Comparison of two Citizen Scientist Methods for Collecting Pond Water Samples for Environmental DNA Studies

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Abstract

The use of environmental DNA (eDNA) for the survey of aquatic species offers a wide range of benefits over conventional surveys and has begun to be used by citizen scientists. One advantage of eDNA over conventional survey protocols is the comparative ease with which samples can be collected over a wide geographic area by citizen scientists. However, eDNA collection protocols vary widely between different studies, promoting a need to identify an optimum method. Collection protocols include ethanol precipitation and various filtration methods including those that use electronic vacuum or peristaltic pumps, hand pumps or syringes to capture eDNA on a membrane. We compare the effectiveness of two eDNA collection methods suitable for use by citizen scientists: glass-microfiber syringe filtration and ethanol precipitation. Paired samples of water were analysed for great crested newt (*Triturus cristatus*) DNA using (1) a laboratory tank experiment using different dilutions of water inoculated with newt DNA; and (2) by sampling naturally colonised ponds. Although syringe filters consistently yielded greater DNA extract concentrations in the tank experiments, this was not the case in samples collected from the field where no difference between the two methods was identified. Clearly, properties within the water – such as algae and particulate matter - can influence the amount of DNA captured by the two methods, so the sampling protocol of choice will depend on the design and goals of the study.

1. Introduction

With threats to biodiversity increasing (Barnosky et al. 2011; Pimm et al. 1995), rapid biodiversity assessment and the ability to reliably detect rare species and species with patchy distributions is imperative for effective evidence-based conservation actions to be implemented (Ficetola et al. 2008; Magurran 2004). Citizen science generated data is widely used for species distribution assessments and other ecological research (Bonney et al. 2009; Van Strien, Van Swaay and Termaat 2013). However, various challenges exist in working with ecological citizen science data, most notably error and bias due to variation between observers (Dickinson, Zuckerberg and Bonter 2010). Accuracy of visual based species identification may be as low as 60% for non-experts (Austen et al. 2016).

Citizen science schemes for the monitoring of amphibians are active within the UK, for example the National Amphibian and Reptile Recording Scheme (NARRS) (Wilkinson and Arnell 2013). Surveys for amphibians, particularly the great crested newt (*Triturus cristatus*) within the UK, require the use of multiple methods and multiple survey visits to achieve a reasonable probability of detection (Griffiths, Raper and Brady 1996; Langton, Beckett and Foster 2001; Wilkinson and Arnell 2013). Surveys require a combination of torchlight visual searches of ponds at night, overnight bottle trapping, sweep netting to catch individuals, and searches of vegetation for eggs. Each of these survey methods requires a considerable amount of time at a pond; has health and safety implications for surveyors (for example working at night); can have variable reliability of detection influenced by environmental factors; and may require extensive training and licencing to ensure that it is carried out with minimum risk to target and non-target organism (Langton, Beckett and Foster 2001). Using all four of these methods combined in a single survey visit yields a probability of between 0.41-0.68 of detecting the presence of great crested newts. To improve confidence that a site is unoccupied to the 95% level requires between 3-6 visits using traditional methods (Sewell, Beebee and Griffiths 2010). As a result, a regional or landscape-wide survey programme can be logistically difficult, prohibitively expensive and require multiple visits and skilled surveyors with

58 taxonomic training (Biggs et al. 2015; Sewell, Beebee and Griffiths 2010). The intensity of the
59 survey methodologies and the number of visits required to achieve such high levels of
60 confidence in the results has the potential to lead to low volunteer retention (Pers. comm. Dr
61 John Wilkinson).

62 Given the issues with traditional survey methods for amphibians, there is a demand for
63 developing simple yet reliable survey methods that can be carried out by citizen scientists.
64 Environmental DNA (eDNA) surveillance is a technique where DNA is isolated from a sample
65 of environmental material and used to identify the presence of a species or community of
66 species through detection of DNA shed into the environment by the target species (Jane et al.
67 2015). Since 2008, eDNA has become a widespread tool for the detection of invasive aquatic
68 species (Ficetola et al. 2008; Jerde et al. 2011; Wilson et al. 2014) and species of conservation
69 importance (Biggs et al. 2015). Laboratory analysis based on DNA circumvents variation in
70 species identification between surveyors and should reduce inaccuracies in data sets
71 contributed to by many individuals.

72 Despite wide use by the research community there has been limited uptake of eDNA within
73 citizen science studies. However, its utility with citizen scientists, for the detection of great
74 crested newts, has been demonstrated by the Freshwater Habitats Trust Pond Net scheme
75 (Biggs et al. 2015; Freshwater Habitats Trust 2017) and the “great crested newt detectives”
76 project of Amphibian and Reptile Conservation in Scotland (Minting 2016) both within the UK.
77 The method has wide applicability with citizen science based studies, allowing assessments
78 of species distribution at scales that would make conventional or commercial surveys
79 prohibitive (Biggs et al. 2015; Gibson et al. 2015). Collecting environmental samples for eDNA
80 analysis requires little training and can be carried out quickly. Samples can be collected by
81 citizen scientists in the field at a time that suits them, with water samples returned to a central
82 location for shipment to a laboratory for analysis. DNA is then isolated and identified using
83 molecular techniques such as real-time quantitative PCR (qPCR) (Thomsen et al. 2012) or
84 metabarcoding (Valentini et al. 2016).

eDNA sample collection requires a much shorter time at the pond than traditional methods, and is often conducted using only a single sample collected during one visit, with detection probabilities much greater than for a single visit using conventional methodologies (Buxton, Groombridge and Griffiths 2018). The reduced number of visits required and lower intensity of sample collection will increase the number of sites a single volunteer can survey for the same effort, and may increase volunteer retention, facilitating an increase in scale for the study as a whole, and increasing statistical rigor (Wilkinson and Arnell 2013). Additionally, surveys targeting eDNA reduce disturbance to the studied species by reducing the number of visits required to the pond, removing the need to disturb the structure of a pond with sweep netting and removing any animal welfare concerns associated with trapping. eDNA also increases the accuracy of results by removing any ambiguity arising from visual species identification. As such eDNA offers a new tool for use by NGOs and other bodies to work with citizen scientists to generate large, accurate species distribution data sets. Nevertheless, if managed inappropriately, removal of contact with the target species in the field may reduce participant engagement.

A variety of methods have been used for the collection of aquatic environmental samples and when using eDNA it is vital to choose sampling methodologies that are appropriate for the goals of the research. To date, most studies have focused on how best to detect a target species from samples rather than the reliability of the sample collection protocol itself (Deiner et al. 2015; Goldberg et al. 2016). The most popular protocols use one of two approaches. Firstly, precipitation in ethanol, where a sample of water is preserved within a large volume of absolute ethanol and a small volume of a salt (Biggs et al. 2015; Deiner et al. 2015; Eichmiller, Miller and Sorensen 2016; Ficetola et al. 2008; Spens et al. 2016). Alternatively, a sample of water is passed across a micropore membrane to concentrate and preserve the DNA; however, the volume of water, membrane substrate and membrane pore sizes used vary considerably between studies (Goldberg et al. 2016). A summary of methods used by different studies has been collated by Rees et al. (2014b). The different sample collection and

112 extraction methodologies may have advantages and disadvantages, but few studies have
113 assessed how they perform against each other, or within different environments i.e. in ponds,
114 lakes, rivers and the marine environment. However, some studies have found filtration
115 recovers more DNA than ethanol precipitation (Deiner et al. 2015; Eichmiller, Miller and
116 Sorensen 2016; Spens et al. 2016); these however, focus on stream and lake environments
117 and do not take into account environmental conditions unique to ponds.

118 Likewise, not all sampling protocols are suitable for citizen science initiatives (Biggs et al.
119 2015). For example, many filtration protocols require the transport of large volumes of
120 unpreserved sample water, on ice, to a central location for filtration (Pilliod et al. 2013) while
121 others use expensive electronic pumping equipment in the field (Pilliod et al. 2013). Neither
122 approach is easily adopted by - nor practical for - citizen science studies, where volunteers
123 may each be expected to collect a small number of samples in a time frame fitted around other
124 commitments. Due to the very low concentrations of target DNA, it is imperative that eDNA
125 sample collection and extraction methods recover the highest amount of DNA possible in a
126 sample. Here we assess two eDNA collection methods that would be applicable to a citizen
127 science study with a large number of surveyors each collecting a small number of samples.
128 We evaluated these methods for their suitability for citizen science studies as they do not
129 require bulky, expensive or electronic pumping equipment or the transport of large volumes of
130 water to a central location for filtration, and can be supplied as individual sealed kits for each
131 sample.

132 We test the precipitation in ethanol method (Biggs et al. 2015), and syringe filtration (Deiner
133 et al. 2015) with glass microfiber syringe filtration method, aiming to identify whether eDNA
134 extract concentrations varied between the two methods as they would be used in the field. We
135 did this in both laboratory tanks and ponds, using water volumes applicable to the different
136 methods. We target the great crested newt, a semi-aquatic amphibian that has been widely
137 used as a study species within eDNA research and citizen science (Biggs et al. 2014, 2015;
138 Buxton et al. 2017; Buxton, Groombridge and Griffiths 2017; Minting 2016; Rees et al. 2014a,

2017; Thomsen et al. 2012), and one in which commercial eDNA analysis has been pioneered (Natural England 2014).

2. Methods

2.1. Field Samples

Two eDNA collection methods, precipitation in ethanol (hereafter referred to as 'ethanol precipitation'), and glass-microfiber filtration using syringe filters (hereafter referred to as 'filtration') were compared using water samples from a naturally colonised pond system. A network of eight small (600 L, 1 m by 2 m and up to 0.6 m deep) ponds used by great crested newts, at the University of Kent, Canterbury Campus (UK) were utilised (Buxton et al. 2017). Sixty-one pairs of eDNA samples were collected from the eight ponds, by experienced researchers, using the ethanol precipitation and filtration methods between March and September 2015, covering the period when adults and larvae are in their aquatic phase.

Prior to field collection all equipment was sterilised in 10% bleach and thoroughly rinsed with water, sterilised in an autoclave or UV-Crosslinker, and then sealed prior to transport to the study site. Due to the small size of the ponds each sample consisted of a single 1 L surface water sample from the pond centre. The order the two samples were taken in was randomised so as to remove sampling bias. A fresh set of disposable gloves were used for each of the samples to prevent contamination.

Sample collection followed the protocol outlined in Buxton et al. (2017). In brief, to collect the filtration samples a sterilised 1 L water bottle was unsealed and rinsed with pond water prior to being filled. A disposable 100 mL syringe was used to pass the sample water across a Sterlitech Corporation® glass-microfiber syringe filter (0.7 µm pore size, 30 mm diameter), refilling the syringe until 1 L had been filtered or the 2 filter units had become blocked. Two syringes of air were then passed through each filter to reduce the amount of residual water in the sealed unit. Filters were sealed in plastic bags and transported to the laboratory; the

maximum time between sample collection and reaching the laboratory was three hours, with samples then maintained at -20 ℃ until extraction.

Ethanol precipitation sample collection followed a protocol outlined in Buxton et al. (2017), originally from Biggs et al. (2015). In brief, six, 50 mL centrifuge tubes, each containing 33 mL of absolute ethanol and 1.5 mL of 3 M sodium acetate solution were filled to the 50 mL gradation with sample water using a disposable plastic pipette. This volume equates to approximately 15 mL of sample being placed into each of the 6 sample tubes and a total sample volume of approximately 90 mL. The lid to each tube was sealed, and the tube contents mixed by inversion. All six sample tubes were then placed in a sealable bag for transport to the laboratory, the maximum time between sample collection and reaching the laboratory was three hours, with samples then maintained at -20 ℃ until extraction.

2.2. Serial Dilution of Tank Water

A laboratory experiment was carried out using great crested newts under controlled conditions. Plastic boxes, dimensions 490 mm x 360 mm x 240 mm deep, were set up in a temperature controlled room (18 ºC ± 2 ºC), containing 10 L of tap water. The water was allowed to stand for a minimum of 24 hours to allow the water to naturally dechlorinate. Great crested newts were collected using the standard bottle trapping method (Langton, Beckett and Foster 2001) from a pond within the campus of the University of Kent and taken into captivity under licence from Natural England (Licence number 2015-10937-SCI-SCI). The newts were allowed to acclimatise to the temperature of the room in tanks containing water from their original pond before one newt was introduced to each experimental tank. The individuals were left in the study tanks for 24 hours before being removed and released into their original pond. Five replicate tanks were used between the 28th and 29th of April 2015, and an additional three replicates between the 14th and the 15th of May 2015.

Prior to sample collection all equipment was sterilised in 10% bleach and thoroughly rinsed with water, sterilised in an autoclave or UV-Crosslinker, and then sealed. Once the individuals

had been removed from the tanks, a 1 in 2 dilution series was performed on the tank water to create samples at 100%, 50%, 25%, 12.5%, 6.25% and 3.125%, of the starting concentration of the tank water. Dilution was undertaken with tap water, making 1 L of sample water at each dilution for each tank. The dilutions were made using the lowest concentration first in order to prevent contamination between levels. An ethanol precipitation eDNA sample of 0.09 L was initially taken, with the remainder of the water, totalling 0.91 L, then passed through two Sterlitech Corporation® glass-microfiber syringe filters (0.7 µm pore size, 30 mm diameter), in equal proportions, following the protocols described above. In total 48 pairs of samples were collected. Samples were then stored at -20ºC until DNA extraction.

2.3. Extraction Protocols

DNA extractions were conducted following the protocol outlined in Buxton et al. (2017). In brief, all extractions were undertaken in a UV sterilisable work station, with dedicated equipment, and were based on the Qiagen® DNeasy® Blood & Tissue kit with amended protocols as outlined. Periodic extraction blanks for both methods were undertaken through the laboratory phase of the project to check for equipment contamination.

2.3.1. Syringe Filtration

In a fume hood sterilised with a 10% bleach solution and UV-light the filter paper was removed from the sealed syringe filter holder using sterilised wire cutters and sterilised forceps. Once removed the filters were cut into strips approximately 3 mm in width with each filter placed into a separate 1.5 mL microcentrifuge tube. Thus, in the digestion step each sample consisted of two microcentrifuge tubes, one for each of the two filters. 675 µL of the ATL buffer from the DNeasy® Blood & Tissue kit (Qiagen®) was added to each tube; it was then vortexed for 15 seconds to mix before 20 µL of Pro K was added and again vortexed. The samples were then incubated on a rotating block, for 3 hours at 56 ℃ or overnight at 37 ℃. Following incubation the two digestion reactions for a sample were combined in a fresh microcentrifuge tube. DNA extraction continued as per the DNeasy® Blood and Tissue kit manufacturers' protocol, eluting into 200 µL of the elution buffer.

2.3.2. Ethanol Precipitation

eDNA extraction from ethanol precipitation samples was undertaken using a modified protocol from Biggs et al. (2014). The six centrifuge tubes were centrifuged at 10,020 g, (8500 rpm) for 35 minutes and the supernatant discarded. The remainder of the extraction protocol followed the modified Qiagen® DNeasy® blood and tissue kit protocol, from Biggs et al. (2014).

2.4. qPCR analysis

Following extraction, samples were stored at -20℃ until real-time qPCR could be undertaken. qPCR plate set up was conducted in a separate dedicated laboratory, also within a separate UV-sterilisable work station. qPCR was performed using qPCR primers and hydrolysis probe and assay designed by Thomsen et al. (2012) and validated by Biggs et al. (2014, 2015), using TaqMan® Environmental Master Mix 2.0 (Applied Biosystems®). Samples were run on a BIO-RAD® CFX Connect Real-Time PCR detection system, under thermal cycling conditions from Biggs et al. (2014, 2015). Eight qPCR replicates were performed on each sample (Ficetola et al. 2014). qPCR standards were created from a serial dilution of a great crested newt tissue extract, quantified using a Qubit® 2.0 fluorometer (Life Technologies®) with the double stranded DNA high sensitivity kit following manufacturers' instructions. Three standards were used in each assay, acting as positive controls and to allow quantification using a standard curve, negative qPCR controls were also included.

2.5. Analysis

All statistics were undertaken using R version 3.1.3. (R Development Core Team 2016), and the tests used are indicated within the results section. Median values were used in the analysis over arithmetic mean to prevent outlying qPCR replicates from affecting the extract concentration. Linear regression was conducted for both of the eDNA collection methods comparing dilution level and extract concentration. An analysis of covariance (ANCOVA) was then performed on the linear regression models to compare the effect of collection method on the DNA extract concentration. Wilcoxon-Pratt signed rank tests were then used to compare

filter and ethanol precipitation samples to ascertain whether there was a statistically significant difference between the two methods. Wilcoxon-Pratt signed rank tests were also used to identify any difference between the sampling methodologies between paired samples from the real ponds, in terms of eDNA concentration and eDNA score (i.e. the proportion of positive qPCR replicates).

3. Results

3.1. Samples from naturally colonised ponds

All positive field samples were found to be above the limit of quantification for this study, the minimum concentration that can be reliably quantified by the qPCR assay (Buxton et al. 2017). There was no difference in extract concentration from filters and ethanol precipitation paired samples collected from ponds (Wilcoxon-Pratt signed-rank test z=-1.03; p=0.30; Figure 1).

Figure 1: Paired eDNA samples collected from natural ponds. The black line indicates the point at which the two extract concentrations have equal DNA concentration. Sample pairs with greater extract concentration from the glass-microfibre filter collection method appear above the line and sample pairs with greater extract concentration from the precipitation method appear below the line. Due to logarithmic scale where one or both of a pair are a negative sample, no result is shown.

We analysed the eDNA score (proportion of positive qPCR replicates) for the two collection methods for all 61 paired eDNA samples. Again we found no significant difference between the sample collection methods (Wilcoxon-Pratt signed-rank test z=-1.0; p=0.319).

3.2. Experimental Serial Dilution

All samples from the experimental serial dilution fell above the qPCR minimum concentration for reliable quantification (limit of quantification) (Buxton et al. 2017). Regression analysis of the level of dilution on the final extract concentration was highly significant for both the ethanol precipitation ($t = 5.0$; $df = 46$; $p<0.0001$) and filter ($t = 6.3$; $df = 46$; $p<0.0001$) collection

methods. A significant interaction (ANCOVA: $F = 33.3$; $df = 1, 93$; $p<0.0001$) was found between the collection method and the sample dilution level. In addition to the significant difference in slope between the two eDNA collection methods, the intercept was also found to be different (precipitation = 2.541×10^{-5} ngul^{-1}; glass-microfiber filter = 0.003892 ngul^{-1}). This indicates that the effect of initial sample concentration on the final extract concentration depends on the collection method used (Figure 2).

Figure 2: Relationship between DNA extract concentration and the level of dilution. Each point represents the median qPCR concentration value for an eDNA sample for two collection methods at the different dilution levels. Open circles represent samples collected via filtration, closed circles represent samples collected via ethanol precipitation.

For each of the paired samples at all dilution levels the filtered samples yielded a greater extract concentration than the corresponding sample collected using the ethanol precipitation method (Figure 3). This result was highly significant (Wilcoxon-Pratt signed-rank test: $z = 6.03$, $p<0.0001$).

Figure 3: Paired eDNA samples collected from serial dilution of tank water. The black line indicates the point at which the two extract concentrations would have equal DNA concentration. Sample pairs with greater extract concentration from the glass-microfibre filter collection method appear above the line and sample pairs with greater extract concentration from the precipitation collection method appear below the line. Note the logarithmic scale for clarity.

4. Discussion

In experimental tanks where water contained no organic or particulate matter, filtration of 0.91 L of sample water using 0.7 µm glass-microfiber syringe filters recovered larger amounts of eDNA than ethanol precipitation with a sample volume of 0.09 L across the range of sample

293 water concentrations tested. However, when assessed in the field with real pond water no
294 significant difference between the collection methods was observed.

295 We compared two methodologies considered to be suitable for use within widespread citizen
296 science projects, where provision of equipment such as peristaltic pumps would not be
297 logistically or financially viable (Biggs et al. 2015). However, we recognise that other filtration
298 methodologies are available that require the use of pumping equipment, which may allow for
299 increased eDNA capture rates (Minamoto et al. 2016; Spens et al. 2016).

300 The concentrations of target DNA in the extracts from the laboratory tanks were within or
301 above the range of that observed in the natural ponds. Our results from the laboratory tanks
302 support previous work on lake water, which showed that filtration recovers greater amounts of
303 total and target eDNA than the ethanol precipitation method (Spens et al. 2016). However,
304 Spens et al. (2016) used different filters and sampling volumes from the present study.
305 Increased sample volumes used in the filtration method are likely to have been responsible
306 for the greater concentrations of eDNA recovered, when compared with the ethanol
307 precipitation method, in the tank experiment.

308 The difference in extract concentrations between the two sampling methods observed in the
309 laboratory tanks was not repeated in the field samples. This result may reflect the composition
310 of pond water compared to tap water. When processing natural pond water filters may become
311 blocked by suspended solids and algae which were not in the samples from laboratory tanks.
312 We found that that 0.91 L of water from laboratory tanks could easily be passed through two
313 filters. In contrast, in some field samples it was not possible to pass 500 mL of pond water
314 through two glass-microfiber filters disks. The lower water volume is likely to reduce the
315 amount of eDNA captured and therefore the quantity available for extraction. In contrast,
316 during the initial precipitation and centrifugation step with the ethanol precipitation samples,
317 suspended solids and algae precipitated out of solution with the eDNA collecting as a pellet
318 on the side of the tube. This additional material may have assisted in securing the eDNA in

319 the pellet, preventing it being discarded with the supernatant and increasing the amount of
320 DNA within the extract.

321 Pond water can differ from water found in rivers, lakes or the marine environment. Pond water
322 is more stagnant, allowing the build-up of algae and suspended solids to a greater extent than
323 lotic water or large lakes where stratification and wind action allow for water movement. The
324 results from our field experiment do not fully support the conclusions of Spens et al. (2016) or
325 Deiner et al. (2015), both of whom conclude that filtration outperforms precipitation. In our
326 experiment we observed no difference between the sampling methods when they are applied
327 to pond water. However, these two studies utilised lake and river water respectively and so
328 may not have faced the same limitations found with ponds.

329 Deiner et al. (2015) show that different combinations of sampling and extraction protocols are
330 appropriate when targeting different taxa, and conclude that it is imperative to pick the
331 combination best suited to the specific study, advice also advocated by Minamoto et al. (2016).
332 Our results suggest that this approach should be extended to environment type as well as to
333 taxon, given that the difference in recovery between the sample types, which we observed in
334 tank experiments, disappears with pond water.

335 Within the laboratory tank experiment there was a significant relationship between the level of
336 dilution and eDNA extract concentration, with more diluted samples showing a reduced extract
337 concentration. However, some samples exhibited greater extract concentration than more
338 heavily diluted samples collected from the same tank. Although this may result from sampling
339 error, it was apparent with both collection methods, but was more prominent in ethanol
340 precipitation samples. This finding suggests that even though concentration of eDNA extracts
341 are related to the amount of DNA within the water sampled, extraction efficiency between
342 samples may not be consistent, or the amount of eDNA within a sample may be heavily
343 influenced by the form that the eDNA takes (extracellular, single cells or aggregations of cells).
344 Assuming that the majority of eDNA collected is intracellular (Deiner et al. 2015; Rees et al.

345 2014b), concentration may be influenced by aggregations of cells within samples, with larger
346 water volumes used with the filtration samples helping to mitigate for this.

347 To detect statistically meaningful changes in pond occupancy by amphibians on a regional or
348 national scale using traditional visual based survey methods, the number of sites needed to
349 be visited and the survey effort required may be prohibitive (Biggs et al. 2014; Wilkinson and
350 Arnell 2013). This would be the same for citizen scientists or professional surveyors. New
351 survey methods, such as the targeting of eDNA, require fewer visits to a pond, less time on
352 site and more sociable working hours. This may allow surveys to be undertaken on a larger
353 scale, thereby improving estimates of occupancy and population change, which have been
354 goals of citizen science led national monitoring projects such as the Amphibian and Reptile
355 Conservations Trust (ARC) NARRS project (Wilkinson and Arnell 2013). However, there is a
356 financial cost associated with processing eDNA samples and the laboratory analysis would
357 need to be budgeted from the outset. The use of the eDNA technique by citizen scientists
358 within national or regional distribution assessment projects has been demonstrated within the
359 ARC Great Crested Newt Detectives project in Scotland (Minting 2016) and the Freshwater
360 Habitats Trust PondNet project (Biggs et al. 2015). These projects are managed centrally by
361 nature conservation charities working closely with a commercial laboratory. The laboratory
362 provides eDNA sampling kits to the volunteer surveyors and undertakes the genetic analysis.
363 The availability of eDNA survey methods for projects utilizing samples collected by citizen
364 scientists has the potential to be utilised for a wide range of taxa of conservation concern.

365 Citizen scientists clearly have options for eDNA sample collection. However, the collection of
366 filtered samples from natural ponds with filtration was challenging as filters can easily become
367 blocked and this may not lend itself to participant engagement and retention. Citizen scientists
368 are likely to vary in the level of perseverance when trying to pass water across a filter. This
369 may cause differences in the amount of water collected between individuals under the same
370 conditions, impacting the consistency of the results and reducing the efficiency of the filtration
371 method. Although filtration outperformed ethanol precipitation under experimental conditions,

citizen scientists would be sampling natural ponds. Given that no difference in performance was observed between the methodologies in the field, either methodology would seem to be equally applicable under the conditions encountered here. However, different methods may recover different amounts of eDNA in different situations. We recommend pilot studies are undertaken to identify the most appropriate method for individual studies; with decisions on the most appropriate method taking into account practical considerations relating to the two methods, and the specific study needs.

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