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Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader

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Abstract. Effective management of alien species requires detecting populations in the early stages of invasion. Environmental DNA (eDNA) sampling can detect aquatic species at relatively low densities, but few studies have directly compared detection probabilities of eDNA sampling with those of traditional sampling methods. We compare the ability of a traditional sampling technique (bottle trapping) and eDNA to detect a recently established invader, the smooth newt *Lissotriton vulgaris vulgaris*, at seven field sites in Melbourne, Australia. Over a four-month period, per-trap detection probabilities ranged from 0.01 to 0.26 among sites where *L. v. vulgaris* was detected, whereas per-sample eDNA estimates were much higher (0.29–1.0). Detection probabilities of both methods varied temporally (across days and months), but temporal variation appeared to be uncorrelated between methods. Only estimates of spatial variation were strongly correlated across the two sampling techniques. Environmental variables (water depth, rainfall, ambient temperature) were not clearly correlated with detection probabilities estimated via trapping, whereas eDNA detection probabilities were negatively correlated with water depth, possibly reflecting higher eDNA concentrations at lower water levels. Our findings demonstrate that eDNA sampling can be an order of magnitude more sensitive than traditional methods, and illustrate that traditional- and eDNA-based surveys can provide independent information on species distributions when occupancy surveys are conducted over short timescales.

Key words: amphibian; Australia; bottle trap; detection probability; eDNA; invasive species; sampling effort; smooth newt, Lissotriton vulgaris vulgaris; *survey design.*

Introduction

Invasive species impose significant stress on terrestrial and aquatic ecosystems globally (Strayer 2010), threatening ecosystem integrity and biodiversity (Ricciardi 2007) and inflicting substantial management and mitigation costs and loss of ecosystem services. Successful eradication or containment of invasive species hinges on detecting populations in the early stages of invasion, which is often challenging, as newly established populations are typically patchily distributed with few individuals (Dodd et al. 2014).

This problem is not unique to invasive species. In reality, it is unlikely that all individuals or species are ever detected during single-occupancy surveys (Yoccoz et al. 2001, Mackenzie and Royle 2005). In aquatic systems, collecting accurate occupancy data with traditional sampling methods (e.g., trapping, netting, electrofishing) often requires large amounts of effort. Indeed, using these methods to survey some aquatic systems or species may require sampling under challenging environmental conditions or times of day. Additionally, many traditional sampling methods involve handling and detaining target and nontarget organisms, which can cause stress or mortality, and potentially bias study conclusions. Surveys based on detecting species-specific DNA from environmental samples (eDNA) can overcome these limitations under some circumstances (Thomsen et al. 2012).

The eDNA method is still in its infancy, but quantitative comparisons with traditional sampling techniques have already indicated superior sensitivity of eDNA-based sampling in some systems. For example, recent studies have found higher detection probabilities of eDNA sampling compared to traditional methods for Asian carp *Hypophthalmichthys molitrix* and *Hypopthalmichthys nobilis* (Jerde et al. 2011) and American bullfrogs *Lithobates catesbeianus* (Dejean et al. 2012). However, most studies to date have simply investigated whether eDNA can detect species at sites where occupancy has been confirmed with other methods (Goldberg et al. 2011, Dejean et al. 2012, Hyman and Collins 2012, Thomsen et al. 2012*b*, Pilliod et al. 2013). Such comparisons, while informative, do not account for the fact that a species may go undetected with both traditional and eDNA methods (Schmidt et al. 2013). Detection probabilities of eDNA sampling estimated in this way are therefore likely overestimates. Additionally, no studies have repeatedly sampled multiple sites to

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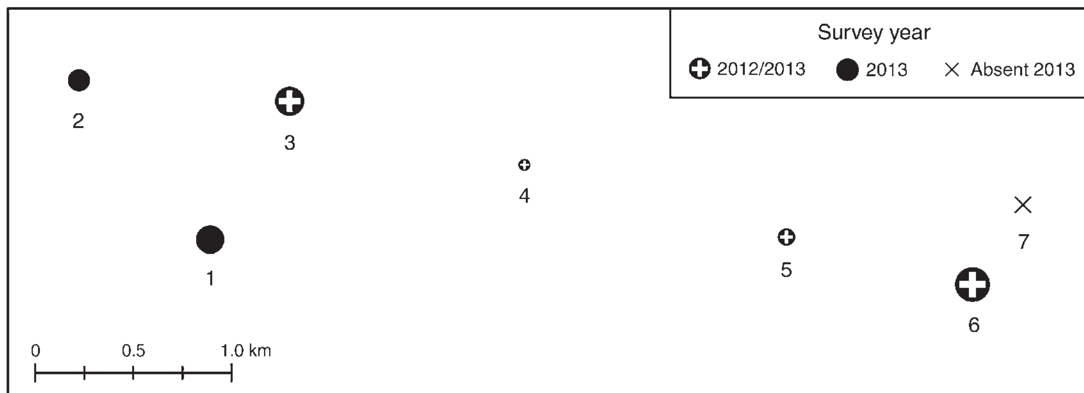

Fig. 1. Locations where *Lissitriton v. vulgaris* was detected in Melbourne, Victoria, Australia. Site size is proportional to the number of individuals captured (shown in six size classes: 1, 2, 3, 4, 5, and 23 individuals). Also shown is a site that we surveyed over 16 nights in 2013 but where the species was not detected. Distinctive landscape features have been removed as illegal trade in this species has been implicated in the establishment of *L. v. vulgaris*.

investigate spatial and temporal correlations in detection probabilities between sampling methods. Understanding whether traditional and eDNA methods are spatially and temporally correlated is not only important for testing the validity of eDNA sampling, but can also help determine cost-efficient survey designs. For example, when sampling techniques are not strongly correlated through time or space, a combination of methods may produce the most cost-effective and robust sampling regime.

The recent introduction of a European newt (*Lissotriton vulgaris vulgaris*, see Plate 1) to Melbourne, Australia (Tingley et al. 2015), provides an ideal opportunity to explore these unresolved issues regarding the relative sensitivity of eDNA-based sampling. *Lissotriton v. vulgaris* was first detected in Melbourne in 2011 but lack of knowledge of the species' detectability currently hinders containment and eradication. We use this system to (1) compare detection probabilities between eDNA and a traditional sampling method (bottle trapping) via repeat sampling at multiple sites, (2) examine correlations between spatial and temporal variation in detection probabilities from eDNA sampling and trapping, and (3) investigate how environmental factors influence detection probabilities of both sampling methods.

Methods

Study area

This study was undertaken in Melbourne, Victoria, Australia, a temperate city, with average monthly temperatures ranging from 7°C to 25°C, and rainfall averaging 650 mm/yr. The study area is located within the outer suburban fringe of Melbourne, in an area composed of interconnected roadside ditches and open-plan drainage channels constructed for agriculture. While our study occurred within this connected landscape, each site was independent, with no continuous connection or flow of water between sites over the course of the study period (Fig. 1).

Study species

Lissotriton vulgaris has an extensive native geographic range that extends throughout the United Kingdom, southern Scandinavia, central Europe, and east into Ukraine and Russia. In 2011–2012, the subspecies *L. v. vulgaris* was detected at a number of sites in an outer suburb of Melbourne, Australia. While the mode of introduction is unknown, the species' historic presence in the Australian pet trade suggests that this introduction resulted from the release or escape of captive animals (see Tingley et al. [2015] for details).

Lissotriton v. vulgaris inhabits a variety of habitat types, including woodlands, meadows, and disturbed habitats (Griffiths 1984). Like many amphibians, *L. v. vulgaris* has a life cycle with aquatic eggs and larvae that metamorphose into semi-aquatic juveniles. In the species' native range, adults spend most of the breeding season (typically spring or autumn, depending on location) in water but return to land soon afterward. Breeding occurs in slow-moving shallow waterbodies and irrigation ditches, where eggs are attached to aquatic vegetation. Males become sexually mature at two to three years of age, while females mature approximately one year later (Griffiths 1984).

Field sampling

From September to December 2013 (the suspected breeding season), we sampled seven roadside drains for the presence/absence of *L. v. vulgaris* (Fig. 1). *Lissotriton v. vulgaris* was detected at four of the surveyed sites in 2012 (Tingley et al. 2015). The three remaining sites were located in suitable habitat in close proximity to sites where the species had previously been detected. Sites were surveyed using bottle traps constructed from 2-L soda bottles, with the tapered mouth section removed and inverted (see Griffiths 1984). Bottle traps

are generally the preferred method for detecting newts (Griffiths 1997, Griffiths et al. 2010). Traps were fastened partially underwater with a cane (at least 50% of the intake), had an air pocket inside, and were baited with 10 × 100 mm glow sticks. Baiting traps with glow sticks increases the probability of detecting *L. v. vulgaris* by attracting prey (Bennett et al. 2012).

Traps were set for four consecutive nights each month, providing a total of 16 days at each site to assess *L. v. vulgaris* presence/absence. At each site, we set 11 traps at 13.5-m intervals (although numbers were sometimes lower due to variation in water depth). Site six (Fig. 1) was considerably smaller than the others, and so only four traps were set at that site. Traps were checked within 16 hours of being set. Any traps containing *L. v. vulgaris* were discarded and replaced with a sterile trap to minimize the chance of DNA contamination on subsequent visits. All captured newts were identified, sexed, and euthanized on site in accordance with The University of Melbourne animal ethics protocols (Permit ID 1212627.1) and state laws regarding handling of invasive species.

eDNA sampling

Environmental DNA sampling occurred concurrently with bottle trapping on the first and last sampling day of each month. A 500-mL water sample was taken from the beginning, center, and end of each site. Preliminary field trials in August 2013 revealed that collecting water samples by submerging the 500-mL bottles resulted in high sediment loads, leading to high rates of PCR inhibition. We therefore used disposable syringes (60-mL sterile luer slip syringes; Livingstone International, Sydney, Australia) to draw water into the 500-mL bottles. As site six was considerably smaller than the other sites, only a single 500-mL sample was taken each sampling period. Sterile gloves, syringes, and 500-mL bottles were used to collect each water sample, and all field equipment was sterilized in 10% bleach solution and thoroughly dried between sites. Water samples were placed in a dark container of ice immediately after collection until DNA extraction could be performed one to four hours later (grab-and-hold method; Goldberg et al. 2011). A total of 152 water samples were collected from the seven sites for DNA extraction and amplification over the course of the study. A double blind assessment was undertaken for water samples; water samples were coded and the person who collected the samples was different from the person who processed these samples in the laboratory. Results of both trapping and eDNA were not compared until all sampling and DNA analyses were completed.

Environmental correlates of detection

We investigated whether three environmental factors influenced the detection of *L. v. vulgaris*: ambient temperature, water depth, and precipitation (measured over the 12-h period preceding sampling). These variables were uncorrelated, and have been shown to influence detection in other amphibian species (Canessa et al. 2011). Ambient temperature and water depth were measured at night after traps were set. Ambient dry bulb temperatures were measured on site using a sling hygrometer. Depth was measured with a 1-m rule at three points throughout the site and averaged to give a single reading. Rainfall was recorded from the nearest Australian Bureau of Meteorology station.

DNA extraction and qPCR analysis

Water samples (~500 mL) were passed through a filter (0.45-μm cellulose nitrate; Nalgene, Sigma-Aldrich, Sydney, Australia) using a filter funnel (250 mL and 47 mm; Thermo Fisher Scientific, Melbourne, Australia) and peristaltic pump with filters then placed in a sterile petri dish in the dark at 4°C prior to DNA extraction. A control sample (sterile water) was also filtered and carried through the extraction process. DNA was extracted from filters using bead beating and a Qiagen DNeasy Blood and Tissue Kit (using spin-column protocol; Qiagen, Chadstone, Victoria, Australia). Filter membranes were cut into 1-mm slices and placed in 2-mL microcentrifuge tubes. 0.3 g of 1.0–1.5 mm glass beads (Cat. No. 22.222.0005; Retsch GmbH, Haan, Germany) and 720 μL ATL Buffer (Qiagen) were added to each tube and shaken in a Mixer Mill (MM300, Retsch GmbH, Haan, Germany) at 30 oscillations/s for 2 min. Tubes were then incubated at 56°C for 30 min, followed by another beating and incubation step as above before adding 40 μL of Proteinase K (Qiagen) to each tube and incubating at 56°C for 1 h. Samples were then vortexed for 15 s and centrifuged for 1 min (6000 g). Each supernatant (500 μL) was transferred into new 2 mL tubes and processed using the Qiagen DNeasy Blood and Tissue Kit using the manufacturers protocol with the following minor volume adjustments: 500 μL AL buffer, 500 μL ethanol, and final elution in 50 μL AE buffer (Qiagen).

Species-specific primers and a TaqMan (Life Technologies Australia, Mulgrave, Australia) minor groove binding (MGB) probe were developed to target a 99 base-pair (bp) fragment of the mitochondrial cytochrome *b* (CytB) gene in *L. v. vulgaris* based on sequences from Tingley et al. (2015). Forward and reverse primers were 5′– CCTACTTCTCCTACAAAGACATGCT–3′ and 5′–TTTCTGGGTCTCCTAAAAGGTTTGG–3′, respectively. The MGB probe (labeled with FAM) was 5′–AAGGAGCATAAGTAAGAAACC–3′. The primers and probe were unique to this species based on NCBI blast searches (database *available online*).[4] To further assess the specificity of the primers/probe, we also obtained tissue samples and extracted DNA (using the Qiagen kit) from the only other amphibian species detected in our study area during surveys (*Limnodynastes peronii*, *L. tasmaniensis*, *Litoria ewingii*, and *Crinia*

[4] http://blast.ncbi.nlm.nih.gov/Blast.cgi

signifera). Template DNA from each species did not amplify in quantitative PCR (qPCR), confirming specificity of the primers/probe.

TaqMan qPCR assays were conducted using a Roche LightCycler 480 system (Roche Diagnostics Australia, Castle Hill, New South Wales, Australia) in a 384-well format. Reaction volumes were 10 μL containing 5 μL of 2× Qiagen multiplex PCR Master Mix (Qiagen), 0.5 μL 20× TaqMan Gene Expression Assay (final primer and reporter concentration 0.9 μmol/L and 0.25 μmol/L, respectively), 2.5 μL ddH_2O, and 2 μL of DNA. Each reaction was prepared in quadruplicate and included a TaqMan exogenous internal positive control probe (VIC labeled) to test for the presence of inhibition. Included in each 384-well assay plate were *L. v. vulgaris* DNA extracted from tissue (positive control) and a no-DNA template reaction (negative control). PCR amplification conditions were 15 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 60°C. The amplification profiles of each PCR were used to determine the crossing point (Cp) value using the Absolute Quantification module of the LightCycler 480 software package. Assay efficiency was determined by quantifying *L. v. vulgaris* DNA extracted from tissue using a Qubit fluorometer (Invitrogen Australia, Mount Waverley, Victoria, Australia) and running an assay on a dilution series. The efficiency of the quantitative PCR was 98%. Specificity of the TaqMan assay was confirmed by sequencing PCR products from three sites (all sequences were identical to the *L. v. vulgaris* sequence from which the probe was developed). Water samples were labeled positive for the presence of *L. v. vulgaris* DNA if one out of four qPCR reactions resulted in DNA amplification (see the Appendix for results using 2/4, 3/4, and 4/4 PCR positive reactions).

All extractions and qPCR analyses were undertaken in a room that is dedicated to low-quantity DNA sources, with qPCR setup undertaken in a laminar flow hood. No DNA from *L. v. vulgaris* had been handled in the room previously. Positive controls and standards were added immediately prior to placing in the Roche LightCycler 480 (separate room). Negative controls were included at all stages (DNA extraction, qPCR) so that laboratory contamination could be identified if present; no contamination was found.

Model description

Detection probabilities of trapping- and eDNA-based sampling methods were modeled using hierarchical Bayesian generalized linear models (GLMs). Generalized linear models are composed of (1) a random component that describes the distribution of the response, in this case a Bernoulli distribution appropriate for modeling detection/non-detection data, (2) a linear predictor, which combines the independent variables in a linear equation, and (3) a link function describing the relationship between the random and systematic components, in this case a "cloglog" function, that rescales the linear predictor to the interval [0, 1]. The cloglog link was used because the linear predictor can be interpreted in terms of the logarithm of the rate of detection (McCarthy et al. 2013), while other link functions cannot. Random effects were included in all models to account for variation due to sampling month, day, and site. Environmental variables were incorporated as fixed effects. We modeled the cloglog of the probability of detection by either trapping or eDNA as

$$\text{cloglog}\Big(P(Y_{ij}=1)\Big) = \alpha_{j_0} + \beta_{j_1}R_i + \beta_{j_2}T_i + \beta_{j_3}D_i + \varepsilon_{\text{d}[i],j} + \varepsilon_{\text{m}[i],j} + \varepsilon_{\text{s}[i],j} + \varepsilon \tag{1}$$

where α controls the average detection probability for method j; $\beta_{j,n}$ are the model coefficients describing the effects of rainfall (R), temperature (T), or depth (D) for sample i and method j; and $\varepsilon_{\text{m}[i],j}$, $\varepsilon_{\text{s}[i],j}$ and $\varepsilon_{\text{d}[i],j}$ are random effects for month, site, and sampling day within month, respectively.

When these random effects are independent between detection methods in the model, prior distributions for each random effect are drawn from independent normal distributions with a mean of 0 and a precision (τ) to be estimated from the data

$$\begin{aligned} \{\varepsilon_{\text{m}[i],j}\} &\sim \text{dnorm}(0, \tau_\text{m}) \\ \{\varepsilon_{\text{s}[i],j}\} &\sim \text{dnorm}(0, \tau_\text{s}) \\ \{\varepsilon_{\text{d}[i],j}\} &\sim \text{dnorm}(0, \tau_\text{d}). \end{aligned} \tag{2}$$

As temporal and spatial variation are likely to be correlated between eDNA and trapping methods, we allow the temporal and spatial random effects to covary, in which case, bivariate normal distributions are used as priors. These joint distributions are characterized by a mean of 0 and a precision defined by a variance covariance matrix, Σ

$$\begin{aligned} \{\varepsilon_{\text{m}[i],j}\} &\sim \text{dmnorm}(0, \Sigma_\text{m}) \\ \{\varepsilon_{\text{s}[i],j}\} &\sim \text{dmnorm}(0, \Sigma_\text{s}) \\ \{\varepsilon_{\text{d}[i],j}\} &\sim \text{dmnorm}(0, \Sigma_\text{d}) \end{aligned} \tag{3}$$

where the variance covariance matrix is expressed as

$$\Sigma_k = \begin{bmatrix} \sigma^2_{k,j_1} & \sigma_{k,j_1,j_2} \\ \sigma_{k,j_1,j_2} & \sigma^2_{k,j_2} \end{bmatrix}. \tag{4}$$

Here the diagonal elements are the variances of the random effects for the two methods, j_1 and j_2, and the off diagonals are the covariance for the random effect k (k = m for months, d for days, and s for sites). The correlation between each random effect, r_k, is then

TABLE 1. Survey metrics and raw and modeled detection probabilities of *Lissotriton v. vulgaris* using trapping and environmental DNA (eDNA) surveys across seven sites in Melbourne, Australia.

Survey	1	2	3	4	5	6	7
Positive samples†							
eDNA	7/24	9/24	12/24	9/24	7/24	8/8	0/24
Trapping	2/171	3/171	4/176	2/159	2/103	13/50	0/176
Raw estimate							
eDNA	0.29	0.38	0.50	0.38	0.29	1.00	0.00
Trapping	0.01	0.02	0.02	0.01	0.02	0.26	0.00
Model estimate							
eDNA	0.28	0.36	0.50	0.35	0.30	0.97	0.02
Trapping	0.01	0.02	0.02	0.01	0.02	0.25	0.00
n for $P > 0.95$							
eDNA	9	7	5	7	9	1	-
Trapping	299	148	148	299	148	10	-

Note: The number of samples (n) required such that the detection probability (P) exceeds 0.95 is based on the raw estimates.

† Values are the number positive out of the total number of samples.

$$r_k = \frac{\sigma_{k,j_1,j_2}}{\sigma_{k,j_1}\sigma_{k,j_2}}. \quad (5)$$

Drawing random effects from a bivariate normal distribution in this way allowed us to examine the spatial and temporal correlation between sampling methods. For example, the detection probability for each of the two methods would be positively correlated among sites if the site-based correlation coefficient (r_s) was positive. Temporal correlations in detection among days or months would occur when the relevant correlation coefficients were positive (r_d and r_m).

The estimated single-site detection probability (p) can be used to estimate an overall detection probability given any number of repeat visits. We used the raw site-specific detection probabilities for each survey method (Table 1) to estimate the number of repeat visits required to achieve a detection probability >0.95 using the equation

$$P = 1 - (1 - p)^n \quad (6)$$

where P is the overall estimated probability of detection after n surveys (Kéry 2002).

Model fitting

Models were fit with Markov chain Monte Carlo (MCMC) Bayesian modeling software JAGS v.3.4.0 run through R v.3.0.2 via the package R2jags v.0.03-11 (R Core Team 2013). Three model chains were run over 30 000 iterations, with the first 3000 discarded as a burn-in, which was sufficient for the MCMC chains to converge. The remaining samples were thinned by a factor of 27, resulting in 1000 retained samples per chain for post-processing. Prior distributions for the intercept (α) and environmental coefficients (β_n) were specified as normal with a mean of zero and standard deviation of 1000. Distributions for temporal and spatial random effects were characterized with a mean of zero and standard deviation to be estimated from the data. Prior distributions for the standard deviations of each method were uniform over the range [0, 10]. The prior distributions of the correlations between methods were uniform in the range [−1,1]. In order to check for convergence, we used the coda v.016-1 (Plummer et al. 2006) package in R. All chains produced potential scale reductions of <1.1, indicating convergence of the chains.

RESULTS

We detected *L. v. vulgaris* with bottle traps and eDNA at six of the seven sites surveyed. Adults were detected in traps at all six sites, whereas larvae were only observed at three sites (from October through December). Observed detection probabilities per trap ranged from 0.01 at the site where *L. v. vulgaris* was detected least frequently to 0.26 at the site where it was detected most frequently. In contrast, per-sample eDNA estimates were four times higher than trapping estimates at the site where the species was most frequently observed (detection probability, $P = 1$), and an order of magnitude greater at the five remaining sites (detection probability, $P = 0.29–0.50$; Table 1). The number of traps required to produce a detection probability >0.95 varied from 10 to 299 across sites, whereas the number of water samples required to achieve a similar level of detection was 1 to 9.

Predicted detection probabilities from our model closely matched observed estimates (Table 1). Random effect coefficients revealed strong variation in detection probabilities between sites (Fig. 2), and estimates of this spatial variation were highly correlated between sampling methods (mean r_s [95% CI] = 0.89 [0.39, 0.99]). Detection probabilities varied between days and months for both sampling methods (Fig. 2); however, estimates of temporal variation were very uncertain, and did not appear to be strongly correlated between the two approaches (daily variation, r_d = 0.03 [−0.95, 0.95];

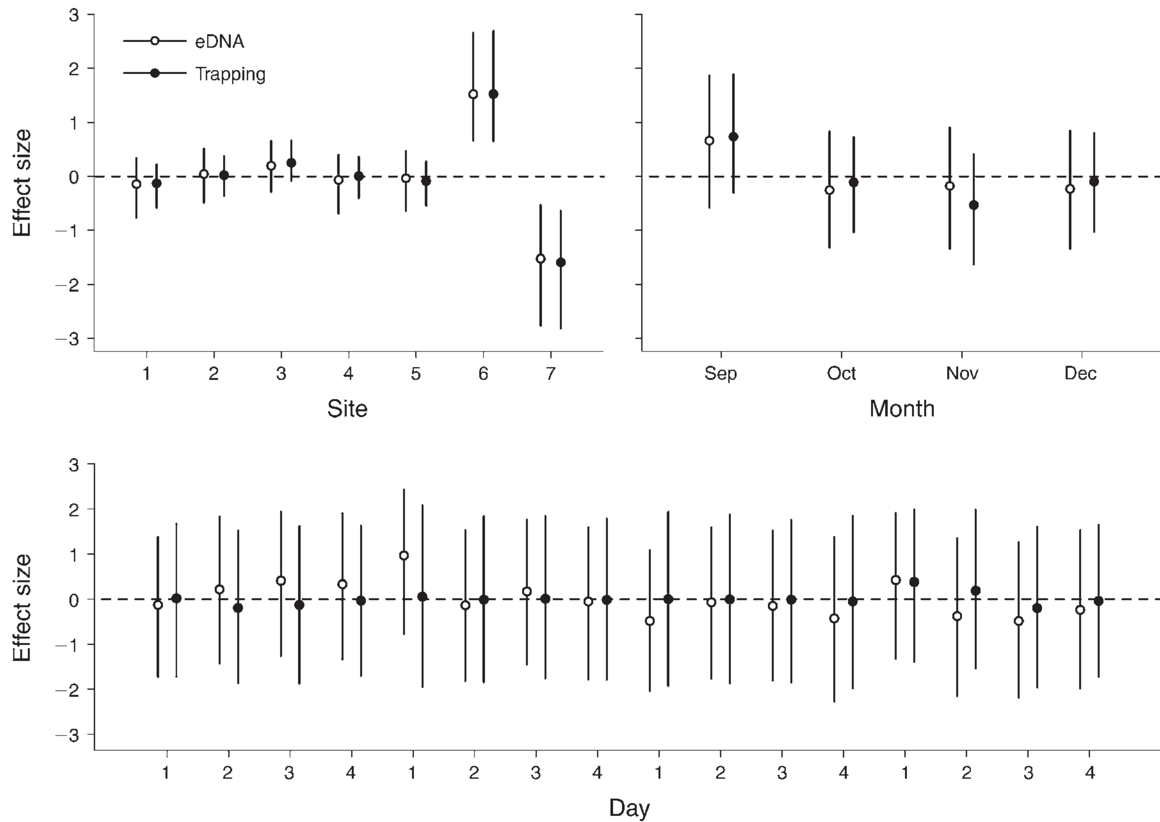

FIG. 2. Mean and 95% credible intervals of the random effect for site, month, and day within month (four consecutive days roughly one month apart), for trapping and environmental DNA methods.

monthly variation, $r_m = 0.27$ [–0.88, 0.98]). Estimated effects of environmental variables on detection probabilities of both sampling methods were generally weak and highly uncertain, with 95% credible intervals for most variables encompassing zero (Fig. 3). The only exception was that eDNA detection probabilities were negatively correlated with water depth.

Using more stringent qPCR thresholds (2/4, 3/4, or 4/4 qPCRs had to test positive for the presence of *L. v. vulgaris* DNA) reduced the sensitivity of eDNA sampling (Appendix: Table A1). However, estimates of spatial and temporal variation (Fig. A1), and the influence of environmental covariates on detection probabilities (Fig. A2) were similar when the 2/4 threshold was used. Results were also qualitatively similar for the other two more stringent qPCR thresholds (results not shown).

DISCUSSION

Early detection of invasive species increases the probability that control and eradication efforts will be successful (Anderson 2005). Additionally, economic costs associated with early detection and subsequent response efforts are far less than those of long-term management programs aimed at controlling establishment or spread (Vander Zanden et al. 2010). However, as the probability of detecting rare and cryptic species is often low, current monitoring techniques typically require large effort to detect species reliably. Therefore, many invasive species are only detected once they are abundant and widespread, limiting potential management actions and increasing costs.

Our results demonstrate that using eDNA to detect invasive species may reduce sampling effort and increase detection probabilities relative to current monitoring techniques. In fact, we found that per-sample eDNA detection probabilities were up to an order of magnitude greater than with bottle trapping. Previous studies have similarly demonstrated that eDNA can provide higher detection probabilities when compared with other sampling methods (Dejean et al. 2012, Thomsen et al. 2012*a*, Schmidt et al. 2013). However, only Schmidt et al. (2013) used an inferential statistical model to estimate detection probabilities with eDNA, and no studies have investigated spatial and temporal concordance between detection probabilities estimated with eDNA and a traditional sampling technique.

Our results revealed considerable spatial heterogeneity in detection probabilities using trapping and eDNA surveys, and this heterogeneity was strongly correlated between methods. Abundance often influences detection probabilities (Royle and Nichols 2003, McCarthy et al.

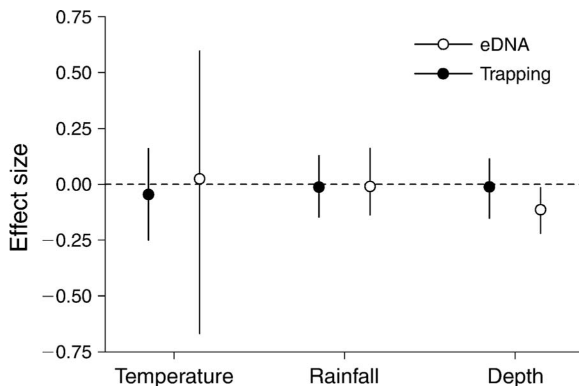

FIG. 3. Mean and 95% credible intervals for regression coefficients describing effects of water depth (measured in cm), rainfall (measured in mm), and temperature (measured as °C) on detection probabilities of *L. v. vulgaris* using bottle traps and environmental DNA (eDNA) sampling.

2013), so differences in abundance across sites can drive spatial variation in detection. Indeed, previous studies have found effects of local densities on successful amplification rates using eDNA (Dejean et al. 2011, Jerde et al. 2011, Thomsen et al. 2012*a*).

Detection probabilities estimated with both methods varied substantially across days and months. This temporal variation suggests, at least for our case study, that resources would be more efficiently allocated by visiting sites on multiple occasions, as opposed to spending one's budget visiting sites all at once. Interestingly, estimates of daily and monthly variation did not appear to be strongly correlated between trapping and eDNA sampling. Thus, trapping and eDNA-based surveys may provide different estimates of species occupancy when studies rely on a single visit, or are conducted over short time scales.

Previous studies have documented strong effects of environmental variables on amphibian detection probabilities estimated with traditional sampling methods (Bailey et al. 2004*a*, *b*, Heard, Robertson and Scroggie 2006, Canessa et al. 2011), but we found that effects of temperature, rainfall, and water depth on detection probabilities estimated with bottle traps were weak and extremely uncertain. However, our study was conducted over the species' breeding season, when individuals are primarily aquatic and environmental conditions are relatively favorable for activity. Expanding our study to include cooler time periods when *L. v. vulgaris* is more terrestrial may have revealed more pronounced environmental effects. Similarly, eDNA detection probabilities did not appear to be significantly correlated with rainfall or temperature. We did, however, find a negative correlation between water depth and eDNA detection probabilities, possibly reflecting higher eDNA concentrations and thus greater PCR amplification success at lower water depths.

Examining spatial and temporal correlations between sampling methods can help inform the most cost-efficient allocation of survey resources. For example, when both spatial variation and temporal variation are positively correlated between methods, surveys can simply rely on the most cost-effective method. When variation is uncorrelated between methods, however, using both techniques might be beneficial. In our case study, temporal correlations between methods were highly uncertain, but eDNA had a much higher detection probability than trapping. Thus, it would probably only be cost efficient to use traps when sampling costs can be shared between survey methods (e.g., when eDNA sampling is done on several occasions, allowing traps to be set and checked on subsequent days).

Our results suggest that eDNA sampling detects *L. v. vulgaris* more reliably than trapping, but the relative utility of eDNA sampling will depend critically on the costs of the methods and different types of errors. The reliability of any sampling method can be characterized by false positives (Type I errors, a species is detected where it is absent) and false negatives (Type II errors, a species is not detected where it is present), and eDNA methods are no exception. For example, our results show that eDNA sampling can reduce false negative errors. However, surveys must consider false positives arising with eDNA-methods through non-specificity of the primers and probes used for detection, unhygienic field protocols, within laboratory contamination, transfer of target DNA from nontarget species, or protracted DNA persistence after the death of the target organism (Darling and Mahon 2011). In contrast, trapping methods are far less prone to false positive errors. Thus, while eDNA methods have greater sensitivity than current methods, this potentially comes at a cost of lower specificity. However, primer specificity was not an issue in our study, as our primers failed to amplify the DNA of the only other amphibian species found within our study area. Additionally, we are confident that field contamination did not influence our results, as water samples from the site where *L. v. vulgaris* was not detected ($n = 24$ water samples, 96 qPCRs) did not test positive for *L. v. vulgaris* DNA. We also included negative controls during DNA extraction and qPCR, and these were always negative, ruling out the likelihood of laboratory contamination. Future studies should similarly ensure primer specificity and include negative controls at all stages of eDNA sampling to reduce the probability of false positives. Nonetheless, managers need to consider the relative costs of false positives and false negatives when deciding between sampling methods. In some cases, simultaneously deploying both methods might be valuable. Alternatively, eDNA might be used as an early detection tool that triggers surveys with multiple methods (Jerde et al. 2011, Mahon et al. 2013).

Plate 1. Captive *Lissotrion v. vulgaris* taken from the wild in Melbourne, Australia. Photo credit: David Paul/Museum Victoria.

Worryingly, there is currently no guidance for deciding which threshold to use when designating a water sample as positive. Indeed, some studies have labeled a water sample as positive if one out of three PCRs successfully amplify target DNA (Dejean et al. 2012), whereas others have used one out of eight (Mahon et al. 2013). As expected, our results demonstrate that using more stringent decision thresholds reduced the sensitivity of eDNA sampling. Nevertheless, sensitivity of eDNA sampling will depend on a variety of additional factors, including the analytical methods used, temporal heterogeneity in detection probabilities, and the available survey budget. Future work could investigate how these factors influence cost-efficiency under optimal survey designs (e.g., Moore et al. 2014).

Our results demonstrate that eDNA sampling can provide considerably higher detection probabilities than a traditional survey method. However, we have also shown that the relative sensitivity of eDNA can vary across space and time, and that temporal variation is not necessarily correlated between sampling techniques. Ultimately, the utility of eDNA as a monitoring tool will depend on the strength of such correlations, the relative costs of false positive and false negative errors given the management problem at hand, and the cost-efficiency of traditional and eDNA methods. Further understanding these correlations and constraints will help clarify the relative utility of eDNA methods in management and research, facilitating more robust monitoring and management outcomes.

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