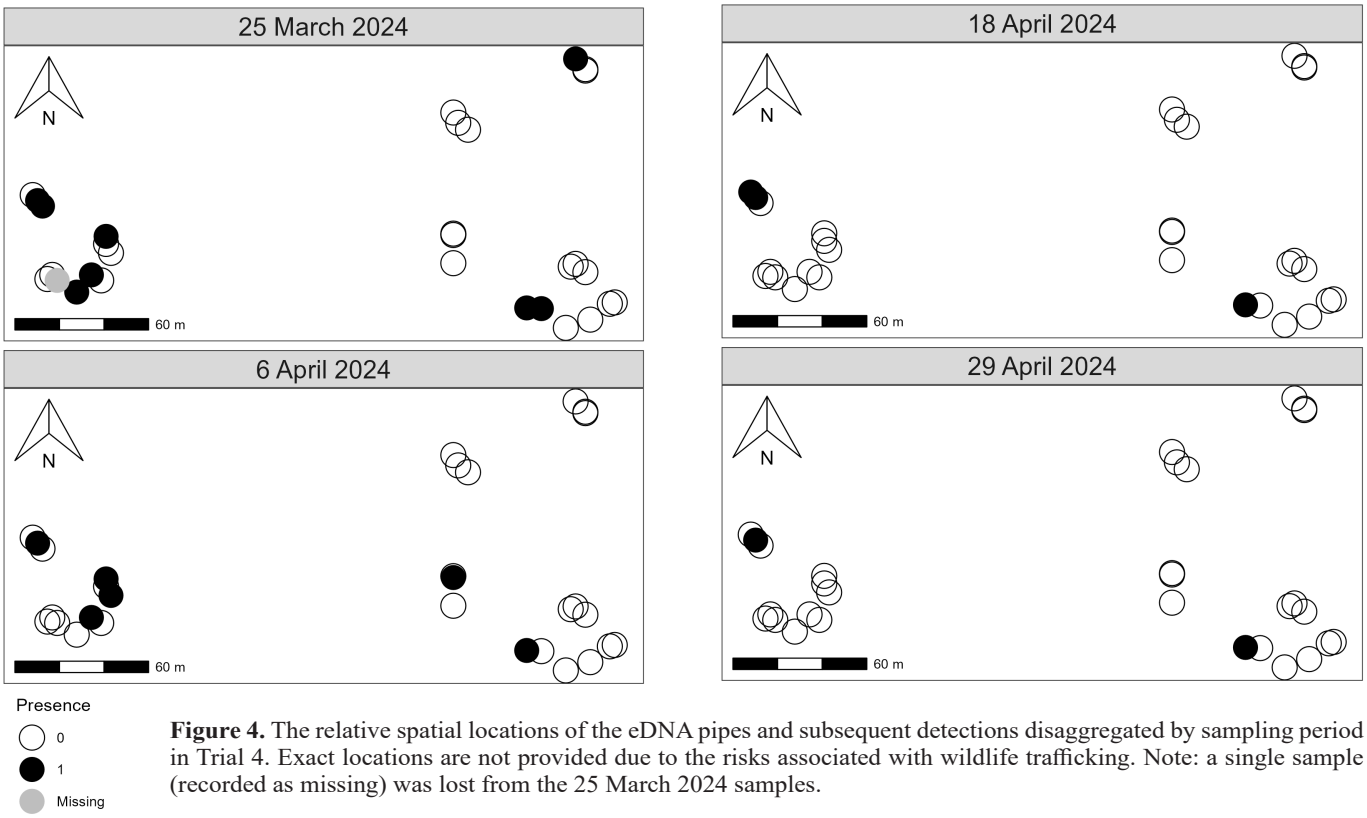

Figure 4. The relative spatial locations of the eDNA pipes and subsequent detections disaggregated by sampling period in Trial 4. Exact locations are not provided due to the risks associated with wildlife trafficking. Note: a single sample (recorded as missing) was lost from the 25 March 2024 samples.

Table 3. Model selection table: single-season occupancy analysis for the eDNA trial at Awa Nohoaka Conservation Area (Trial 4) using RPresence. Models ranked by AIC. Key: K = number of parameters; Δ AIC = difference between the AIC of the current model and that of the top-ranked model; Model weight = the relative likelihood of the model; Log likelihood = maximised value of the log-likelihood function. Model parameters: ψ(.) = constant occupancy probability, *p*(.) = constant detection probability, *p*(trend) = detection probability exhibits a linear trend with survey session, *p*(survey) = detection probability varies by survey session.

Model name	K	AIC	Δ AIC	Model weight	Log likelihood
ψ(.) *p*(trend)	3	46.59	0	0.68	−20.30
ψ(.) *p*(survey)	5	49.39	2.80	0.17	−19.69
ψ(.) *p*(.)	2	49.66	3.06	0.15	−22.83

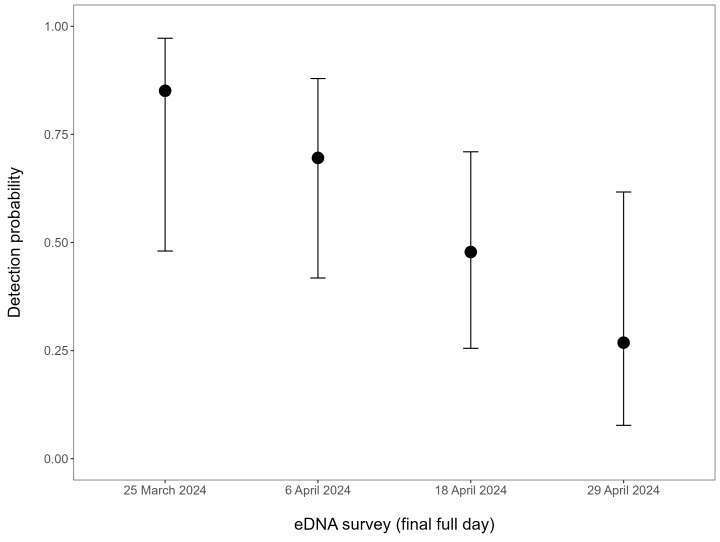

Figure 5. eDNA detection at Awa Nohoaka Conservation Area in 2024 declined with the deepening of autumn based on the top-ranked single season occupancy model ψ(.) *p*(trend). Key: error bars = 95% confidence intervals.

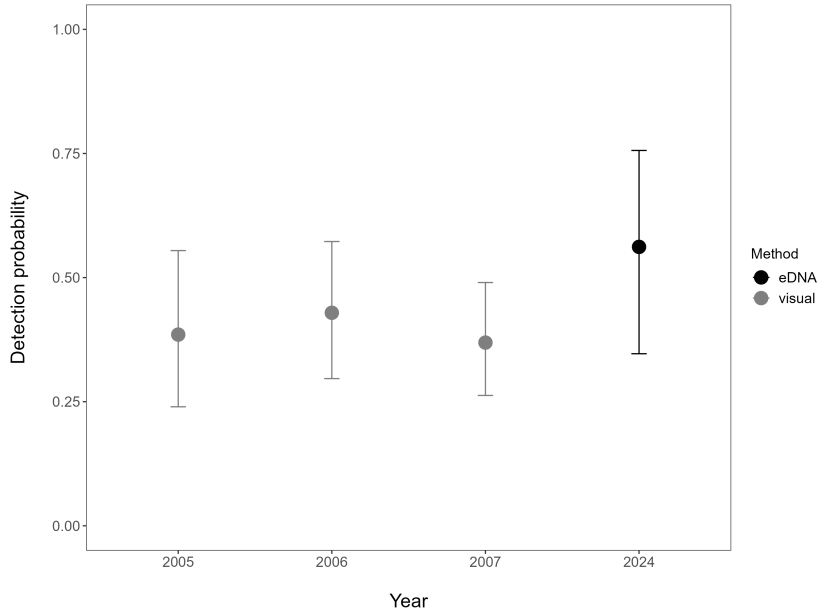

Figure 6. The variation in the naïve seasonal probability of detection at Awa Nohoaka Conservation Area given the different detection methods (based on survey-specific single season constant detection occupancy models). Key: error bars = 95% confidence intervals.

Table 4. Model selection table: multi-season occupancy analysis for the eDNA trial (trial 4) at Awa Nohoaka Conservation Area using RPresence. Models ranked by AIC. Key: K = number of parameters; Δ AIC = difference between the AIC of the current model and that of the top-ranked model; Model weight = the relative likelihood of the model; Log likelihood = maximised value of the log-likelihood function. Model parameters: ψ(.) = constant occupancy probability, $\gamma(0)$ = colonisation probability set to zero, $\varepsilon()$ = singular extinction probability, $p(.)$ = constant detection probability, p(trend) = detection probability in eDNA surveys exhibits linear trend, p(method) = detection probability varies by field method, p(survey) = detection probability varies by survey, p(method + survey) = detection probability varies by both field method and survey, p(method + trend) = detection probability varies by field method while the eDNA survey session exhibits a linear trend with time.

Model name	K	AIC	Δ AIC	Model weight	Log likelihood
ψ(.) $\gamma(0)$ $\varepsilon()$ p(method + trend)	6	311.58	0	0.63	−149.79
ψ(.) $\gamma(0)$ $\varepsilon()$ p(method + survey)	8	314.37	2.80	0.16	−149.18
ψ(.) $\gamma(0)$ $\varepsilon()$ p(method)	5	314.63	3.06	0.14	−152.32
ψ(.) $\gamma(0)$ $\varepsilon()$ $p(.)$	4	315.80	4.23	0.08	−153.90

method, but only c. 25% if the first session's eDNA detection rate was representative of a typical field season (as opposed to a higher cost later in the field season as detectability drops).

Discussion

We have demonstrated that eDNA pipes can be used to monitor occupancy of wild Otago skinks effectively. Detection rates using our eDNA tool were approximately 30–50% higher than those recorded by field teams historically in the Awa Nohoaka Conservation Area. Furthermore, our findings show that the Awa Nohoaka Otago skink population persists, but likely occupies less habitat in 2024 than it did between 2005–2007 (equivalent to an overall retraction in occupancy of 38%). By chance, all ten rock outcrops included in the study happened to be occupied in 2005—a situation which helped to simplify our interpretation, as colonisation in the intervening period was effectively removed as a possibility. The decline in area of occupancy aligns with expectations following a large salvage operation to supplement a captive breeding programme in February 2014 (which saw the removal of 33 animals from the wider site; Lynn Adams, DOC, pers. comm.) and earlier operations which removed five animals in 2009, three animals in 2010 (Hutcheon et al. 2009; Hutcheon et al. 2010), and nine animals in 2012 (Karin Ludwig, formerly of DOC, pers. comm.). In addition, throughout this period it is likely there was ongoing predation by a suite of invasive predators. Given the punctuated nature of this human-induced decline, the range retraction we recorded will not be indicative of background mortality by predation alone and is therefore unlikely to be a reliable indicator of the future trajectory at this site.

Our novel eDNA technique allowed more nuanced models to be tested than the visual occupancy technique. We attribute this to the elevated detection probabilities the former provided. Indeed, our ability to simultaneously determine a localised extinction rate using historic data from Awa Nohoaka Conservation Area and detect a temporal trend in detection probability exceeded our initial expectations given our small sample size. However, as demonstrated by Trial 1, the eDNA pipes are not infallible and produced false absences c. 17% of the time.

Prior to our monitoring research, the status of the Otago skink population at Awa Nohoaka Conservation Area was unclear. A single Otago skink was sighted during a reconnaissance mission on 14 November 2022 (during optimal

weather conditions), indicating that a residual Otago skink population had persisted. Despite our eDNA monitoring method detecting Otago skinks in multiple locations, no Otago skinks were encountered by field staff in the process of deploying or retrieving the eDNA pipes at Awa Nohoaka Conservation Area. This highlights the problem of relying on incidental monitoring methods, as even when skinks are present they may not be readily encountered. Interestingly, the spatial distribution of detections in the Awa Nohoaka Conservation Area trial corresponded with the historic hotspots (outcrops memorable for the reliability at which Otago skinks were detected), which further strengthened the validity of our findings.

Concerns over the viability of the population in Awa Nohoaka Conservation Area led to a salvage operation for Otago skinks to supplement a captive breeding/translocation program, which resulted in 33 individuals being removed from the greater area in February 2014 (Lynn Adams, DOC, pers. comm.) for eventual release into the Mokomoko sanctuary (Central Otago Ecological Trust 2024). Between April 2013 and May 2017, a DOC predator trapping programme operated over 608 ha of the surrounding landscape, which removed 305 hedgehogs, 79 ferrets, 42 stoats, 29 mice, 23 cats, 15 rats, 14 possums, and 9 weasels using 71 modified Timms traps and 334 DOC200 traps (Ian Turnbull, Central Otago Lakes Branch of Forest & Bird, pers. comm.). However, the trapping programme was eventually discontinued due to concerns about its effectiveness, the absence of outcome monitoring, and the inability to allocate sufficient resources for monitoring at an adequate resolution (James Reardon, DOC, pers. comm.). Prior photo-resight monitoring in this area was also temporarily halted in 2010 by DOC due to perceived health and safety risks associated with potential falls from the rock outcrops (NW, pers. obs.). In later years this monitoring was abandoned due to limited resources and an inability to generate precise estimates (James Reardon, DOC, pers. comm.).

The monitoring period for Trial 4 was likely outside the typical birthing period for skinks in Awa Nohoaka Conservation Area, as neonates were captured in the 2014 salvage operation in mid-February (Lynn Adams, DOC, pers. comm.). Potentially, if neonates were dispersing during the study period onto uninhabited outcrops, the assumption of population closure (as it relates to the estimation of the occupancy rate) would be violated. If this scenario was true, the occupancy estimate would be overestimated, and the extinction rate underestimated. However, given that the spatial extent of detections appeared to be contracting rather than expanding through time in Trial 4 we suspect that such a violation has not taken place.

Based on our results, we believe that eDNA occupancy monitoring could be useful for periodic long-term monitoring of other hard to detect lizard populations. We suspect it will be particularly effective for saxicolous species occupying rock outcrops, due to the ease of inferring probable movement routes, thereby improving the chances of interception, but less effective in scenarios where there is more homogenous habit. However, our analysis of cost-effectiveness indicates that the costs of our eDNA method are currently not cost competitive for the monitoring of Otago skinks based on cost per detection given our current design. Consequently, this method is likely to be financially prohibitive for single species use unless the costs associated with eDNA assays reduce (e.g. with technological advances) or the method can be shown to be effective with fewer eDNA pipes (noting that the use of fewer replicates in our trial would result in poorer estimates given c. 47% of detections were picked up in only one of the three pipes installed on each outcrop). In a multi-species occupancy scenario, however, an eDNA occupancy approach would likely be a cost-effective monitoring solution. Given that modern eDNA assays can screen for thousands of taxa and multi-species occupancy models allow sophisticated modelling of co-occurrence (MacKenzie et al. 2018), eDNA pipes could form the basis for understanding deeper ecological questions that have management implications, for example, resolving the spatial impacts of mice on native herpetofauna (Norbury et al. 2023).

Occupancy monitoring using eDNA pipes has many non-economic advantages over traditional means of occupancy monitoring for lizards. Researchers can deploy eDNA pipes in most weather conditions (ideally in advance of favourable weather) and collect data over an extended period including days with poorer weather. In contrast, visual surveys for most diurnal species are largely restricted to survey days when the species is likely to be active (i.e. sunny, warm days). Furthermore, the deployment of eDNA pipes reduces the time exposure of field staff to potential field hazards as the eDNA pipes only have to be placed and retrieved. The use of passive eDNA pipes also removes several sources of heterogeneity by minimising disturbance issues associated with human observers which may cause skinks to flee and remain undetected, and eliminating the variability between human observers.

Currently, we estimate that the analysis cost will need to reduce by a minimum of 25% for the eDNA method to be cost effective on a cost per detection equivalent. However, it should be noted that increasing field effort in the visual occupancy method by using more observers might not ever yield a detection rate approaching that of the eDNA pipe method (e.g. it could potentially result in greater disturbance and lower detection). Given that higher detection probabilities require fewer sampling occasions to achieve optimal occupancy results (MacKenzie & Royle 2005; Sanderlin et al. 2014) the decision to employ a method with a lower detection probability simply based on a linear cost-benefit analysis could prove suboptimal. Higher detection probabilities enable more nuanced (complex) models to be explored while lower detection probabilities, as a consequence of fewer detections, have less information to reliably estimate the additional parameters of more intricate models and therefore favour less informative models. Such characteristics suggests that assessing cost-effectiveness on a cost per detection equivalent, while tangible, oversimplifies a more nuanced situation regarding the quality of information. Consequently, if model complexity, staff safety, field schedule certainty, and the ability to schedule field monitoring outside of the classic spring-summer period are prioritised then eDNA pipes might already be a pragmatic solution despite their higher cost. During the trials our method utilised a moderate amount of disposable paraphernalia which generated waste and added to costs. Following our trials, Wilderlab have developed sterilisation protocols and easy pull filters which allow the reuse of the pipes (SW, unpubl. data). If eDNA costs are prohibitive, a low-cost alternative for a single species occupancy analysis could involve simply substituting the Dacron filters with footprint-tracking ink cards, provided the footprints of the target species are readily distinguishable. However, while some gecko species and genera can be reliably distinguished by their footprints (Jarvie & Monks 2014; Harker et al. 2017), skink species cannot (Jarvie & Monks 2014; Lettink et al. 2022). Since three species of skink (*O. otagense*, *O. maccanni*, and *O.* aff. *polychroma* Clade 5; Appendix S3) were detected from our eDNA results at Awa Nohoaka Conservation Area a

footprint-based method of detection is not likely to be feasible at this site.

We note that some conservation managers remain reticent about using occupancy methods, preferring instead to work with abundance estimates or indices, which they find more tangible. Such is the strength of this professional attachment that Nichols (2014) has pointed out that abundance monitoring is often inadvertently viewed as a prerequisite for conservation management. We accept that mark-recapture (and mark-resight) methods are generally more sensitive to change than occupancy methods (e.g. Conner et al. 2016; Berigan et al. 2019). However, when dealing with sparse populations, mark-recapture is often: (1) impractical due to high labour and processing costs (Field et al. 2005; Turlure et al. 2018), and (2) imprecise due to very low recapture probabilities (e.g. Couturier et al. 2013). Even if the unit cost for mark-recapture (or photo-resight) can be improved, the low recapture probability issue is often not resolved, and therefore the estimates will remain imprecise unless the amount of resampling can be dramatically increased, which entails additional expense (Couturier et al. 2013; Lieury et al. 2017). A different problem is encountered when using indices based on counts from cryptic lizard species. In this situation, the counts tend to be variable, and typically the analyst will need to adequately account for the underlying variation in detectability by modelling covariates (e.g. Lardner et al. 2015). Additionally, the analyst will usually need to employ a statistical tool that accounts for an excess of zero counts (i.e. zero-inflation), which adds complexity to the models and necessitates modelling true zeros (i.e. when animals are truly absent) separately from false zeros (i.e. the animal was there but not detected or was temporarily absent) (Martin et al. 2005; Dénes et al. 2015). Under such conditions, counts move from being an easily analysable index to one arguably requiring as much statistical expertise as an estimator approach.

Some authors have suggested that occupancy can be problematic as it can conflate the presence of established residents with transient movements from non-residents (Berigan et al. 2019). However, Conner et al. (2016) suggest that occupancy methods are likely to be suitable for species which maintain stable territories when the units of the occupancy study represent viable territory, in which case occupancy dynamics should be strongly correlated with population dynamics. We believe that this situation likely applied to the Otago skinks of Awa Nohoaka Conservation Area during the study period as the observed detections appeared to be spatially consistent across the four eDNA surveys.

Ultimately, the strength of inference for any given monitoring study should be a function of the available budget. While rarely discussed explicitly, budget, not inference, is the primary driver of study design (Nuno et al. 2015). Therefore, if a mark-recapture study is not within budget, occupancy may be a superior monitoring option (Mazerolle et al. 2007). Indeed, Conner et al. (2016) have noted that the use of occupancy monitoring has increased in recent times, as shrinking budgets have incentivised the use of less expensive approaches to monitoring. Furthermore, we argue that budgetary constraints often limit the acquisition of abundance estimates to areas so small that they are not representative of the population under management. Under such circumstances, it may be more strategic to opt for a monitoring method which is representative of the wider population over an extensive area, but less sensitive to change, over a method which is spatially confined and sensitive to change but potentially not representative of the greater population.

Our ability to obtain a moderately precise occupancy estimate at Awa Nohoaka Conservation Area with eDNA pipes, with a detection rate c. 30–50% higher than the historic visual occupancy method, suggests that this may be a viable technique for monitoring other populations of cryptic or sparsely distributed saxicolous lizards. This study has not only demonstrated that eDNA occupancy monitoring is practical, but that it also has advantages over visual occupancy monitoring. These include reduced observer heterogeneity, higher detectability, more nuanced modelling, field scheduling certainty, and improved field team safety. While eDNA methodologies currently remain expensive, our expectation is that a c. 25% reduction in consumable and analytical costs will be required to make the eDNA pipe technique more-or-less cost competitive with visual occupancy methods (in a single-species monitoring scenario). However, if multi-species monitoring is needed or non-economic factors are prioritised, then an eDNA occupancy array incorporating a tool similar to ours may already be the pragmatic monitoring solution.

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Additional information and declarations

Conflicts of interest: SW is the director of Wilderlab, a commercial eDNA testing laboratory based in Wellington, NZ.

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Ethics: Wildlife research permits (in the form of Wildlife Act Authorisations) were not required for this research, as it was (a) deemed non-invasive, (b) did not involve handling/disturbing wild animals, and (c) did not entail habitat modification. Captive lizards in Trial 1 were handled by Kiwi Park Queenstown staff. eDNA research at Awa Nohoaka Conservation Area was authorised by the 2024 community agreement (DOC 7840564) between DOC and the Central Otago Lakes Branch of Forest

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Data availability Copies of the data and R code for the analyses can be found here: https://osf.io/z6ubq/. Date and code which could reveal precise locations were not uploaded.

Author contributions: TR conducted and coordinated all fieldwork and stakeholder liaison and contributed to project design with NW. SP assisted with the field work and design of Trial 3. SW oversaw the manufacture of the eDNA pipes and developed the eDNA protocols and analysis. NW and SP drafted the text with contributions from TR and SW. NW undertook the data analysis and led initial conceptualisation.

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Supplementary Material

Additional supporting information may be found in the online version of this article.

Appendix S1. Locus-specific sections of fusion-tag metabarcoding primers.

Appendix S2. Coefficient table of the top-ranked logistic regression model from the Trial 3 bait experiment.

Appendix S3. A phylogenetic wheel representing taxa identified from the eDNA samples in Trial 4 at Awa Nohoaka Conservation Area.

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