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METHODS AND RESOURCES

Does mesocosm validation of environmental DNA methods translate to natural environment monitoring applications? A case study detecting a high-profile invader; the red eared slider turtle, *Trachemys scripta elegans*, in Australia

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Abstract

Environmental DNA (eDNA) surveys have gained popularity as a highly sensitive detection tool that generally outperform traditional detection techniques. eDNA surveys can provide a cost-effective means to identify species' distributions and recent incursions, informing the control or containment of invasive species. The red-eared slider turtle, *Trachemys scripta elegans*, is one of the world's most invasive species and is listed as a priority pest species for management in Australia. In this study, we validate two eDNA assays to detect this invasive turtle in Australia. We demonstrate high sensitivity in a laboratory setting and perfect detection rates in mesocosms for one of these eDNA assays but show that this does not translate to high detection rates in urban waterbodies at sites of known occupancy. In fact, our results suggest eDNA surveys provide sub-optimal performance compared to traditional detection methods for *T.s. elegans*. We suggest the capacity for eDNA surveys to provide a highly sensitive detection tool must be evaluated in natural environments on a species-by-species basis to understand any limitations and to avoid high error rates from eDNA surveys leading to wasted resources or inappropriate management decisions. For management of *T.s. elegans* in Australia, clearly defining the utility of certain eDNA based approaches to detect *T.s. elegans* and their incursions is vital for effective management of this pest species.

Keywords eDNA · Invasive species · Low detection rates · Turtles

Introduction

Environmental DNA (eDNA) can be defined as DNA that is deposited into the environment by organisms including from sloughed skin, waste and gametes (Ficetola et al. 2008; Pawlowski et al. 2020). The capture and isolation of DNA from environmental samples coupled with real time PCR provides a non-invasive approach to biomonitoring (Thomsen and Willerslev 2015). A common application of eDNA based approaches is for invasive species surveillance, both as an early detection and monitoring tool, and as a way of providing data when conventional approaches are more difficult to implement (Darling and Mahon 2011; Goldberg et al. 2013). eDNA based surveys can be more cost effective and sensitive than conventional approaches and this is further increasing their popularity (Thomsen and Willerslev 2015). However, while detection from contrived conditions is feasible for many taxa, the value of eDNA surveys to conservation management is often dependent on sufficient

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detection in natural environments (Cristescu and Hebert 2018; Wood et al. 2020). Thus, even though laboratory and mesocosm studies reveal the useful potential of eDNA approaches, poorer detection probabilities and quantitative data resulting from eDNA sampling in more complex and natural environments may reduce the utility of eDNA surveys for certain taxa (Raemy and Ursenbacher 2018; Yates et al. 2019). For invasive species management, this utility requires consideration, where the timing, location, and density of an incursion can remain unknown.

A key invasive species worldwide is the red-eared slider turtle, *Trachemys scripta elegans*. This species is native to southern United States and northern Mexico but is a popular pet worldwide and has become the most commonly traded turtle species (Herrel and van der Meijden 2014). The potential for *T.s. elegans* to outcompete and spread disease to native species (Cadi and Joly 2004) has seen it recognised as one of the world's most impactful invasive species (GISD 2021). *Trachemys scripta elegans* are a priority pest species for management in Australia, where incursions have been recorded for over 30 years with breeding populations found in eastern states (Henderson and Bomford 2011; Mo 2019; Robey et al. 2011). Current management efforts for *T.s. elegans* are time and resource-intensive and include the use of scent detector dogs, visual observation and reporting, opportunistic hand capture, trapping and removal. eDNA surveillance has the potential to inform the distribution of *T.s.elegans*, improving management efforts for this invasive species by providing a highly sensitive and cost-effective detection tool (Kakuda et al. 2019). eDNA surveys could be also conducted by persons already implementing management operations and complement existing monitoring strategies for this high profile invasive.

We aim to develop eDNA methods to detect the red-eared slider turtle (*Trachemys scripta elegans*) in Australia. Two previously published marker sets have been designed to detect *T.s. elegans* and validated for use in eDNA surveys in their respective countries: PCR primers targeting the Cytochrome Oxidase I (CO1) gene region were developed by Davy et al. (2015) to detect the invader in Canada, and a qPCR assay targeting the Cytochrome b (Cytb) gene region was developed and tested for use in Japan by Kakuda et al. (2019). Rather than create yet another *T.s. elegans* marker set, we aim to extend the utility of these existing markers by evaluating their potential to provide highly specific and sensitive eDNA detection of *T.s. elegans* in Australia. We first validate the specificity of these markers for use Australia-wide. Following the development of a probe to compliment the Davy et al. (2015) primer set, we directly compare the sensitivity of detection of these two assays. We then select the most optimal assay for application to eDNA detection surveys of *T.s. elegans* from mesocosm and field sites where traditional detection methods are being employed. We discuss the utility of eDNA sampling methods for monitoring of *T.s. elegans* in Australia, and discuss considerations for detection of turtles more broadly through eDNA analysis of water samples. This data will be useful to inform current and future conservation and management strategies of turtles using eDNA based approaches.

Materials and methods

Sensitivity and specificity testing

Two marker sets developed by Davy et al. (2015) and Kakuda et al. (2019) for use in eDNA applications in Canada and Japan respectively (named Davy and Kakuda from hereon in) were selected for validation and testing for eDNA applications in Australia. These marker sets targeted two different mitochondrial gene regions: Cytochrome Oxidase I (CO1) and Cytochrome b (Cytb) respectively (Table 1). The Kakuda marker contained a TaqMan™ probe for use in qPCR analyses while the Davy marker did not.

Molecular interactions of both primers sets were assessed using OligoAnalyzer® software (IDT, Coralville, Iowa,

Table 1 Details of primers and hydrolysis probes designed to amplify DNA of *Trachemys scripta elegans* (red-eared slider turtle) and sensitivity of each assay based on standard curve calculations. *qPCR probe for this assay was designed during this study

Assay	Sensitivity		LOD (copies/reaction)	Label	Nucleotide sequence (5′–3′)	Fragment size (bp)	Reference
Davy – CO1	Efficiency (%)	84.375	100	CO1-TSc-01-F	GGGAACTGACTCGTGCCATTA	178	(Davy et al. 2015)
	R^2	0.975		TS_COI_Probe*	(FAM)-CAGGCACAGGCTGAACTGTA-MGB		
	Slope	-3.764					
	Error	0.082		CO1-TSc-01-R	GGGCTAAATTTCCGGCTAAT		
Kakuda - Cytb	Efficiency (%)	95.517	10	Tse-Kako-A-F	CCTCCAACATCTCTGCTTGA	153	(Kakuda et al. 2019)
	R^2	0.996		Tse-Kako-A-MGB-P	(FAM)-CGGAATTTTCTTGGCTATAC-MGB		
	Slope	-3.434					
	Error	0.028		Tse-Kako-A-R	ATTGTACGTCTCGGGTGATG		

United States), Primer3 and Primer3 Plus (Version 4.1.0) (Untergasser et al. 2012). The specificity of the primers to successfully target *T.s. elegans* and avoid amplification of non-target organisms found throughout Australia was tested *in silico* against the National Centre for Biotechnology Information (NCBI) BLASTn database. Ten *T.s. elegans* sequences and 23 of the 25 Australian native turtle species (Van Dyke et al. 2018) were downloaded into Geneious Prime (Version 10.2.6) for alignment (Table S1). MAAFT (Version 1.4.0) alignments were constructed at both the COI and Cytb mitochondrial gene regions to assess primer sequence matches to *T.s. elegans* ($N=10$) and mismatches against the Australian native species. Two species (*Elseya flaviventralis and Emydura s. worrelli*) could not be included in the analysis due to lack of data availability. However, four other species within the same genera were represented in these *in-silico* alignments.

To increase the specificity of the Davy et al. (2015) primers, a TaqMan™ probe was developed. Probe selection was conducted by forcing Primer3Plus (Untergasser et al. 2012) to determine a candidate probe sequence in the amplicon region. IDT's OligoAnalyzer (IDT, Coralville, Iowa, United States) was then used to assess the melting temperatures, molecular structures, and dimerization properties of all oligo sequences. Specificity of this and the Kakuda et al. (2019) probe was also tested as described above.

Assays were tested for specificity in vitro on *T.s. elegans* (11 individual tissue samples, collected from Queensland and New South Wales, Australia) and 13 non-target freshwater turtle species distributed throughout Australia and spanning seven genera (Van Dyke et al. 2018) (Table S2). DNA was extracted from tissues using either the Qiagen DNeasy blood and tissue kit (Qiagen) following the manufacturer's instructions, or a modified salting out method (Cawthorn et al. 2011) and diluted to between 5 and 20 ng/µL. Each extract was then tested for specificity in duplicate qPCR reactions. qPCR reactions consisted of 10 µL of TaqPath ProAmp Master Mix (Applied Biosystems, Vic, Australia), 1 µL of TaqMan™ assay (Applied Biosystems, Vic, Australia), 5 µL of DNA and made up to a final volume of 20 µL with RNase/DNase free water (Invitrogen, Carslbad, CA, United States). Quantitative PCR was performed using a Viia™ 7 Real-Time PCR System (Applied Biosystems, Vic, Australia) with cycling conditions set at 50 °C (2 min), 95 °C (5 min), followed by 55 cycles of 95 °C (15 s) and 30 °C (30 s) with a final extension for 2 min at 60 °C. qPCR reactions were considered positive if exponential amplification was observed with a Ct value below 45. Negative controls were included for each qPCR run and positive amplicons were sent to the Australian National University's Biomolecular Resources Facility (ANU-BRF) for Sanger sequencing on an AB 3730xl DNA Analyser for confirmation. The only Australian native genus not included in in vitro testing was *Pseudemydura*: This genus is represented by a single species, the critically endangered Western Swamp tortoise, *Pseudemydura umbrina*, found only in a small pocket of Western Australia (Burbidge 1981) for which tissue samples could not be obtained.

A synthetic double-stranded gene fragment matching *T.s. elegans* was designed for each amplicon region, incorporating a small inversion to control for possible contamination (gBlocks, IDT, Iowa USA) (Table S3). This gBlock was used throughout sensitivity testing of each assay to enable direct comparison of the efficiency, R^2, slope, error and limit of detection (LOD). The LOD was defined as the concentration that can be detected with 95% certainty (Bustin et al. 2009).

qPCRs were performed as described above with 1 µL of gBlock for each 20 µL reaction. Standard curves across 10 replicates were established using a dilution of copy/reaction concentrations ranging 10^6 copies/reaction to 10^{-1} copies per reaction. The LOD was further assessed by diluting the gBlock to 80, 60, 40 and 20 copies/reaction for the Davy assay and 8, 6, 4 and 2 copies/reaction for the Kakuda assay. Eight no-template controls were included on each PCR plate. Y-intercept, R^2, efficiency, error values and LOD were then summarised for each assay. The most optimal assay was selected for subsequent testing on eDNA samples.

Environmental DNA surveys

An enclosure that housed surrendered and/or trapped red-eared slider turtles recovered during management operations served as a controlled mesocosm site for testing the assay on eDNA water samples. The mesocosm shared characteristics with urban ponds and water bodies inhabited by *T.s. elegans* (i.e., limited flow, vegetation) but was greatly reduced in scale at 24m^2. *T.s. elegans* were placed inside the mesocosm over time with the final three of 10 turtles placed in the mesocosm approximately one month prior to eDNA sampling but were restricted to a sub-section of the enclosure (Figure S1). Sampling events took place on the 23rd of November 2020 (Day 1), and 9th (Day 15) and 16th of December 2020 using an eDNA sampler (Smith-Root, Vancouver, WA, USA) with self-preserving filter papers (5.0 µM) (Smith-Root) which allow for immediate filtration and preservation with minimal contamination risk. Six 1 L eDNA samples were collected on each occasion following the manufacturer's guidelines at approximately evenly spaced intervals around the enclosure including two samples within the sub-section housing turtles and four samples in the broader enclosure. This enabled monitoring of eDNA dispersal by comparing eDNA concentrations within the sub-section housing the turtles to eDNA concentrations

within the broader enclosure. Field controls were processed for all sampling events which consisted of a pre-sterilised (i.e., rinsed and cleaned with bleach and UV sterilised water) 1 L bottle filled with nearby tap water. Turtles were removed from the enclosure immediately following the second sampling event on 9th December 2020, enabling the final sampling event to monitor eDNA degradation after seven days.

Environmental DNA surveys were conducted on the 23rd and 25th of November 2020 at three urban waterbodies in Sydney, NSW, Australia (Table 2). Sites were selected opportunistically based on confirmed occupancy by *T.s. elegans* several days prior to sampling. Sites were locations where traditional monitoring (visual surveys and trapping) were being employed to detect the invasive species. Immediately prior to eDNA sampling, each site was visually inspected for the presence of *T.s. elegans* (which are easily distinguishable from other turtle species) by circumnavigating the perimeter of the waterbody for 10 min. Water was sampled on-site using the Smith-Root eDNA sampler with self-preserving filter papers (5.0 µM) (Smith-Root). Samples were collected by lowering the sampling pole into the water and filtering until either the filter paper was blocked, or the target 1 L volume was achieved. Samples were collected at roughly even locations around the entire waterbody, except for De Fraites Wetland where a subsection of preferred habitat (based on observations from ongoing monitoring) was sampled. Field controls were processed for all sampling events which consisted of a pre-sterilised 1 L bottle filled with UV sterilised water.

eDNA extraction and PCR

All eDNA extractions were performed in the University of Canberra's Trace DNA Laboratory - a designated PCR-free environment - to limit contamination. Filter papers were first removed from their housings and placed in new tubes for extraction using sterilised forceps [see Thomas et al. (2019)]. A modified Qiagen DNeasy Kit protocol was used to extract genetic material from filter papers following protocols described in Hinlo et al. (2017) with a final elution of 100 µL to maximise potential yield. A negative extraction control was included with each batch of extractions. Quantitative PCR was then used to determine the presence/absence of target DNA in eDNA samples following setup and PCR conditions described above with the addition of 0.75 µL universal fish endogenous control assay in the field-collected samples to assess inhibition (Furlan and Gleeson 2017). Triplicate reactions were performed for each extract. Each qPCR plate included three non-template controls (NTC), three positive control (consisting of tissue derived *T.s. elegans* DNA) and a 5-point standard curve of a gBlock fragment in concentrations ranging from 1,000,000 to 100 copies/µL to estimate eDNA concentrations. Approximately 10% of positive eDNA amplicons from the mesocosm site and all positive amplicons from field sites were sent to the Australian National University's Biomolecular Resources Facility (ANU-BRF) for Sanger sequencing on an AB 3730xl DNA Analyser.

Results

Assay design and validation

Primers and probes for both assays showed sufficiently stable molecular structures within the tolerable range (i.e., delta g values >-7) except for the reverse primer sequence of the Davy et al. (2015) assay, which generated high self-dimering scores (delta g value −9.75). To avoid the possibility of

Table 2 Details of the sampling sites, sampling effort and detection results for the universal fish and species-specific environmental DNA assay for *Trachemys scripta elegans* (red-eared slider turtle). Sites include a controlled mesocosm site and three urban waterbodies located in Sydney, NSW, Australia. Detection results are given as the total number of PCR replicates performed, the number of amplifications for the universal fish assay, and species-specific positive PCR replicates for each site

Location	Sampling Date	Approximate size	Latitude	Longitude	Concurrent visual sighting	Total no. samples (replicates)	Positive Uni-Fish samples (replicates)	Positive *T.s. elegans* samples (replicates)
Mesocosm	23/11/2020 9/12/2020 16/12/2020	24m^2			Yes	18 (54)	N/A	18 (54)
Bonnyrigg Wetland	23/11/2020	4500m^2	-33.887	150.884	No	6 (18)	6 (15)	0 (0)
De Fraites Wetland	23/11/2020	2800m^2 (subsampled preferred habitat area of 760m^2)	-33.873	150.961	No*	5 (15)	5 (15)	2 (3)
Wiley Park Upper	25/11/2020	690m^2	-33.926	151.074	Yes	6 (18)	6 (18)	0 (0)

*Three individual *Trachemys scripta elegans* were captured in cage traps the same day as eDNA sampling took place

dimerization creating a false positive detection in SYBR™ Green qPCR assays, a fluorescently labelled TaqMan™ probe was developed for use in qPCR analyses (Table 1).

Analysis of the *in- silico* data revealed that both assays are highly specific to *T.s. elegans* in Australia and dissimilar to all non-target species tested here. The ten *T.s. elegans* individuals displayed very little sequence variability across the target gene regions with three nucleotide differences observed for the COI and two differences in the Cytochrome b gene regions respectively. No variation was detected in the primer/probe region with both assays providing a perfect sequence match to this target species. Both assays showed high variability to non-target species with a minimum of 10 nucleotide mismatches across primer-probe bindings sites for the Davy assay, and 11 for the Kakuda assay (Table S1). In vitro testing showed successful PCR amplification in all replicates of the target species and failed to amplify any non-target species, validating both assays for eDNA applications throughout Australia (Table S2). Although in vitro amplification against individuals of the *Pseudemydura* genus could not be tested, *in silico* analyses revealed numerous (more than 10) primer nucleotide mismatches, making amplification of this genus extremely unlikely.

Sensitivity testing showed that both assays were able to amplify low quantities of target molecules. Standard curves for the Kakuda assay indicated an average PCR efficiency of 95.517% and an r^2 value of 0.996 (Figure S2) while the Davy assay indicated an average PCR efficiency of 84.375% and an r^2 value of 0.975 (Figure S3). Serial dilutions of the gBlock revealed a LOD of 100 copies per reaction for the Davy assay, and 10 copies per reaction for the Kakuda assays (Table 1, Table S4 and Figures S2 and S3). Although we have shown that both assays are likely to be highly specific for DNA detection of *T.s. elegans* in Australia, the Kakuda assay showed better performance with higher sensitivity [a lower LOD, increased efficiency, reduced error] and reduced dimerization. Therefore, the Kakuda assay was selected for subsequent testing and eDNA validation.

Environmental DNA surveys

The Kakuda assay successfully amplified eDNA from *T.s. elegans* in all water samples and replicates from the mesocosm (Table 2). During the first two sampling occasions (i.e., while turtles were present), eDNA concentration was 843.5 ± 939 and 1089.3 ± 764 copies per reaction. On both sampling occasions, concentrations were slightly higher in samples collected within the smaller subsection housing the turtles (1924.9 ± 583 versus 302.8 ± 431 on Day 1 and 1135.6 ± 1045 versus 1066.1 ± 778 on Day 15) although differences were not statistically significant. On the final sampling occasion seven days after turtles had been removed, eDNA concentration decreased throughout the entire enclosure to just 110.4 ± 173 copies per reaction.

Field sampling and conventional monitoring

Analysis of eDNA samples collected from field sites returned positive results in two samples (three replicates total) collected at one site; De Fraites Wetland (Table 2). The presence of multiple individuals was confirmed at this site with three *T.s. elegans* caught in traps on the day of eDNA sampling. Amplification did not occur at the two remaining sites, including Wiley Park Upper where the presence of *T.s. elegans* was visually confirmed at the time of eDNA sampling. The universal fish assay amplified in all samples at all sites (excluding negative controls), providing confidence in the eDNA sampling and laboratory workflows. All Sanger sequence results were confirmed as a match to the target species and all controls (i.e., non-template, negative extraction, positive and field controls) performed as expected.

Discussion

We evaluated the suitability of two previously designed PCR primers for use in eDNA surveillance of the invasive red-eared slider turtle, *T.s. elegans* in Australia. Following development of a probe to compliment one primer set, we show that both assays are highly specific to detecting *T.s. elegans* to the exclusion of all other native turtle species found throughout Australia. Both assays were found to be reasonably sensitive (i.e., perfectly detecting 100 copies per reaction), making them well-suited to eDNA applications, although the Kakuda assay showed increased efficiency and was able to reliably detect lower eDNA concentrations. The Kakuda assay was further tested on water samples collected from a mesocosm site housing *T.s. elegans*, achieving 100% amplification success in all eDNA samples even one week after turtles had been removed. This highlights the ability of the eDNA assay to detect the species in high densities, even when individuals are no longer present in the system.

eDNA surveys conducted in this study at field sites also confirmed the ability of the assay to detect *T.s. elegans*, although detection rates were low. In the wild, turtle densities will be lower compared to that of our mesocosm study. Despite implementing robust and validated eDNA water sampling approaches (Hinlo et al. 2017), eDNA surveys failed to detect *T.s. elegans* at two out of three field sites. Although only a limited number of water samples (i.e., five samples and 15 technical replicates) were collected for eDNA analysis, traditional detection methods appeared to be more successful: visual detection of the species was confirmed at one site where eDNA methods failed to detect the

species (Wiley Park Upper) and for the site where eDNA detection did occur (De Fraites Wetland), eDNA amplification success remained low (occurring in 3/15 PCR replicates), despite trapping confirming the presence of multiple individuals on the day of sampling. At our final field site (Bonnyrigg Wetland), traditional detection methods had previously confirmed the presence of *T.s. elegans*, although it is possible that turtles were not present at the time of eDNA sampling: as a semi-aquatic species, they may have been situated out of the water or may have relocated to another waterbody, thereby reducing eDNA detection probabilities. Alternatively, *T.s. elegans* may have still been present in the waterbody but eDNA either failed to have been collected or successfully amplified in the water sampled.

eDNA detection probabilities can be influenced by the concentration of eDNA in the water which, in turn, is strongly influenced by the physiology and traits of species (Barnes and Turner 2016; Stewart 2019). Turtles pose a particular challenge to eDNA approaches (Adams et al. 2019; Raemy and Ursenbacher 2018). The ability of turtles to shed material may be compromised by their lack of a mucous layer, and hard keratinized skin (i.e., the 'shedding hypothesis' see Adams et al. (2019)), and consequently, turtles shed eDNA in a different way to taxa such as fish and amphibians (Harper et al. 2020; Raemy and Ursenbacher 2018). Turtle excretions have also been found to contribute less to eDNA than those from fish and amphibians (Akre et al. 2019; Harper et al. 2020). Some turtle species also exhibit traits such as brumation (decreased metabolic rate), which contributes to varied seasonal activity and will likely decrease eDNA concentrations (De Souza et al. 2016). Turtles can also wander on land or between sites, which influences the temporal and spatial heterogeneity of DNA across landscapes (Tarof et al. 2021; Troth et al. 2021). Furthermore, lentic systems such as urban ponds present further challenges to eDNA water sampling because the spatial configuration of eDNA in such systems is already potentially patchy and unevenly distributed (Harper et al. 2019; Lacoursière-Roussel et al. 2016). In combination, these traits and environmental factors are likely to influence and limit the production and deposition of eDNA into the water and affect the consistency of its availability for sampling. Despite eDNA water surveys successfully detecting turtles from contrived conditions (Davy et al. 2015; Raemy and Ursenbacher 2018) and across a range of environmental conditions (Feng et al. 2020; Kakuda et al. 2019; Tarof et al. 2021), detection in natural environments is often reported as being higher using conventional approaches (i.e., visual identification and trapping) (Adams et al. 2019; Akre et al. 2019; Fyson and Blouin-Demers 2021; Kakuda et al. 2019; Raemy and Ursenbacher 2018).

The non-invasive and cost-effective benefits of eDNA based approaches, however, could still prove useful for detecting turtle species as it would avoid the invasive nature of current survey methods, which can also be time and resource intensive (García-Díaz et al. 2017; O'Keeffe 2009). To increase the utility of eDNA surveys for challenging species like turtles, attempts can be made to increase eDNA detection probabilities. This can be done in several ways: (i) eDNA sampling strategies can be improved by devoting more resources to effort i.e., collecting more samples from a site, (ii) altering methods like choice of filtration and extraction method (Deiner et al. 2015), or (iii) by considering species-specific traits that may influence the deposition or accumulation of eDNA such as seasonality, and sampling location within each water body (Andruszkiewicz Allan et al. 2021; Wittwer et al. 2018). For example, crayfish eDNA detection probabilities were shown to be highest after egg hatching (Troth et al. 2021) while detection of European carp was shown to be greater in vegetated areas of water (Furlan et al. 2019). Proximity to the shedding source has also been found to affect eDNA detection (Deiner and Altermatt 2014; Dunker et al. 2016; Pilliod et al. 2014) – a pattern that the mesocosm results presented in this study suggest may also be occurring in *T.s. elegans*, even across the small distances investigated here. Alternatively, adopting an alternate eDNA sampling approach, such as through the collection of soil or swab samples can have a drastic effect on detection probabilities and has proven effective for other reptile taxa (Matthias et al. 2021).

Although eDNA has proven highly successful for the detection of multiple species world-wide, we must also consider that its application to certain species or taxa may be limited. In the context of management of *T.s. elegans* in Australia, densities of this priority pest species are likely to be extremely low at incursion sites, and therefore highly sensitive detection tools are required (Mo 2019). As a result of invasive pressures associated with the pet trade and other avenues, incursions could occur in a range of habitats and at any time of year, requiring effective and robust approaches across varying environments and regardless of temporal factors such as seasonality (Henderson and Bomford 2011). Our failure to reliably detect the highly invasive *T.s. elegans* from urban pond sites despite concurrent confirmation of their presence suggests that eDNA surveys conducted through analysis of water samples may add little value to current monitoring efforts for this invasive species. Hence, resources devoted to turtle surveillance may be better utilised elsewhere (e.g., visual surveys or trapping). With finite resources available for management efforts, establishing which detection survey approaches are to be implemented and how resources can be effectively utilised is essential to the overall conservation of biodiversity (Morisette et

al. 2021). For taxa such as turtles, which are challenging to detect with eDNA based-approaches, further investigation of eDNA shedding, availability and behavioural traits is needed to determine whether eDNA surveys can be targeted appropriately to sufficiently increase detection probabilities.

Conclusion

The management of invasive species is dependent on collaborative and effective use of limited resources. Species-specific detection of eDNA from water samples has been shown to be an extremely valuable tool for detection and monitoring a range of invasive species, providing a cost-effective and highly sensitive alternative to traditional detection methods. For certain taxa however, species-specific characteristics may reduce eDNA detection rates and render eDNA surveys a less appropriate option for monitoring species presence or absence than traditional surveys. Even though validated detection in mesocosms is necessary for eDNA workflow development, it is imperative that we consider eDNA detection rates in the field and evaluate whether eDNA surveys provide a cost-effective option for low-density applications. The high success rates generated from controlled experiments conducted on high density populations will rarely be translated to the wild where densities are likely to be lower. Evaluating which species present a challenge for eDNA based approaches is important to ensure resources are appropriately allocated and to avoid false negative detections leading to misguided management action. Continued exploration of alternative approaches that harness eDNA methods to achieve greater detection probabilities will facilitate more effective monitoring of reptiles.

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Declarations

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