Invertebrate traps and DNA – literature review

Survey of sample preservatives compatible with DNA analysis

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Invertebrate traps and DNA – literature review

Helen C. Rees

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Natural England Project manager

Debbie Leatherland debbie.leatherland@naturalengland.org.uk

Contractor

RSK ADAS Ltd.

Author

Dr Helen C. Rees helen.rees@adas.co.uk

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Further information

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Foreword

DNA – based methods offer a significant opportunity to change how we monitor and assess biodiversity. However, for most techniques, there is still much development required before they can be used in routine monitoring. Natural England has been exploring the further use of these methods for environmental monitoring for several years, delivering a series of reports which focus on the development of DNA-based methods with potential in a particular area.

Proof of concept studies have demonstrated that DNA has the ability to change the way some of our terrestrial invertebrate monitoring is carried out, but that a thorough review of peer reviewed, and grey literature was required around sampling techniques (particularly focused on traps) and subsequent laboratory processes for DNA analysis, to allow field guidance to be produced with recommended techniques, preservatives and length of time in traps.

This project carried out the literature review, and also provides an understanding of the associated strengths and weaknesses of the different techniques and provides options for practical testing and gap filling.

Executive summary

A targeted literature review was carried out to investigate methods for terrestrial invertebrate sampling for use with DNA analysis techniques. The aim of the review was to investigate the current state of knowledge for the sampling and preservation of terrestrial invertebrates and compare and assess these methods. The review included studies that had been subject to peer review along with grey literature and personal communications from individuals within organisations known to carry out terrestrial invertebrate sampling. The findings of the review indicate that propylene glycol may better preserve DNA for downstream applications than ethanol despite general consensus that 100% ethanol is the best preservative, although this statement is made with caution as the sample number was small. In general, there was considerable variation between methods at every stage in the process and information which could have been useful for example biotic and abiotic factors was most often not included. Several studies did not state the concentration of the preservative used, the sampling duration, or included information on long term storage conditions, all of which would have provided valuable insight into the potential reproducibility of the studies. Options for practical method testing are given with a view to the standardisation of trapping methods currently used by Natural England.

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1. Introduction

Natural England is the Government's advisor for the natural environment. It provides practical advice on how to safeguard England's natural wealth for the benefit of everyone. ADAS is an environmental consultancy which exists to provide ideas, specialist knowledge and solutions to secure our food and enhance the environment.

DNA based methods could significantly change how biodiversity monitoring and assessment is carried out with potential benefits including making the process more efficient and removing potential ambiguity that might be associated with conventional taxonomic classification. There are well over 30,000 different species of invertebrates in the UK (Key *et al.,* 2000) and it can take many years to become an expert on species identification. Natural England would therefore, like to evaluate the different methods that have been reported for terrestrial invertebrate sampling for use with DNA techniques by way of a targeted literature review of both peer reviewed and grey literature. Sampling and preservation methods need to be compatible with downstream DNA analysis techniques such as species and community identification via DNA barcoding and metabarcoding.

1.1 Invertebrate Identification

Insects are one of the most diverse and numerous animal groups and their identification can be both painstaking and resource-demanding (Hernandez 2020). Traditionally, taxonomic research projects or biomonitoring campaigns would involve collection of invertebrates, sorting, and species identification followed by an analysis of the results. Sorting is time consuming, however, it is the species identification which causes bottlenecks as this requires taxonomic expertise for each insect group requiring examination. DNA based methods have the potential to address this identification bottleneck and is usually based on analysis of a short (658bp) fragment of the Cytochrome oxidase I (COI) gene, known as DNA barcoding (Hebert *et al.,* 2003). DNA barcoding can help with the identification of cryptic species and different life stages and can greatly reduce the sample processing time (Hebert *et al.,* 2004; Janzen *et al.,* 2009; Telfer *et al.,* 2015). To be able to use DNA for species identification global reference databases such as BOLD (Hebert and Ratnasingham 2007) have been developed in collaboration with taxonomists covering all groups of organisms. More recently, the development of high throughput sequencing methods, or metabarcoding, has allowed the identification of whole communities such as that found in a trap catch (Pompanon *et al.,* 2011; Riaz *et al.,* 2011; Taberlet *et al.,* 2012).

To be able to identify species by their DNA, the specimens need to be collected in such a way that prevents the DNA from degrading from the time the specimen is caught to the time the sample can be analysed – a period that can be measured in days to weeks and - usually entailing the use of a preservative solution. Traditionally, insects have been preserved in 70% ethanol for taxonomic identification (Martin 1977), however, it has been shown that long term storage (many years) in 70% ethanol can lead to DNA degradation

making DNA extraction and PCR amplification problematic (Baird *et al.,* 2011, Carew *et al.,* 2017). Preservation of insects in 95-99% ethanol which would preserve the DNA well can lead to insects becoming brittle and more difficult to work with if required for taxonomic identification as well (King and Porter, 2004). There is therefore a balance that needs to be found between being able to preserve good quality DNA with a method that also allows the preservation of delicate specimens for conventional taxonomic identification. It is hoped that the need for this balance will be infrequent as sequence libraries are improved and DNA techniques are further developed. This review identifies current practices in insect collection and preservation and evaluates the suitability of these for the isolation of good quality non-degraded DNA for further analysis.

1.2 Invertebrate Traps

Invertebrates are often collected to track shifts in community composition and temporal succession for example such as the Global Malaise Trap Program (Geiger *et al.,* 2016) a worldwide collection and DNA barcode identification program using this sampling method. There are a variety of methods used for the collection of terrestrial invertebrates including: hand collection; sweep netting; and trapping. Currently, Natural England employs the use of pitfall traps, vane traps (flight interception traps), and on occasion, Malaise traps in their current biodiversity monitoring and assessment as well as sweep nets and other hand collecting.

Pitfall Traps

A pitfall trap is a simple device used to collect small animals, usually invertebrates. The simplest format of a pitfall trap is a buried container with its top level to the ground. Animals wandering by fall into the trap and are unable to climb back out. There are many modifications that can be made, including the use of roofs – leaving a gap to allow animals to fall in – the use of walkways (lollipop sticks) across the trap, and fences to stop larger animals wandering into the trap. Pitfall traps are either wet – containing a solution designed to kill and preserve the specimens – or dry but can also contain a lure/bait.

Vane Traps

Vane traps in the style generally used by Natural England consists of intersecting panels of Perspex, connected through a funnel to a screw-on collecting bottle. A Perspex roof slots on top of the panels and a layer of chicken wire is laid between the bottom of the panels and the top of the funnel to stop unwanted objects falling into the bottle and act as a barrier to larger animals (Figure 1C). Rope is used to attach the vane traps to suitable trees providing a vertical barrier to insect flight that is thought to be invisible to them. On collision with the panel, beetles and other insects will often drop down and fall into the collection bottle.

Malaise Traps

Malaise traps (Malaise, 1937) are widely used for insect collection and are based on a large tent like structure for the trapping, killing, and preserving of flying insects. Insects fly into the wall of the tent and are then funnelled into a collection bottle at its highest point. Traps must be located such that a maximum number of flying insects will pass through the opening, for example high vegetation around this opening can limit the number of flying insects that can enter the trap. A well-placed trap can collect up to 1000 insects per day (Gressitt and Gressitt 1962).

Figure 1. Example of: A. Malaise trap (© Jo Hackman); B. a pitfall trap (© Adrian Gardiner), and C. a vane trap (© Jon Webb) installed within a hollow in an oak tree.

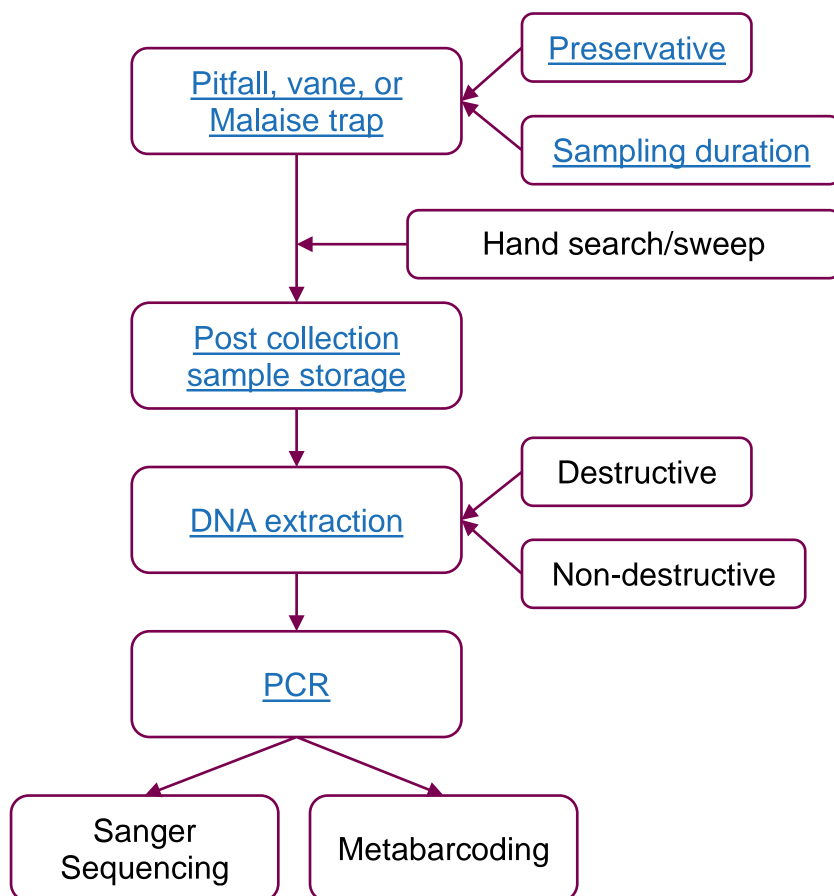

Figure 2. Flow chart showing the steps involved in invertebrate sampling and DNA analysis.

1.3 Aims and Objectives

This study set out to perform a targeted literature review that would explore the current state of knowledge and best practice regarding the different steps involved in terrestrial invertebrate sampling for DNA monitoring especially in the context of pitfall traps (Figure 2). This information was used to perform an assessment and comparison of the studies found where the likelihood of errors, confidence in results and potential for optimisation and standardisation for different applications. Suggestions for practical method testing and validation including risks and benefits and estimated costs have been made. This report details the methodology employed in this study and the results obtained before comparison and assessment of these results along with recommendations for studies to test any potential optimisation and standardisation of the methods found.

2. Methods

2.1 Database selection

Several literature databases were used, including: Pubmed; Web of Science; Science Direct; and Google Scholar. Grey literature was sourced by applying search terms to the Google search engine. Various individuals within organisations known to carry out terrestrial invertebrate sampling and subsequent DNA analysis were also contacted with a list of questions to determine the methods that they use.

2.2 Defining the questions to be addressed during the review.

The following broad questions were used to define the literature search terms.

a. Does the study involve samples collected via trapping?
b. Does the study contain DNA methodology?
c. Does the study involve invertebrates?

2.3 Search Terms

A preliminary search of the databases using search terms: DNA; invertebrate; and terrestrial was carried out. Individual terms and combined terms were used to assess how many results each would return to gauge how focused the results would be and how many results would be returned. During the literature review search terms were modified and additional searches performed to ensure that all relevant articles were found. Therefore, when sourcing articles that would be used in the final review the following searches were applied:

1. 'DNA AND (invertebrate OR insect) AND (pitfall trap OR vane trap OR malaise trap) NOT bacteria NOT microbial NOT mammal NOT virus NOT yeast'
2. 'DNA AND (invertebrate OR insect) AND (ethanol or ethyl alcohol) NOT bacteria NOT microbial NOT mammal NOT virus NOT yeast'
3. 'DNA AND (invertebrate OR insect) AND (propylene glycol OR propane-1,2-diol) NOT bacteria NOT microbial NOT mammal NOT virus NOT yeast'

Google Scholar and the Google search engine require the use of additional search terms to reduce the number of results being returned. For Google Scholar and the Google search engine the search terms are entered as follows noting that for the Google search engine the file type selected was PDF and the language 'English' to reduce the number of results:

1. DNA AND (invertebrate OR insect) AND ("pitfall trap" OR "vane trap" OR "malaise trap") -bacteria -microbial -mammal -virus -yeast'
2. 'DNA AND (invertebrate OR insect) AND (ethanol or "ethyl alcohol") -bacteria -microbial -mammal -virus -yeast'

3. ‘DNA AND (invertebrate OR insect) AND (“propylene glycol” OR “propane-1,2-diol”) -bacteria -microbial -mammal -virus -yeast’

Search Terms	PubMed	Web of Science	Science Direct	Google Scholar	Google	Total
DNA AND (invertebrate OR insect) AND (pitfall trap OR vane trap OR malaise trap) NOT bacteria NOT microbial NOT mammal NOT virus NOT yeast	23	145	270	777 selected for inclusion*	292	1,507
DNA AND (invertebrate OR insect) AND (ethanol or ethyl alcohol) NOT bacteria NOT microbial NOT mammal NOT virus NOT yeast	199	631	440	676 selected for inclusion*	95	2,041
DNA AND (invertebrate OR insect) AND (propylene glycol OR propane-1,2-diol) NOT bacteria NOT microbial NOT mammal NOT virus NOT yeast	3	21	198	280 selected for inclusion*	118	620
Total	225	797	908	1,733	505	4,168

Table 1. Results returned from a search of various databases and the Google search engine (databases accessed 21/10/2021).

* Google Scholar search results were manually selected for inclusion into Endnote as there is no feature to export all results.

2.4 Title Screening

Once results were generated from each database, search results titles were exported into Endnote reference manager software, except for Google Scholar where results were manually selected as there is no feature to export all results. The duplicate removal feature was applied followed by manually reading titles to remove any duplicates that were missed. The remaining titles were read to determine if each individual manuscript/piece of

literature would be of use in the review using a set of inclusion and exclusion criteria (Table 2).

Inclusion criteria	Exclusion criteria
Manuscripts that contain primary data.	Manuscripts which are published in languages other than English.
Studies that focus on terrestrial invertebrates.	Review papers.
Studies that contain DNA methodologies.	Any studies which are based on vertebrates, bacteria/fungi/algae.
Studies that use trapping for sample provision.	

Table 2. Inclusion and exclusion criteria used within this review.

2.5 Abstract/Methods Screening

Once the inclusion/exclusion criteria had been applied to the titles, the abstracts/methods of each article was read using both key words and the inclusion/exclusion criteria to decide which to retain. Methods were also screened because trapping was not always mentioned in the abstract. This process allowed for the removal of articles where titles suggest they were suitable for review but are outside the bounds of the inclusion/exclusion criteria. Key words included: DNA; invertebrate; pitfall trap; Malaise trap; vane trap; and preservative.

2.6 Literature screening

The full articles were critiqued by assessing specific variables. Using Thalinger et al., (2021) as a guide, a list of variables was generated to allow relevant information to be extracted from each study to allow for comparison and assessment of each method. Thalinger et al., (2021) describes the measures and tests required for successful validation of targeted eDNA assays some of which are relevant here.

a. Does the study state the type of trap used?
b. Does the study state what preservative was used in the trap?
c. Does the study state how long were specimens in traps before analysis/how often are samples collected?
d. Does the study state what preservative/storage conditions are used post sample collection?
e. Does the study contain any information on biotic (abundance, biomass, life stages etc.) or abiotic (temperature etc.) factors?
f. Does the study use destructive sampling?
g. Does the study state what DNA extraction methods are used?

h. Does the study contain control samples?
i. Does the study utilize mock communities?
j. Does the study state what primers (probes) are used?
k. Does the study state what gene is being targeted and amplicon size?
l. Does the study contain PCR set up and cycling information?
m. Does the study detail PCR product purification/quantification?
n. Does the study state the success rate of amplification?
o. Does the study involve metabarcoding?
    i. Does the study contain information on library preparation?
    ii. Does the study contain information on the sequencing?
    iii. Does the study state the sequencing depth/number of reads per sample?
    iv. Does the study contain bioinformatics?
    v. Does the study provide the bioinformatic workflow (names/methods)?
    vi. Does the study use a custom DNA database?
    vii. Does the study provide open access to their data?
p. What are the conclusions of the study?

3. Results

3.1 Literature Review

The in-depth literature search (including grey literature) revealed a total of 4,168 results which were obtained from PubMed, Web of Science, Science Direct, and Google Scholar. From this 2,293 were removed as duplicates by the Endnote reference manager, with a further 28 being removed manually. Screening of the titles led to 649 results being removed and a further 1,110 from screening of the abstract/methods. Peer reviewed papers and other documents found during the grey literature search were removed during the abstract/methods screening for the following reasons: no information on preservatives used despite use of trapping; no DNA analysis; use of museum specimens and/or reared or cultured specimens; use of netting; despite stating the preservative, no information on trapping supplied or no trapping performed for example specimens placed directly into preservative; and finally documents that were superseded by peer reviewed papers for example conference presentations or MPhil/PhD theses and dissertations. Two papers were kept despite not using trapping - rather they used hand searching - as they discussed the use of different preservatives prior to DNA analysis. This left 88 result to be assessed (Figure 3). Personal communications from contact with various individuals within organisations known to carry out terrestrial invertebrate trapping are not included within this figure, but their responses can be found within Supplementary data file 1 and are included in section 3.3 below and within the discussion section.

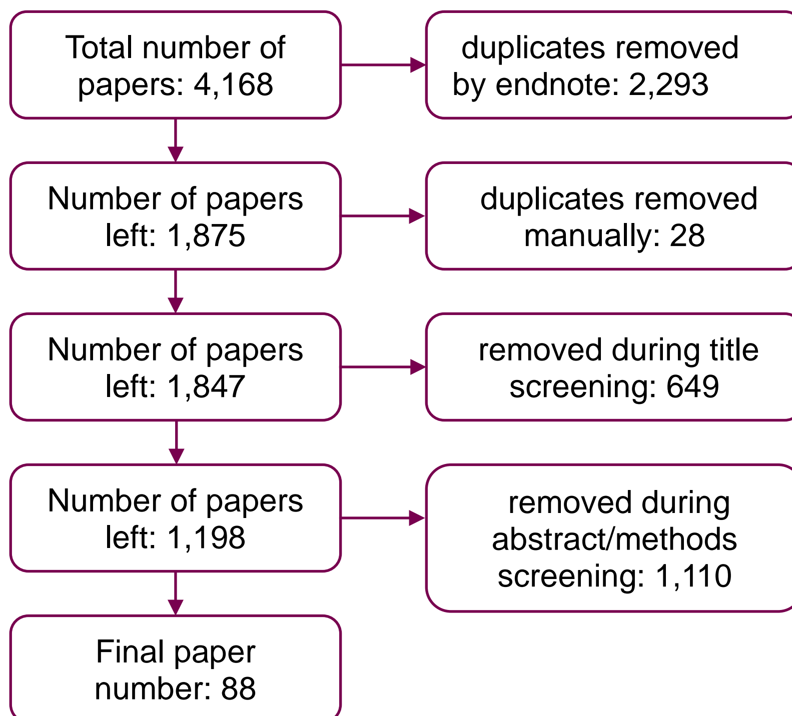

Figure 3. The collection of the literature to be reviewed. Initial collection of 4,168 papers was reduced to a final number of 88 through removal of duplicates and screening of titles, abstracts, and methods sections.

3.2 General outcomes of the literature review

Of the 88 papers found during the literature review, 48% used Malaise traps, 30% pitfall traps, 8% vane/flight interception traps, and the rest other traps such as funnel traps and pan traps (some studies used more than one trapping method). The preservatives used in the studies are summarised in Table 3 (see also Supplementary data file 1) with the majority using ethanol (of varying concentrations); followed by propylene glycol (varying concentrations), followed by ethylene glycol (varying concentrations); then soapy water or other preservatives (which in practice are rarely used – personal communications).

Preservative		Malaise Trap	Pitfall Trap	Vane/flight interception Trap	Other	Totals
Ethanol	20-50%	0	0	1	1	2
	70-84%	12	2	0	2	16
	85-94%	6	1	0	1	8
	95-100%	18	4	1	3	26
	Concentration not stated	6	1	0	0	7
Total using ethanol						**59**
Propylene glycol	20-50%	1	0	1	1	3
	50-77%	0	5	1	1	7
	95-100%	1	4	0	4	9
	Concentration not stated	2	7	3	4	16
Total using propylene glycol						**35**
Ethylene glycol	5%	0	1	0	0	1
	10%	1	1	0	0	2
	50%	0	0	1	0	1
	100%	0	0	0	1	1
	Concentration not stated	1	2	1	4	8
Total using ethylene glycol						**13**
Soapy water		2	2	1	1	6
Total using soapy water						**6**
Other	Not stated	1	0	0	0	1

	Glycol	1	0	1	0	2
	2.5% Acetic acid	0	1	0	0	1
	5% Acetic acid	0	1	0	0	1
	30% Acetic acid	0	0	0	1	1
	3% formalin	0	0	0	1	1
	10% sodium benzoate	0	0	0	1	1
	Distilled water	0	1	0	1	2
	Low salt	0	1	1	1	3
	Saturated salt solution	0	0	0	1	1
	10% Glycerin	0	0	0	1	1
	50% vinegar	0	1	0	0	1
	3% copper sulphate	0	0	1	1	2
	20% DMSO	0	0	0	1	1
	RNA later	0	0	0	1	1
	Dry/No preservative	1	0	1	0	2
Total using other preservatives						**22**

Table 3. Summary of the different sampling methods and the preservatives used within them. Note that some documents used more than one trapping method and/or more than one preservative.

The most common length of time samples were left in the traps prior to sample collection when using ethanol or propylene glycol as the preservative was 1 or 2 weeks (Figure 4), however, samples were collected in as little as one day (Gruber *et al.,* 2013; Koch *et al.*, 2015; Thormann *et al.*, 2016) or as long as 90 days in a simulated pitfall trapping experiment (Rubink *et al.*, 2003).

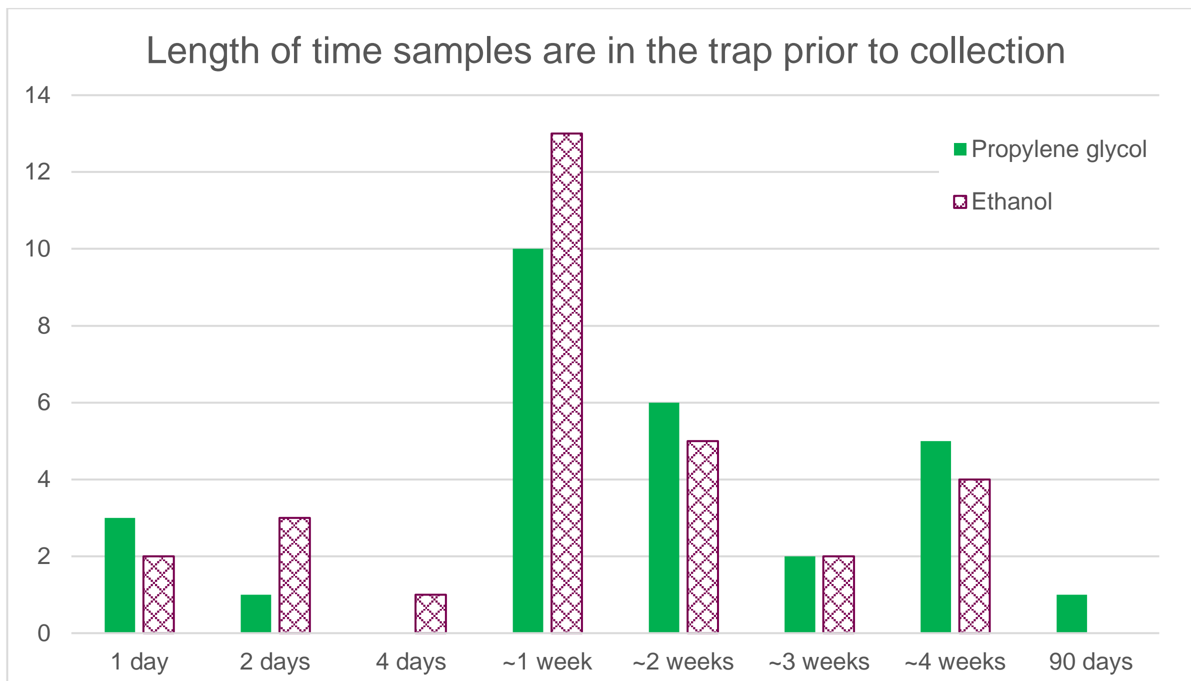

Figure 4. Bar chart showing the length of time that samples were left in the traps prior to sample collection.

Post collection 92% of all samples were either kept in the ethanol used in the trap or where other trapping preservatives were used - such as propylene glycol - were transferred into ≥70% ethanol for storage prior to molecular analysis. Storage ranged from room temperature (9%), in the fridge at 4°C (11%), or at -20°C (27%), with the remainder not stating storage temperature (53%).

71 of the studies employed destructive sampling with one of these also analysing the ethanol preservative. Two studies specifically stated using non-destructive sampling via a tissue lysis method and the remainder did not state whether destructive sampling was used or not. 28 of the studies carried out DNA extraction using the DNeasy blood and tissue kit (Qiagen), 14 did not state what DNA extraction methods were used, and the remainder were other DNA extraction methods most of which were used by only one study.

In terms of the primers used for PCR amplification (note that some studies use multiple target genes), most studies use primers for mitochondrial genes: COI (68, most for 658bp barcode); 16S (10); 28S (6); 18S (6); other mtDNA (9). A few studies used microsatellite DNA (3), and a few did not state what primers were used (7).

Although few studies stated the PCR and/or sequencing success rates (21 of 88), there was considerable variation in PCR success rates for both those samples initially collected in ethanol (60.5%-100%) and in propylene glycol (65%-100%). Two additional studies that used either ethylene glycol (Lucas *et al.*, 2016) or soapy water (Villalta *et al.*, 2021) as initial preservative, recorded 72% and 83% PCR success rate respectively. For those studies that stated their PCR and/or sequencing success rates, propylene glycol and ethanol concentrations varied from 50 to 100% and 40 to 96% respectively. Samples left in

the trap for 1 week prior to collection saw success rates of 78-100% for propylene glycol (Dopheide *et al.*, 2019; Gibson *et al.*, 2012; Lantero *et al.*, 2019) and 81-91% for ethanol (Aagaard *et al.*, 2017; deWaard *et al.*, 2019a/b; D'Souza *et al.*, 2021). Samples left in the trap for 2 weeks prior to collection saw success rates of 100% for propylene glycol (Rees *et al.*, unpublished) and 64-76% for ethanol (Uscanga *et al.*, 2020). The lowest stated success rates were for 75% propylene glycol with samples left in the traps for 3 days or 96% ethanol with samples left in the traps for 4 weeks. Of note, was that a 92% solution of propylene glycol (containing 5% water and 3% proprietary additives) left for 90 days was still able to produce success rates of 91-100% (Rubink *et al.*, 2003) although this was under simulated conditions rather than in the field.

The studies mainly used the samples for: sequencing analysis (64); followed by metabarcoding analysis (17); other molecular analysis such as gel electrophoresis (6); or did not use DNA analysis (1). This final study was included as it investigates the use of different preservatives within pitfall traps (McCravy *et al.*, 2007).

3.3 Personal Communications

Several individuals from organisations known to study terrestrial invertebrates were contacted to understand the methods that are currently being used within UK based survey work. Their responses indicated that all use ethanol (of varying concentrations) as the preservative within their traps (soapy water was also used in water traps by FERA). Some respondents were or had investigated the use of propylene glycol stating that it appeared to work well and did not have any of the safety issues of ethanol but that it was more difficult to work with (de Conceicoa, L). Samples were collected within 24 hours to one week and were stored in ethanol in the fridge at 4°C or $^{-}$20°C. Three out of four responders used destructive sampling of the whole specimen or the legs and one performed non-destructive tissue lysis. Again, the DNeasy blood and tissue kit (Qiagen) was used by half of the responders and one group used methods developed within their own team at Sanger. All perform PCR analysis with COI primers for barcoding and/or metabarcoding.

4. Discussion

4.1 Assessment of existing studies

Effective sampling: This review was focused on the preservation of terrestrial invertebrates mainly collected by Malaise, pitfall, and vane/flight interception traps but also included some other methods such as pan trapping (all passive methods of collection). Malaise traps are one of the most widely used devices for collecting insects and this was reflected in the results where nearly half of the studies used this method. It was also noted that small differences in the design of Malaise traps can influence the 'catch' illustrating that where region comparisons are being made a standard trap design needs to be adopted (D'Souza *et al.,* 2021).

Although one of the simplest types of insect collection device, there was a lack of consensus in the size and design of pitfall traps. Some studies used roofs a small distance above the ground to prevent rainfall from entering the trap which could have the effect of diluting the preservative as was likely the case for those studies who did not state the use of a roof, whilst minimising the catch of vertebrates (for example Framenau *et al.*, 2014). Other studies included: a round plastic mesh around each trap (Liu *et al.*, 2020a); or inserted funnels into the traps which also reduced evaporation (Ghofer *et al.*, 2015; Price, B. personal communication), or used snap on lids with holes in (Higgins, 2019) to prevent the catch of vertebrates. One study used a 'constellation' of 5 plastic cups connected by wooden walkways to increase the efficiency of the traps by diverting wandering invertebrates that contact the walkways into the traps (Marrone *et al.*, 2014). Trap design is therefore one variable that can be standardised for all trapping methods for example using an accepted pitfall trap design across all studies with the recommendation of using a roof to prevent rainfall ingress, although this will influence the catch.

Trap design is an important consideration in invertebrate sampling as traps need to be able to catch as wide a range of taxa as possible. As trap design can influence the catch, this could be an important area for further investigation so that the most appropriate trapping devices are deployed. If trap designs could be standardised it would allow for easier comparisons between and within sampling locations, however care would need to be taken to ensure that traps chosen for use were available for all in terms of expense and practicality.

Choice of preservative: The effectiveness of any preservative used at the point of trapping is likely to be one of the most important factors for success in DNA-based invertebrate analysis as it must maintain the integrity of the DNA (Stein *et al.*, 2013). From personal communications with scientists who are currently carrying out such analysis there was a consensus that 100% ethanol will always 'preserve the DNA the best' this is because if the concentration of ethanol is high enough (≥95%) it will denature any proteins (nucleases) that would normally degrade DNA (Flournoy *et al.*, 1996). Ethanol based preservation methods were therefore very common in articles used in this review. A recent review by Liu *et al.*, (2020b), identified >95% ethanol as the preservative used in the

majority of DNA metabarcoding studies carried out between 2015 and 2019. Ethanol is however, rarely used in practice as the licenses for purchase in any quantity are very difficult to obtain and ethanol has more health and safety requirements than other preservatives such as propylene glycol.

The results of this literature review suggest that PCR and/or sequencing success rates are higher when using propylene glycol as a preservative compared with ethanol. However, considering the low number of articles that specifically detail the success rates this should be taken with some caution as this is a very small sample number. Other preservatives which have been used in a small number of the studies include ethylene glycol (antifreeze), ethanol at less than 70%, soapy water (rarely used in practice – personal communications), and dimethyl sulphoxide, these are known to be less suitable for DNA preservation and so should be avoided (Vink *et al.*, 2005; Moreau *et al.*, 2013).

With respect to studies using pitfall traps twice as many used propylene glycol as used ethanol. Propylene glycol is cheap to purchase, easily found, and restriction free. Ethanol use may be more limited because of its higher cost, and the challenges associated when using ethanol especially for larger studies where many litres of preservative could be required. These challenges may include; the volatility of ethanol – ethanol will evaporate over time requiring frequent topping up and the difficulties in procuring and safely transporting ethanol. The evaporation of ethanol can be ameliorated to some extent using a funnel, this has two-fold benefits in reducing evaporation and preventing vertebrates from falling into the trap (Price, B. personal communications).

There are other practical limitations of field use of preservatives as they can have an attraction/repulsion to certain taxa. Depending on the target taxa this effect will either have a positive or a negative effect on the data leading to differences in numbers and species captured (McCravy and Willand 2007). It is therefore important that consideration of catch biases due to chemical attractant/repellent are made prior to preservative choice so that the taxa of interest can be preferentially selected. The choice of preservative could also influence the species complexity during subsequent metabarcoding analysis. It has previously been reported that some species will regurgitate/vomit into the preservative on trapping, in part explaining why some species/groups were only found in the ethanol preservative when compared with bulk samples (Marquina *et al.* 2019).

Further investigation as to the suitability of preservatives could involve the comparison of ethanol and propylene glycol to answer the following questions: 1) are UK found specimens sufficiently intact for taxonomic identification; 2) is good quality long length DNA present for downstream DNA analysis; 3) is there any bias in the preservation of material i.e. is there good penetration of the preservative whether a large or a small specimen?; 4) do any gains in points 1-4 justify the expense (approximately 4 times the price of propylene glycol) and inconvenience of a more costly preservative such as ethanol. When using ethanol there are health and safety issues to consider as ecologists would need to be able to safely store and transfer the ethanol. Both preservatives could be tested side by side over the same timescales using multiple traps per timepoint within the same sampling site. Any collection (on a statistically large enough scale) would also be able to compare the species collected to determine if there was likely to be any sampling

bias created by the different preservatives (chemo- attractants or repellents). Ethanol and propylene glycol concentrations could be measured post sampling to ensure that they are high enough to preserve the specimens. Up to 80% ethanol can be measured with a hydrometer, and up to 95% propylene glycol via a refractometer or hydrometer.

Post sampling there was a consensus that samples should be stored in ≥75% ethanol. Those that were collected in ethanol were generally left in the collection solution unless they were being transferred into a higher concentration of ethanol. Samples collected in other preservatives were also generally transferred into ethanol for storage. It has been demonstrated that samples that have been stored in 95% ethanol and subsequently stored for 8 months in either 75 or 95% ethanol at ambient or -20°C generate good quality DNA (Corriveau *et al.*, 2010). Therefore, we would recommend that ethanol (≥75%) should be used post sampling for sample storage.

A measure of preservation provided by the chosen preservative is the subsequent PCR and/or sequencing success rate. Success rates tended to be stated in studies where barcoding of specimens was carried out, however, the number of studies stating this was relatively low (21 out of 88 studies). If these had been stated more often (PCR and/or sequencing success rate) this would have allowed for a much more robust comparison of trapping methods and their preservatives. Further investigations could measure the PCR success rate by demonstrating the presence of amplifiable fragments of different sizes, the reaction Cq values could also be compared to controls and may give additional information as to the relative amounts of amplifiable DNA that is present between samples collected using different preservatives. The use of different lengths PCR amplicons may be useful in demonstrating the success or otherwise of the methods in preserving the best quality (longer length) DNA samples. Likewise, the use of low percentage agarose gel electrophoresis could check for the integrity of high molecular weight genomic DNA. An indication of degradation (maybe over time and for each preservative types) might be indicated by a smear of lower molecular weight DNA species.

Time: There was a lack of consensus in the length of time traps were left with the most common sampling regimes being 1 or 2 weeks with ethanol or propylene glycol as preservative. Several of the shorter sampling regimes (1-3 days) used soapy water (Ballare *et al.*, 2019; Koch *et al.*, 2015; Pyle 2018; Telfer *et al.*, 2015; and Villalta *et al.*, 2015), primarily as the killing agent. The use of soapy water is not recommended here as a DNA preservation agent and it is not often used in practice, a drop of detergent is often added to the trap preservative to break the surface tension over the preservative solution. For short sampling regimes (1 day) soapy water would be a very cheap and safe alternative to propylene glycol and ethanol and if specimens were transferred to ≥95% ethanol upon collection of the trap, DNA should still be suitably preserved for molecular analysis. In addition, soapy water is unlikely to suffer from biases associated with chemical attractants/repellents. It is not clear from this review whether there would be a difference in preservation/degradation between specimens that fall into the trap early on and those that fall into the trap later during the sampling period. There have been many published studies on preserved museum specimens (not included within this review) which could help to answer this.

The maximum length of time a specimen should sit in the preservative (in field) is an important variable where standardisation of methodologies across different trapping methods could be made. This could be investigated in the field using replicate Malaise, pitfall and vane traps that would need to be set up at several sites and samples collected after appropriate amounts of time (day(s) to weeks). Shorter sampling times could be trialled however, they are not always realistic when it comes to surveyor availability for setting up and collecting samples (Leatherland, D. personal communication). A study such as this could provide information on the size of the catch which could inform the decision on how long to sample for - although different sizes of sampling pots/bottles could be used where there was the potential for overfilling.

DNA extraction: There was consensus for DNA extraction methods. Many of the studies (28) used the Qiagen DNeasy blood and tissue kit (Qiagen) which has been recommended for eukaryotic biodiversity studies (Diener *et al.* 2015). Although 46 other studies did not use the Qiagen kit (the remainder did not state the DNA extraction method) they used a variety of other methods with no single method being used more than a handful of times. This is a potential strength of the existing studies as DNA extraction is therefore not a variable requiring further study, allowing a more direct comparison of the sampling/preservation methods that have been recorded. However, only four of the studies stated that they had performed quantification of DNA after extraction. If this was combined with DNA fragmentation analysis using gel electrophoresis it could be used as a measure of success of DNA preservation.

Despite the consensus on the DNA extraction step itself, there were a variety of methods used to present the sample for DNA extraction. Comparison of destructive methods such as grinding the sample in liquid nitrogen; bead beating; or homogenising with a mortar and pestle should be compared with non-destructive methods for example tissue lysis or using the ethanol preservative directly to find the best way to present samples for DNA extraction. Methods used for barcoding (using a leg or other part of the specimen) may not be easily achievable for bulk specimens being used for community analysis (metabarcoding). Additionally, for bulk samples, an investigation of size sorting could be carried out to find the optimal number of size groupings to give best coverage of the community and how best to pool these groupings back together (if at all) for metabarcoding.

Few studies found during the literature review provided information on the DNA yield. DNA yield per dry weight of specimen could be calculated per sample to determine the effect of the preservative on the total yield of DNA per specimen and again any trend (decrease in amount of DNA extracted for example) over time could be assessed over multiple replicate DNA extractions using several biological replicates per timepoint. To test the amount of DNA extracted, DNA quantification should be performed for example using a Qubit fluorometer (ThermoFisher) and a check of DNA purity made using an A_{260}/A_{280} absorbance measurement. As mentioned previously, the use of low percentage agarose gel electrophoresis could check for the integrity of high molecular weight genomic DNA. Field controls would also be required for comparison, for example hand collected samples that are kept live until DNA extraction to represent a ‘no degradation’ sample

and/or samples collected in soapy water for the same durations as the test samples to represent a ‘no preservative’ sample (killing specimens only) with potentially the highest level of degradation.

Biotic and abiotic factors: One of the questions asked during this literature review was whether the studies gave any information on biotic or abiotic conditions, in the 88 articles reviewed only three recorded this information (Kortmann *et al.,* 2021; Lucas *et al.,* 2016; Villalta *et al.,* 2021). This information can contribute to the choice of preservative. For example, in hot and arid locations ethanol filled traps might dry out if left too long requiring the use of higher volumes of ethanol/regular refilling (adding to the expense of sampling) or shorter sampling regimes. Propylene glycol is far less volatile with an evaporation rate >500 times lower than ethanol (Moreau *et al.*, 2013). Propylene glycol filled traps can therefore be used over several weeks or months (Hohbein and Conway 2018; Rubink *et al.*, 2003). On the other hand, propylene glycol is susceptible to decomposition upon long term UV exposure (Nakahama *et al.*, 2019). It has also been suggested that samples collected in propylene glycol should be thoroughly washed of the glycol preservative before long-term preservation in ethanol as it is possible that it could prevent ethanol from fully penetrating specimens for preservation (Ballare *et al.*, 2019). This study also found that vane trap specimens with no preservative (dry) in high temperatures of up to 40°C often had the best level of preservation. It is thought that the high temperatures may have quickly dried specimens leading to the preservation of higher quality DNA. Rainfall can also play a part in choice of preservative; for example, concentrations of propylene glycol as low as 20% have been shown to be sufficient for preserving DNA over long periods of time (Ferro and Park 2003). This suggests that if 50%-100% propylene glycol was used within pitfall traps and there was dilution from rainfall the DNA should still be sufficiently preserved (Higgins, 2019).

It is unclear how much of an effect biotic and abiotic factors have on sampling as very few studies provided any relevant information. Clearly sampling at different times of year will result in differing conditions (temperature, rainfall). Samples also need to be collected during the months of May-September when adults are likely to be present. Optimal sampling durations could therefore be different depending on the time of year for the different trapping methods. This could vary depending on the preservative used, for example in the warmer summer months if ethanol was being used as the trapping preservative it may be recommended that sampling durations are shorter than in the cooler months or that the ethanol needs to be replaced/topped up at shorter intervals. It is also possible that the optimal trapping preservative may be different at different times of the year or for the type of trap being used due to the different taxa that are collected by the different methods.

In terms of pitfall traps the substrate that they are deployed into may also play a part in suitability of samples for downstream analysis (Heaver, D., personal communications) and again was not revealed in the literature search. Some substrates such as peat might influence the pH of the preservative as it is not uncommon to find substrate in the traps. At a pH of above 9, DNA can be subject to hydrolysis. A study of traps deployed in different substrates would be simple and inexpensive as the only factor requiring study would be

the pH which could be tested in-field by use of a pH test strip. If certain substrates caused an inflation in the pH of the preservative solution, then such areas could be avoided when performing trapping studies.

Potential sources of error: Where cross-contamination of sampling equipment might be an issue, for example metabarcoding, it has been recommended that single use new or decontaminated equipment should be used. Decontamination with 10% bleach for at least 10 minutes is a simple and effective method (Prince and Andrus, 1992) and remaining bleach can be removed with sterilised water or ethanol (Liu *et al.* 2020b). The use of a field negative control is strongly recommended in DNA studies (Dickie *et al.* 2018) and are often used in eDNA studies. Most of the studies did not mention the use of field controls. This is more of an issue for metabarcoding studies where they can help to quantify any potential contamination thus increasing confidence in results and therefore, should be recommended.

4.2 Concluding statements

Careful consideration of sampling and storage procedures is required to ensure DNA preservation. It is important to consider the cost and practicality of trapping methods, as any standard method needs to be accessible to all parts of the collecting community and not end up being unrealistic, expensive, or field-impractical. Although ethanol was the most widely used preservative within the studies presented, propylene glycol is a good alternative due to its low cost, non-toxicity, non-flammability, and low rates of evaporation. Sampling durations were variable, however, propylene glycol and ethanol preservatives at a range of sampling durations gave high PCR success rates suggesting the preservation of good quality DNA for use within molecular techniques. Post sampling, samples should be stored in ethanol and most studies stored samples at below room temperature to reduce DNA degradation. DNA extraction was one area where a standardised method could be employed although how samples are prepared (destructive or non-destructive) prior to DNA extraction requires further study.

DNA techniques are likely to become a standard tool for measuring ecosystem/community diversity. Conventional sampling techniques will require some adaptation to ensure DNA preservation during the field sampling and storage phases. Future work should be focused on trap design, the choice of preservative, and the sampling duration to enable this.

5. Further Work

5.1 Proposed follow up studies

These costs assume a minimum sampling effort but a statistical evaluation of the numbers of traps required should be carried out prior to any formal submission of project costs.

A. Pitfall traps - Comparison of ethanol and propylene glycol

Propylene glycol appears to be a good alternative to the use of ethanol as preservative for invertebrate traps therefore comparison needs to be made between the two to ensure the preservation of DNA suitable for molecular techniques.

We suggest that a minimum of triplicate replicate traps are set up for each preservative and left for a period of 1 week at a site known to have high invertebrate biodiversity. To investigate the sampling duration parallel traps can also be set up and left for periods of 2, 3, and 4 weeks. Hand collected specimens will be required to act as the 'no degradation' or zero hours sampling duration control. A set of traps containing water will also need to be set up to represent the worst case scenario in terms of degradation. Collected samples should be transferred into ethanol straight away and transported back to the laboratory for storage and molecular analysis. Molecular analysis would include DNA extraction followed by size fractionation and PCR analysis. The DNA yield, quality and PCR success rates would be used as measures of preservative suitability.

This study could be repeated at different times of the year between May and September when adults are most active but would incur a larger cost.

B. Vane/flight interception traps

The study would be the same as A. Pitfall traps, using vane traps/flight interception traps in place of pitfall traps. The cost would be lower if existing equipment was used.

C. Malaise traps

The study would use Malaise traps in place of pitfall traps, and follow A. Pitfall traps. NB the cost of Malaise traps are substantially higher than for pitfall and vane/flight interception traps. The cost would be lower if existing equipment was used.

D. Pitfall trap design

A small study could be carried out to investigate the choice of trap in terms of its design. For example, use of a 'roof', may influence the catch but if it prevents rainwater ingress and subsequent dilution of the preservative it could lead to better preserved DNA for downstream analysis.

We suggest that at a minimum, triplicate pitfall traps are set up with and without 'roofs' with ethanol and/or propylene glycol (dependent on the results of A) and left for 1 or 2 weeks,

ideally when rainfall is expected. The ‘catch’ can be numerated and identified via traditional techniques to investigate where differences occur and within which taxa, and the quality and yield of DNA and PCR success rate can be investigated via molecular techniques.

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Figure 2. Flow chart showing the steps involved in invertebrate sampling and DNA analysis.

Figure 3. The collection of the literature to be reviewed.

Figure 4. Bar chart showing the length of time that samples were left in the traps prior to sample collection.

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