Comparison of manual filtration methods for on-site eDNA sample processing

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Foreword

This work was commissioned to review methods for the manual filtration of water samples for eDNA analysis and to assess the most commonly used methods for their ease of use (accessibility) by our own staff and citizen scientists. Feedback from different surveys had highlighted that the use of filters can cause discomfort for surveyors, and where multiple samples are taken in a day, as discomfort increases the volume of water filtered often decreases, reducing the effectiveness of the technique. The findings will be used to inform recommendations for the manual collection of eDNA water samples by both Natural England staff and citizen scientists where ease of use is important.

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

Executive summary

Background

The involvement of citizen scientists is increasingly recognised as a crucial component of public engagement in conservation science projects. The ease of use and wealth of information that can be gathered using environmental DNA (eDNA) samples has made collection of eDNA samples a preferred and popular citizen science activity. However, for the collected samples to be effectively used in species monitoring, there is a need to standardise the volume of water filtered at each sampling location.

In this report, a systematic literature search was combined with a grey literature search to identify the most promising manual filtration methods for the processing of water samples by citizen scientists. The top three methods were subsequently trialled by nine volunteers to assess the manual difficulty of each method and to determine the volume of water filtered.

Results

Three manual filtration methods were identified: (a) Filtration using a syringe attached to a 0.45 um sterivex filter; (b) Filtration using a syringe attached to a 0.45 um sterivex filter assisted by a silicone gun and (c) Filtration using a syringe attached to a Sylphium cartridge.

Nine volunteers trialled the three methods using water samples with three turbidity levels (12 Formazine Turbidity Unit (FTU), 29 FTU and 59 FTU). The results identified method (b) ‘Filtration using a syringe attached to a 0.45 um sterivex filter assisted by a silicone gun’ as having the lowest scores for physical difficulty and pain, whilst also filtering comparable water volumes to the other trialled methods. DNA concentrations and quality were comparable across the three trialled methods.

Recommendations

A number of recommendations can be made following this study:

1. Prior to the start of any citizen science project, a short pilot trial using water from the proposed sites would be valuable in determining the target volume of water to be filtered. This can then be communicated to citizen scientists. A water volume of 250-300 ml has successfully yielded good eDNA based metabarcoding results in samples collected by citizen scientists in the River Severn in 2021 and 2022 (Natural England unpublished report). Standardising the volume of water filtered is important when comparing results across sites; particularly when translating metabarcoding data into species abundance.

2. Sterivex combined with Qiagen DNeasy Blood and Tissue Kits comprise established equipment with reliable availability, facilitating temporal standardisation of current and future methods. Using the same methods for DNA extraction across years reduces method induced variability and improves temporal data comparison. This is important in long-term monitoring programmes.

3. According to this short study, the least painful and simplest method was the silicone gun assisted filtration through Sterivex filters.

4. If Sterivex filtration is used, the use of silicone guns could improve filtered volumes whilst reducing physical discomfort for the volunteers. However, their relatively large size could make sterilisation in the field prohibitive due to larger volumes of disinfectant having to be used on site. It is, thus, recommended that a separate silicone gun per sampling site should be provided to minimise cross-contamination risk.

Contents

Introduction 8

Methods 8

Literature search 8

Selection of manual filtration methods 9

eDNA filtration testing 9

DNA extraction 10

Costs 10

Results 10

Literature search 10

Selection of manual filtration methods 11

eDNA filtration trial 11

DNA extraction and presence of inhibitors 16

Discussion 16

Recommendations 16

References 17

Appendix 1: Advert used for recruiting volunteers 19

Appendix 2: Participant information and agreement forms provided to volunteers (Ethics ID: 46377) 20

Appendix 3: An example of the questionnaire the volunteers completed 27

Appendix 4: Filtration instructions provided to participants 29

METHOD 1: Sampling PROTOCOL 29

Method 2: Sampling PROTOCOL 33

METHOD 3- Sampling protocol 37

Appendix 5: List of studies included following the published literature search 41

Appendix 6: Anonymised participant results 41

Introduction

The involvement of citizen scientists is increasingly recognised as a crucial component of public engagement in conservation science projects (Biggs and others, 2015). Citizen science projects provide opportunities to promote nature conservation whilst also encouraging access to the countryside and open spaces. The ease of use and wealth of information that can be gathered using eDNA samples and eDNA-based species detection methods makes collecting eDNA samples a preferred and popular citizen science activity (Agersnap and others, 2022). The recent development of on-site manual filtration Tøttrup and others, 2021) has further simplified the handling of eDNA water samples as well as their shipping to processing labs. However, for the collected samples to be effectively used in species monitoring, there is a need to standardise the volume of water filtered at each sampling location.

The volume of water filtered is influenced by a combination of water turbidity, ease of filtration and physical abilities of the user. However, despite its importance, the focus in published papers has mainly been on comparing sampling methods solely on the quantity and quality of DNA they can produce, with very little consideration given to the ease of use and consistency in standardising the volume of water filtered.

This work has been designed to fill this knowledge gap through a literature review on manual filtration protocols for eDNA collection from water samples, followed by a trial of three manual filtration methods at differing turbidity levels. In addition, the presence of inhibitors in the three extraction methods was evaluated.

Methods

Literature search

Original research articles relating to environmental DNA were identified using two different electronic databases; Web of Science™ (Clarivate) and Scopus (Elsevier). Both databases include research literature from a broad range of relevant fields, including biological, earth and material sciences.

Within each database, two searches were performed: 1) “eDNA” AND “filter”, and 2) “environmental DNA” AND “filter”. The resulting articles were then screened to identify only those that used environmental DNA techniques with a filtration step, thereby excluding review articles and technical notes. In addition, articles that were inaccessible due to paywalls and/or publications in other languages were also excluded. For the retained articles, a spreadsheet was compiled to facilitate extraction of information that included details on the study system, taxa of interest and several methodological details, including the location of the filtration step, sampling details and the exact equipment used.

The “grey literature” was also searched via Google using the same search strings to identify any additional sample protocols from non-academic sources, collecting similar information as above. The extracted data were then used to identify suitable sampling methodologies for comparison.

Selection of manual filtration methods

The inclusion criteria for method selection were: i) Filtration methods that could be adapted for on-site manual filtration for eDNA collection and ii) Citizen science friendly methods (including the minimal use of chemicals on site and no manual handling or posting of large water volumes). All published papers and grey literature that met the inclusion criteria had further data extracted, such as: information on volume filtered (including a note of the DNA extraction method used per study); study type; location; water type; taxa and species of interest; turbidity levels and reported ease of filtration.

eDNA filtration testing

Three manual filtration methods were selected following the literature search and subsequent consultation with the NE project officer: (a) Filtration using a syringe attached to a 0.45 um sterivex filter; (b) Filtration using a syringe attached to a 0.45 um sterivex filter assisted by a silicone gun and (c) Filtration using a syringe attached to a Sylphium cartridge.

To test the effect of different turbidity levels on the ease of filtration, three turbidity levels were trialled: 12 Formazine Turbidity Unit (FTU), 29 FTU and 59 FTU. These correspond to intermediate and medium turbidity levels as per the Water Framework Directive, 2000/60/EC. Briefly, the three turbidity levels were created by mixing autoclaved soil with 1 L of water, followed by manual vigorous mixing prior to sampling.

An email advert for the work was circulated to students and staff members within the Department for Life and Environmental Sciences, asking for volunteers (Appendix 1). A total of nine volunteers were recruited and trialled three methods for the three turbidity levels in a randomised order. The work was approved by the ethics board at Bournemouth University (Ethics ID: 46377) and was independently approved by Natural England’s ethics board.

During the filtration test, volunteers received health and safety training, and were given time to read and sign the Participant Information Sheet and Participant Agreement Forms (Appendix 2). Following this, the questionnaire (Appendix 3) was explained to volunteers. At each volunteer station, volunteers were presented with the three methods, in their predetermined random order, accompanied with the water samples at the predetermined turbidity level. Each method was accompanied with a detailed protocol (Appendix 4). Once a volunteer was ready to begin, the lead researcher vigorously mixed the water sample, and the volunteer started the filtration.

Upon completion of each individual filtration, volunteers were instructed to rate the physical difficulty of the manual filtration and were encouraged to write comments on any issues/difficulty in following the written protocol in the comments box associated with each filtration method.

DNA extraction

DNA was extracted from the Sterivex filters using the Qiagen DNeasy Blood and Tissue kit (Qiagen) according to the manual published by the Environmental DNA organisation (Access the detailed protocol). DNA from the Sylphium filters was extracted using the Sylphium extraction manual (Access the Sylphium extraction manual) and Sylphium DNA extraction kit. The extracted DNA was quantified using the DNA nanodrop and a subsample of 27 samples were tested for PCR inhibitors using real time PCR. Specifically, each sample was spiked with 5 ng of *Sphaerothecum destruens* DNA which was then amplified in triplicate using the protocol described in Sana and others. (2018). Amplification of *S. destruens* DNA would indicate the absence of PCR inhibitors in the extracted eDNA.

Costs

Average costs per sampling kit were calculated using market prices as of 24th January 2023. The Sterivex kits must be assembled, whereas the Sylphium kits are posted in assembled packages. For the personnel costs of the Sterivex kits, one hour per 10 kits was costed at £20 per hour (this includes preparation and assembly). Costs were calculated per kit. No personnel costs were included for DNA extraction.

The costs per kit (as per January 2023) were:

Sylphium collection and DNA extraction: £24

Sterivex collection and Qiagen DNA extraction: £22

Results

Literature search

A total of 109 and 147 hits in Web of Science and Scopus respectively were recorded using the terms ‘eDNA AND filter’, and 80 and 935 hits in Web of Science and Scopus respectively were recorded using the terms ‘environmental DNA AND filter’.

A total of 124 papers met the screening criteria described in the methods and had full text access. The papers could be broadly categorised into methodological (methods validation) and research-based papers, with neither type reporting turbidity levels or ease of use. All

papers reported volume filtered and resulting DNA quality. Fifteen of the 124 papers described an exclusively manual filtration, with the majority using pump or drill assisted in-field filtration or lab filtration using vacuum suction. Six of the 15 papers that used manual filtration had used Sterivex filters. Sterivex filters also featured in the grey literature search. Volumes filtered manually ranged from 240 ml to 2 L, with water sources including both freshwater (ponds, lakes, rivers) and seawater.

Selection of manual filtration methods

The final selection of methods to be trialled was decided based on equipment accessibility and independence in generating the data. We rejected filter kits that required DNA extraction and analysis being conducted by a specific organisation or business. Sterivex filters were most often used in cartridge-assisted filtration and met the availability criteria (they are used in a number of industries and are available through a number of scientific equipment suppliers). The Sylphium kits met the criteria of manual filtration and availability, as they are supplied by a molecular ecology company and do not require the samples to be processed via the company.

A promising approach using gravity to filter the water was presented by Oka *et al*. (2022). This approach involved filtering water through a Sterivex filter attached to a plastic bag (through an especially printed 3D attachment) carrying the water, lifted at a height of two metres. This approach used gravity to push the water through the Sterivex filter. This method was tested on seawater, brackish and freshwater, with the authors concluding that at a height of two metres, filtration of freshwater was too time consuming. They recommended using either a greater height or vacuum assisted filtration. This method was, thus, rejected for the present study.

eDNA filtration trial

Nine volunteers (Age ranges, Figure 1) participated in the eDNA filtration study, with each volunteer filtering up to 3 L of water (1 L per turbidity).

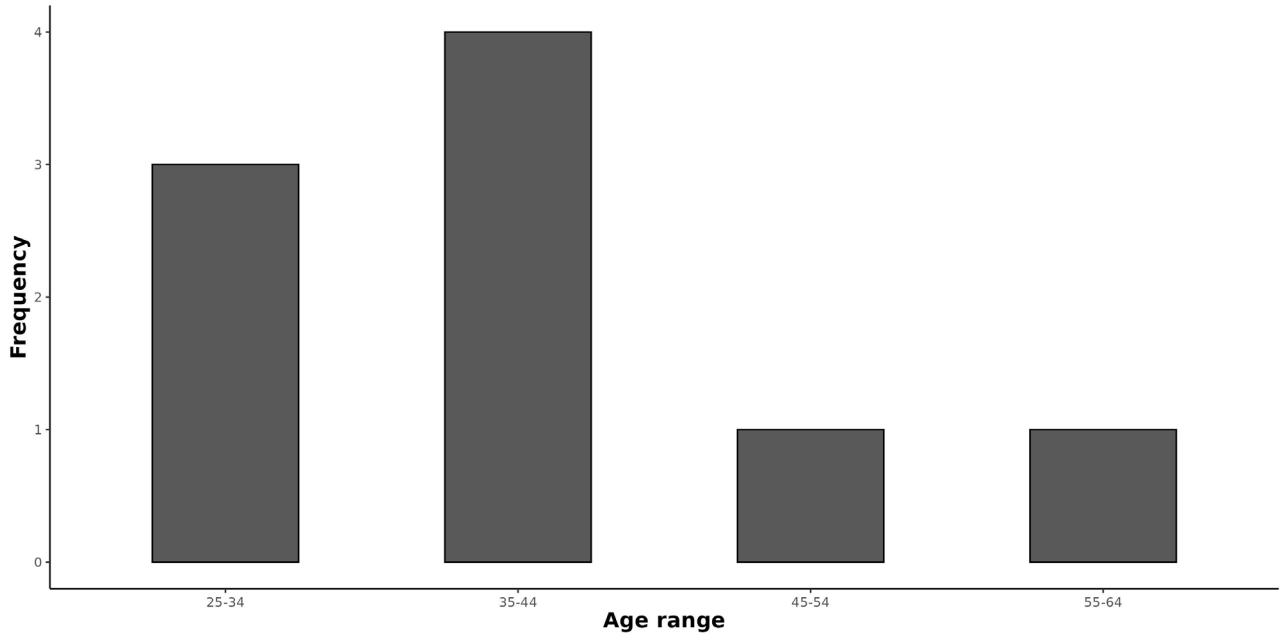

Figure 1 Age breakdown of the nine volunteers completing the filtration test.

Volume of water filtered by method and turbidity combination

A single participant did not record the volume filtered for a single method-turbidity combination (Sterivex with gun, medium turbidity); therefore, these data were excluded. The mean volume of water filtered ranged from 400 to 817 ml (Table 1). Overall, the volume filtered was consistent among turbidity levels, which was unexpected. To investigate this further, a statistical model investigating volume filtered relative to turbidity level and filtration order did not identify a significant interaction (Chi-square, d.f. 4, P = 0.8869). Furthermore, volunteers rated the higher turbidity as easier in terms of difficulty (Figure 1). Turbidity levels were confirmed prior to filtration; therefore, these results could be due to the small number of samples filtered per volunteer (n=3).

Table 1 Descriptive statistics for the volume of water filtered for different method and turbidity combinations. Mean volumes are presented ± Standard Deviation. Water volumes were grouped by method (n=9 per method) and tested for significant differences in filtered volumes using a Kruskal-Wallis test (P=0.21, d.f.: 2)

Method	Turbidity	Number	Mean volume filtered (ml)	Minimum volume (ml)	Maximum volume (ml)
Sterivex	Low	3	650 ± 278	400	950
Sterivex	Medium	3	400 ± 229	200	650

Method	Turbidity	Number	Mean volume filtered (ml)	Minimum volume (ml)	Maximum volume (ml)
Sterivex	High	3	500 ± 218	250	650
Sterivex with silicone gun	Low	3	667 ± 189	450	800
Sterivex with silicone gun	Medium	2	550 ± 283	350	750
Sterivex with silicone gun	High	3	800 ± 132	700	950
Sylphium	Low	3	433 ± 252	200	700
Sylphium	Medium	3	817 ± 231	550	950
Sylphium	High	3	593 ± 221	350	780

Difficulty levels by method and turbidity combination

Difficulty levels were lowest for the Sterivex with silicone gun method, followed by the Sterivex and Sylphium methods (Figure 2).

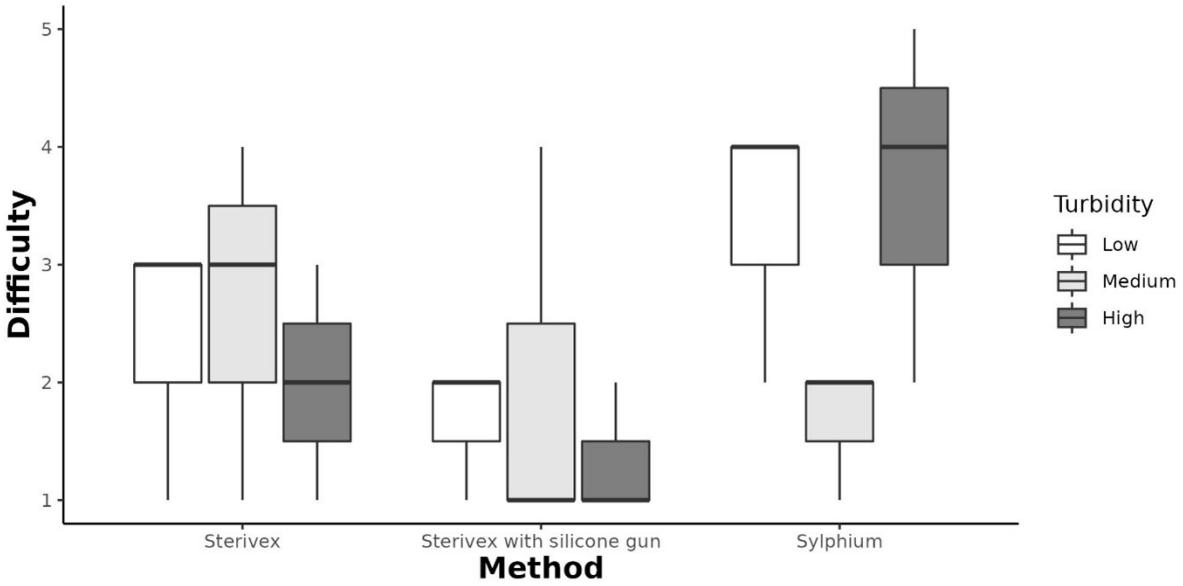

Figure 2 Reported difficulty scores for all method and turbidity combinations.

Pain levels by method and turbidity combination

The number of reported pain areas was lowest for the Sterivex with silicone gun method, followed by the Sterivex and Sylphium methods (Figure 3, Table 2).

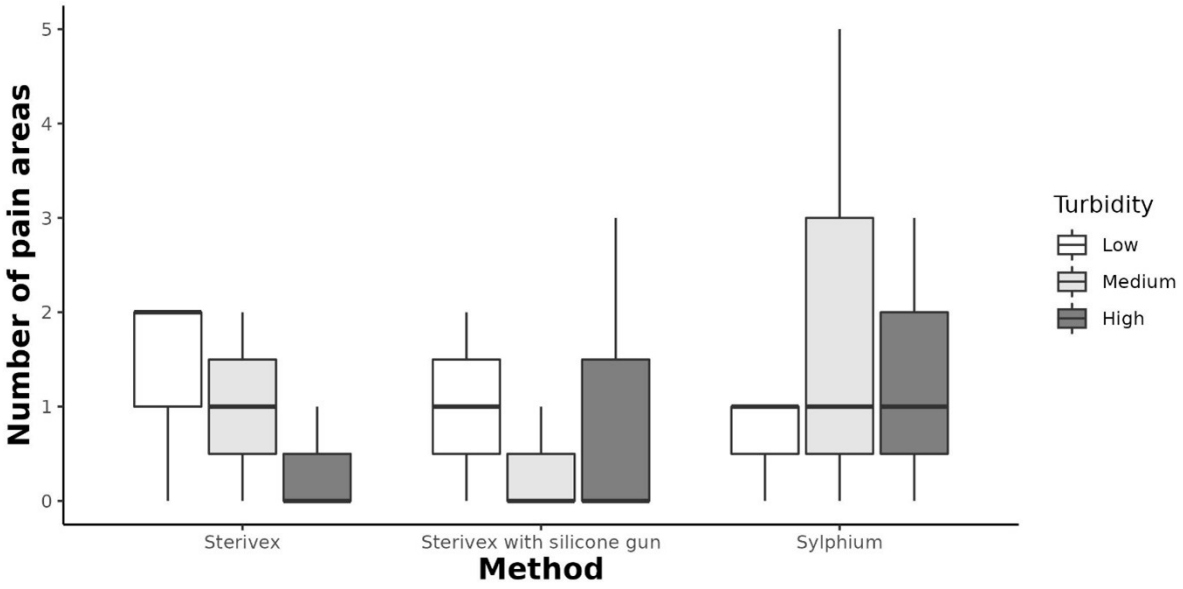

Figure 3 Reported number of pain areas for all method and turbidity combinations during processing.

Table 2 Breakdown of reported pain for combinations of methods and turbidities. The frequency of self-reported pain and the percentage of participants reporting pain within each of the categorised areas are given (n = 3 participants per method and turbidity combination). Thumb pain was categorised within "Digits" and palm pain within "Hands". Where a participant experienced pain within both hands etc. this was counted twice. As information was not gathered on the handedness of each participant, left and right are not differentiated.

Method	Turbidity	Arms	Digits	Elbows	Hands	Shoulders	Wrists
Sterivex	Low	0	1 (33%)	0	1 (33%)	1 (33%)	1 (33%)
Sterivex	Medium	0	3 (67%)	0	0	0	0
Sterivex	High	0	0	0	1 (33%)	0	0
Sterivex with silicone gun	Low	0	1 (33%)	0	2 (67%)	0	0
Sterivex with silicone gun	Medium	1 (33%)	0	0	0	0	0
Sterivex with silicone gun	High	0	1 (33%)	0	1 (33%)	0	1 (33%)
Sylphium	Low	0	0	0	1 (33%)	0	1 (33%)
Sylphium	Medium	0	3 (67%)	2 (33%)	0	1 (33%)	0
Sylphium	High	0	1 (33%)	1 (33%)	1 (33%)	1 (33%)	0

A single participant reported pain within 1 week of sample processing and indicated pain in their left wrist the day after the processing trial. No other participants reported pain after a week.

All anonymised results, including comments, can be found in Appendix 6.

DNA extraction and presence of inhibitors

The average DNA quantity extracted was 18 ng/ul, (SD 5 ng) and 19 ng/ul (SD 8 ng) from the Sterivex and Sylphium filters, respectively. *Sphaerothecum destruens* DNA was amplified from all samples, indicating the absence of PCR inhibition in eDNA extracted using the Sterivex and Sylphium filters.

Discussion

The literature search has revealed that there is little (published) emphasis on the accessibility of eDNA water sampling methods. This knowledge gap needs to be addressed, particularly when sample collection is driven by citizen scientists. The randomisation of methods and turbidity combinations in the current study has meant that each trial by turbidity combination was independently undertaken three times. This small sample size does lead to inherently large variation and it is important to note that differences in filtered volumes were non-significant (Kruskal Wallis test; P= 0.21, d.f. =2).

All three methods resulted in an average of over 400 ml of water being filtered across all three turbidity levels. The standard deviation around the mean is relatively high, which is not unexpected, given that each method-turbidity combination was only repeated in triplicate. This variation is representative of other reported filtered volumes from citizen science eDNA collection projects (Andreou personal communication). A minimal target volume indicated at the start of each citizen science eDNA collection project would allow standardisation of water collection and improve data interpretation (see Recommendations section below).

Of the three trialled methods in this study, the silicone gun assisted filtration through Sterivex filters appeared to be the best in terms of volume filtered, the reported difficulty level and reported discomfort. It is also a highly accessible method, as silicone guns are inexpensive and light and could, thus, be readily deployed to assist water filtration by citizen scientists.

The Sylphium kit had the highest perceived difficulty level and this could be due to the water being pulled through a cartridge using a syringe. This prevents the use of any modifications, such us using a silicone gun. For Sterivex filters, water is pushed through the cartridge using a syringe and the method is, thus, more amenable to modification.

The outcomes of this study can be used to inform citizen science eDNA collection, and these are summarised in the recommendations section below:

Recommendations

1. Prior to the start of any citizen science project, a short pilot trial using water from the proposed sites would be valuable in determining the target volume of water to be filtered.

This can then be communicated to citizen scientists. A water volume of 250-300 ml has successfully yielded good eDNA based metabarcoding results in samples collected by citizen scientists in the River Severn in 2021 and 2022 (Natural England unpublished report). Standardising the volume of water filtered is important when comparing results across sites; particularly when translating metabarcoding data into species abundance.

2. Sterivex combined with Qiagen DNeasy Blood and Tissue Kits comprise established equipment with reliable availability, facilitating temporal standardisation of current and future methods. Using the same methods for DNA extraction across years reduces method induced variability and improves temporal data comparison. This is important in long-term monitoring programmes.

3. According to this short study, the least painful and simplest method was the silicone gun assisted filtration through Sterivex filters.

4. If Sterivex filtration is used, the use of silicone guns could improve filtered volumes whilst reducing physical discomfort for the volunteers. However, their relatively large size could make sterilisation in the field prohibitive due to larger volumes of disinfectant having to be used on site. It is, thus, recommended that a separate silicone gun per sampling site should be provided to minimise cross-contamination risk.

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Appendix 1: Advert used for recruiting volunteers

Evaluating eDNA sampling kits for citizen scientists.

Investigators: Demetra Andreou, Ben Parker

You are invited to participate in a short study comparing three manual filtration methods for collecting eDNA from water samples.

You will be asked to filter water through a filter cartridge attached to a syringe and provide feedback on volume filtered and the ease of the filtration.

You will be required to be available for a maximum of 1 hour.

Participation is voluntary and you must be 18 years old or over.

When: December 13th 2022, 12-2pm

Where: C223

An example of the filtering equipment you will use.

Appendix 2: Participant information and agreement forms provided to volunteers (Ethics ID: 46377)

Participant Information Sheet

The title of the research project

Comparison of manual filtration methods for on-site eDNA sample processing

Invitation to take part

You are being invited to take part in a research project. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part.

Who is organising/funding the research?

The work is organised and conducted by Bournemouth University.

The work is funded by Natural England.

What is the purpose of the project?

The use of environmental DNA in water samples is extensively used to track species distribution and biodiversity. The collection of water samples and on-site filtration has been shown to be an effective and engaging way to include citizen scientists when collecting samples. The ease of filtration influences accessibility as well as the amount of water filtered.

In this project, we will trial 3 different methods of manual filtration of water samples. Volunteers will be asked to filter samples of low, medium and high turbidity using the 3 methods, and then evaluate the ease of use of these methods.

We will require 2 hours of your time to complete this work.

Why have I been chosen?

We are looking for 9 participants for our project.

Participants must be 18 years old or over.

You have been chosen as a representative member of the public to participate in citizen science involving the filtration of water.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a participant agreement form. We want you to understand what participation involves, before you make a decision on whether to participate.

If you or any family member have an on-going relationship with BU or the research team, e.g. as a member of staff, as student or other service user, your decision on whether to take part (or continue to take part) will not affect this relationship in any way.

Can I change my mind about taking part?

Yes, you can stop participating in study activities at any time and without giving a reason.

If I change my mind, what happens to my information?

After you decide to withdraw from the study, we will not collect any further information from or about you.

As regards to the information we have already collected before this point, your rights to access, change or move that information are limited. This is because we need to manage your information in specific ways in order for the research to be reliable and accurate. Further explanation about this is in the Personal Information section below.

What would taking part involve?

You will be asked to filter water using a syringe attached to a filter (please see Fig. 1). Water will have of 3 turbidity levels – low, medium and high. Following each filtration event, you will be asked to fill in a questionnaire answering questions on the ease of filtration, volume of water filtered, or any physical discomfort felt whilst filtering the samples.

One week following the filtration you will receive an email asking whether you have felt any discomfort or pain. We have included this follow-up to make sure that later onset discomfort is accounted for.

Figure 1: Syringe attached to filter (one of the 3 methods to be used in this study).

Will I be reimbursed for taking part?

You will NOT be financially compensated for your time. However, we hope that you find the project interesting and would be willing to participate.

What are the advantages and possible disadvantages or risks of taking part?

Whilst there are no immediate benefits to you participating in the project, it is hoped that this work will help make the filtration of water samples during citizen science events more accessible to all.

Whilst we do not anticipate any risks to you in taking part in this study, you may feel some discomfort whilst manually filtering your samples. If so, you will be asked to stop the process as soon as you feel any such discomfort and provide feedback using the set questionnaire.

What type of information will be sought from me and why is the collection of this information relevant for achieving the research project's objectives?

You will be asked your age group and your perceived physical fitness level. We ask for your age group as we would like the age distribution of our volunteer group to be the representative of the general public. We ask for your perceived fitness level because we will ask you to perform a physically based activity (manual filtration for water, using a syringe and attached filter).

You will also be asked specific questions to assess the ease of use of the three tested filtration methods.

You will be asked for your email address for the 1 week follow up. As soon as we hear back from you after 1 week, all data will be anonymised and your email address will be safely discarded (shredded and securely discarded).

Will I be recorded, and how will the recorded media be used?

You will not be recorded.

We will only hold on to your email address for 1 week in order to perform the 1 week follow up, after which your email address will be securely discarded.

We will not collect any information that will personally identify you.

How will my information be managed?

Bournemouth University (BU) is the organisation with overall responsibility for this study and the Data Controller of your personal information, which means that we are responsible for looking after your information and using it appropriately. Research is a task that we perform in the public interest, as part of our core function as a university.

Undertaking this research study involves collecting and/or generating information about you. We manage research data strictly in accordance with:

- Ethical requirements; and
- Current data protection laws. These control use of information about identifiable individuals, but do not apply to anonymous research data: "anonymous" means that we have either removed or not collected any pieces of data or links to other data which identify a specific person as the subject or source of a research result.

BU's Research Participant Privacy Notice sets out more information about how we fulfil our responsibilities as a data controller and about your rights as an individual under the data protection legislation. We ask you to read this Notice so that you can fully understand the basis on which we will process your personal information.

Research data will be used only for the purposes of the study or related uses identified in the Privacy Notice or this Information Sheet. To safeguard your rights in relation to your personal information, we will use the minimum personally-identifiable information possible and control access to that data as described below.

Publication

You will not be able to be identified in any external reports or publications about the research without your specific consent. Otherwise your information will only be included in these materials in an anonymous form, i.e. you will not be identifiable.

Research results will be published as a report for Natural England in March 2023

Security and access controls

BU will hold the information we collect during the work in hard copy in a secure location and on a BU password protected secure network where held electronically.

We will collect no personal information and the questionnaires will not be identifiable.

Your email address will be linked to your questionnaire for 1 week until the follow up email, after which, your email address will be removed from the questionnaires and will be securely discarded.

Further use of your information

The information collected about you may be used in an anonymous form to support other research projects in the future and access to it in this form will not be restricted. It will not be possible for you to be identified from this data.

Keeping your information if you withdraw from the study

The data collected is anonymised from the start of the project therefore if you withdraw from active participation in the study we will not be able to remove these data.

You can find out more about your rights in relation to your data and how to raise queries or complaints in our Privacy Notice.

Retention of research data

Project governance documentation, including copies of signed **participant agreements**: we keep this documentation for a long period after completion of the research, so that we have records of how we conducted the research and who took part. The only personal information in this documentation will be your name and signature, and we will not be able to link this to any anonymised research results.

Research results:

As described above, during the course of the study we will anonymise the information we have collected about you as an individual. This means that we will not hold your personal information in identifiable form after we have completed the research activities.

You can find more specific information about retention periods for personal information in our Privacy Notice.

We keep anonymised research data indefinitely, so that it can be used for other research as described above.

Contact for further information

If you have any questions or would like further information, please contact Dr Demetra Andreou, dandreou@bournemouth.ac.uk

In case of complaints

Any concerns about the study should be directed to Professor Tiantian Zhang, Deputy Dean for Research & Professional Practice, Faculty of Science & Technology, Bournemouth University by email to researchgovernance@bournemouth.ac.uk.

Finally

If you decide to take part, you will be given a copy of the information sheet and a signed participant agreement form to keep.

Thank you for considering taking part in this research project.

1. Ref & Version: eDNA sample filtration, v1
2. Ethics ID number: 46377
3. Date:18/11/2022

Bournemouth University

Participant Agreement Form

Full title of project: Comparison of manual filtration methods for on site eDNA sample processing

Name, position and contact details of researcher: Dr Demetra Andreou, Principal Academic In Environmental Science, dandreou@bournemouth.ac.uk

To be completed prior to data collection activity

Section A: Agreement to participate in the study

You should only agree to participate in the study if you agree with all of the statements in this table and accept that participating will involve the listed activities.

I have read and understood the Participant Information Sheet (eDNA sample filtration, v1) and have been given access to the BU Research Participant Privacy Notice which sets out how we collect and use personal information (https://www1.bournemouth.ac.uk/about/governance/access-information/data-protection-privacy).
I have had an opportunity to ask questions.
I understand that my participation is voluntary. I can stop participating in research activities at any time without giving a reason and I am free to decline to answer any particular question(s).
I understand that taking part in the research will include the following activities as part of the research:
• Filtering water of different turbidity levels using a syringe attached to a filter.
• Fill in a questionnaire which includes questions on the ease of filtration, volume of water filtered and /or any physical discomfort felt whilst filtering the samples.
I understand that, if I withdraw from the study, I will also be able to withdraw my data from further use in the study **except** where my data has been anonymised (as I cannot be identified) or it will be harmful to the project to have my data removed.
I understand that my data may be used in an anonymised form by the research team to support other research projects in the future, including future publications, reports or presentations.

	Initial box to agree
I consent to take part in the project on the basis set out above (Section A)	

Section B: The following parts of the study are optional

You can decide about each of these activities separately. Even if you do not agree to any of these activities you can still take part in the study. If you do not wish to give permission for an activity, do not initial the box next to it.

	Initial boxes to agree
I agree to providing my email address and being contacted by BU researchers 1 week following the filtration exercise to provide follow up information on any discomfort that I might have experienced after the event	

I confirm my agreement to take part in the project on the basis set out above.

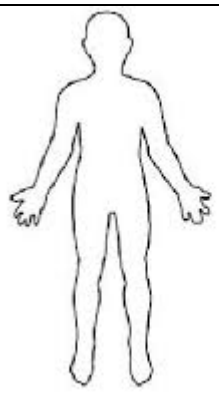
Name of participant (BLOCK CAPITALS)	Date (dd/mm/yyyy)	Signature
Name of researcher (BLOCK CAPITALS)	Date (dd/mm/yyyy)	Signature

Once a Participant has signed, **please sign 1 copy** and take 2 photocopies:

- Original kept in the local investigator's file
- 1 copy to be kept by the participant (including a copy of PI Sheet)

Appendix 3: An example of the questionnaire the volunteers completed

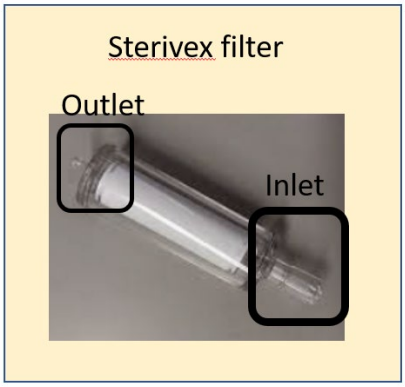
E-mail: You will receive an email to report any discomfort or pain that you might have felt 1 week after the filtering.	

Volunteer number: Date:			
Age group (please circle):	18-24 25-34 35-44 45-54 55-64 65 and over		
Please rate your physical fitness level:	Low Medium High		
		Volume filtered	Please circle the corresponding difficulty level (1 very easy – 5 very difficult)
Filtration method Turbidity	Ease of use		1 2 3 4 5
Please STOP filtering as soon as you feel any discomfort.			
Any pain or discomfort [please circle body part(s)]			
Additional comments?			

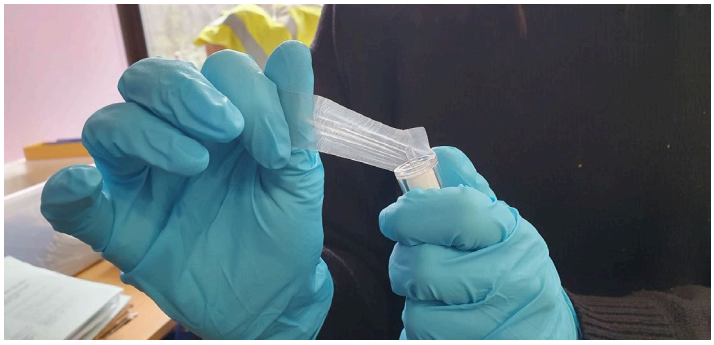
Appendix 4: Filtration instructions provided to participants

METHOD 1: Sampling PROTOCOL

GLOVES, labcoat and protective goggles must be worn at all times of sampling



Step 1: Use the syringe to draw up water up to 50 ml.

Step 2: Attach syringe to Sterivex filter unit (inlet side) Take care NOT to overtighten. Perform pressure filtration in nearby sink. **Repeat steps 1 and 2 until the filter is clogged or you feel discomfort or pain.** **<u>Please note the number of repeats and note the total volume of water filtered.</u>**	
Step 3: When the filtration is finished, remove the Sterivex filter unit and fill the syringe with air. **Reattach the Sterivex filter unit to the syringe, and push out the residual moisture from the filter unit.** **Repeat this procedure several times until no water comes out of the filter unit.**	
Step 4: Seal the outlet post of the Sterivex filter unit with parafilm. **Parafilm:**	

Step 5: Pipette RNAlater from the microcentrifuge tube using a disposable pipette.

Microcentrifuge:

Disposable pipette:

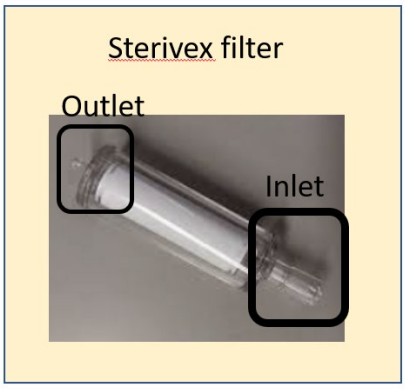
	
Step 6: Inject the RNAlater to the Sterivex filter unit from the inlet port using the disposable pipette.	

Step 7: Seal the inlet port with white cap.	

*Adapted from Chapter 3 of the eDNA society; Environmental DNA Sampling and Experiment Manual Version 2.1 (Miya & Sado, 2019; Minamoto et al. 2020)

Method 2: Sampling PROTOCOL

GLOVES, labcoat and protective goggles must be worn at all times of sampling



Step 1: Use the syringe to draw up water up to 50 ml.

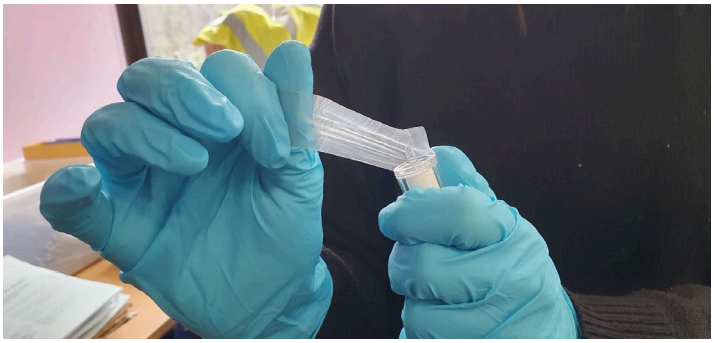
Step 2: Attach syringe to Sterivex filter unit (inlet side). Take care NOT to overtighten.

Place the syringe with sterivex filter within the silicone gun. Secure and filter.

Perform pressure filtration in nearby sink.

Repeat steps 1 and 2 until the filter is clogged or you feel discomfort or pain.

<u>Please note the number of repeats and note the total volume of water filtered.</u>

Step 3: When the filtration is finished, remove the Sterivex filter unit and fill the syringe with air. **Reattach the Sterivex filter unit to the syringe, and push out the residual moisture from the filter unit.** **Repeat this procedure several times until no water comes out of the filter unit.**	
Step 4: Seal the outlet post of the Sterivex filter unit with parafilm. **Parafilm:** 	
Step 5: Pipette RNAlater from the microcentrifuge tube using a disposable pipette. **Microcentrifuge:**	

Disposable pipette:

Step 6: Inject the RNAlater to the Sterivex filter unit from the inlet port using the disposable pipette.

Step 7: Seal the inlet port with white cap.	

*Adapted from Chapter 3 of the eDNA society; Environmental DNA Sampling and Experiment Manual Version 2.1 (Miya & Sado, 2019; Minamoto et al. 2020)

METHOD 3- Sampling protocol

GLOVES, labcoat and protective goggles must be worn at all times of sampling

1.Connect the valve connector to the syringe

2. Place the inlet of the eDNA dual filter in the water. Draw the water into the syringe by pulling the syringe plunger. Avoid air bubbles.

3. Empty the syringe into the adjacent sink by pushing the syringe plunger.

4. Repeat steps 2 and 3 until the filter is clogged or you feel any pain or discomfort. Make a note of how many times you repeat steps 2 and 3.

5. When finished, remove all the water from the eDNA dual filter capsule by pulling the filter from the water and drawing air though the filter. During this step, keep the eDNA dual filter capsule connected to the valve connector and the syringe.

6. Remove the double filter capsule from the silicone tubing connected to the connector.

Place the blue cap of the 5ml syringe on the outlet of the filter capsule.

Connect the 5ml syringe with the preservation solution to the inlet of the capsule.

Hold down the filter capsule and push the preservative solution into the capsule.

7. Pull the plunger back to 3ml to release pressure and remove the syringe from the dual filter capsule.

It is not necessary to remove all the air from the dual filter capsule.

Place the other supplied blue cap on the inlet of the filter capsule.

Appendix 5: List of studies included following the published literature search

This table is available in the excel file ‘NECR491 Appendix 5 and 6.xlsx’. Some of the cells in this table have been deliberately left blank.

Appendix 6: Anonymised participant results

Appenidix 6 is available in the excel sheet ‘NECR491 Appendix 5 and 6.xlsx’. Anonymous participant 6 was the only participant that reported pain at the one-week follow-up point, they reported pain in the left wrist the day after the filtration. Some of the cells in the table have been deliberately left blank.

www.gov.uk/natural-england