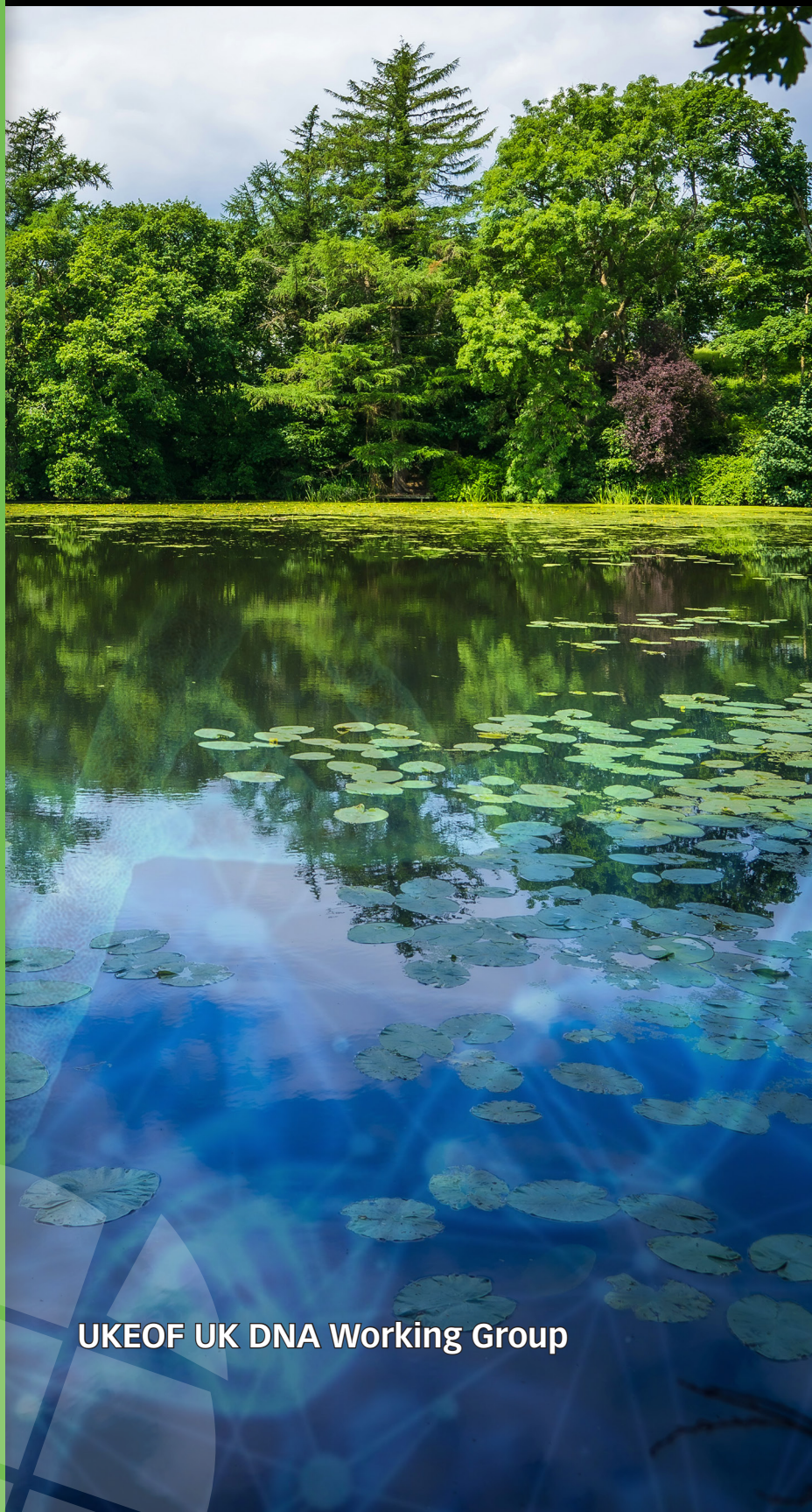
UKEOF
ENVIRONMENTAL OBSERVATION FRAMEWORK

Guide to using DNA-based monitoring with citizen science

Traditional biodiversity monitoring by professionals can be time-consuming and resource-intensive, and many habitats and species remain understudied and data deficient. Citizen Science (CS) and DNA-based monitoring are helping to fill these gaps by expanding the scale and impact of biodiversity monitoring. CS, or community science, involves members of the public in scientific research. It offers opportunities to build skills, improve wellbeing, and foster stronger connections with nature. DNA-based monitoring can detect species that are difficult to survey and help track how species distribution and ecosystem health and function change over time.

This guide introduces CS and DNA-based monitoring, demonstrates how the two approaches can work together (CS x DNA), and provides practical advice on project design, volunteer involvement, training, and communication. The CS x DNA project, Genepools (Rees et al., 2023), is used to illustrate project design from start to finish, and we also explore future opportunities and challenges for this emerging approach.

This document aims to facilitate crossover between these monitoring approaches and provides high-level advice for those wishing to use DNA-based methods in projects with volunteer involvement. We hope it will inspire collaborations between CS and DNA practitioners to drive better outcomes for people and nature.



UKEOF UK DNA Working Group

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Who has created this guide?

Written by scientists at UK Centre for Ecology & Hydrology and Natural England, on behalf of Natural England and the UK Environmental Observation Framework DNA Working Group.

Natural England is here to secure a healthy natural environment for people to enjoy, where wildlife is protected and England's traditional landscapes are safeguarded for future generations.

The **UK Centre for Ecology & Hydrology (UKCEH)** is a leading independent research institute dedicated to understanding and transforming how we interact with the natural world. With over 600 researchers, it tackles the urgent environmental challenges of our time, such as climate change, pollution, and biodiversity loss.

The **UK Environmental Observation Framework (UKEOF)** is a partnership of the major public funders of environmental science and was launched in 2008 to address issues of fragmentation, data access and a lack of strategic direction in environmental monitoring.

The **UKEOF's UK DNA Working Group** facilitates dialogue and collaboration by providing a forum for the wide community of government agencies, academics and other stakeholders to discuss priorities and emerging developments in the use of DNA for environmental monitoring.

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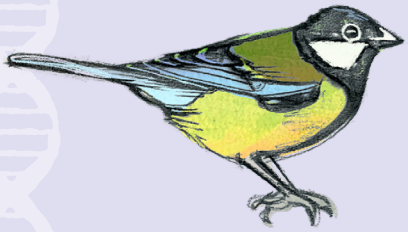
Guide to using DNA-based Monitoring with Citizen Science

Summary

What is a Citizen Science x DNA Project?

1

In a DNA-based monitoring project with Citizen Science (CS), scientists and volunteers come together to study the natural world using DNA analysis. These projects leverage the collective power of volunteers to accelerate scientific discoveries, and advance our understanding of ecosystems and species.



How can volunteers get involved?

- co-designing the project;
- sampling;
- lab work;
- data analysis;
- actions informed by the research outcomes, e.g. tips for looking after their local patch for biodiversity.

Why CS x DNA?

Large-Scale Data Collection: These projects can amass a vast amount of genomic data from a diverse range of sources and at large spatial and temporal scales, which can be valuable for research.
Engaging the Public: These projects engage the public in science and can foster a sense of scientific curiosity and community involvement.
Cost-Effective and Accelerated Research: By utilising volunteers, these projects can reduce the cost of data collection and analysis, allowing researchers to undertake larger-scale studies and accelerate the pace of scientific discovery.

Examples of CS x DNA projects

GenePools: Revealing the hidden biodiversity in UK ponds.
Sampling the Munros: Volunteers hiking up mountains and taking samples down to understand the health of mountain ecosystems through fungal communities.
Environmental DNA Expeditions: Global marine monitoring of vertebrates by UNESCO with recreational sailors and school children.

Considering a CS x DNA Project?

Whether a CS x DNA project will be suitable for your research question depends on the following factors:

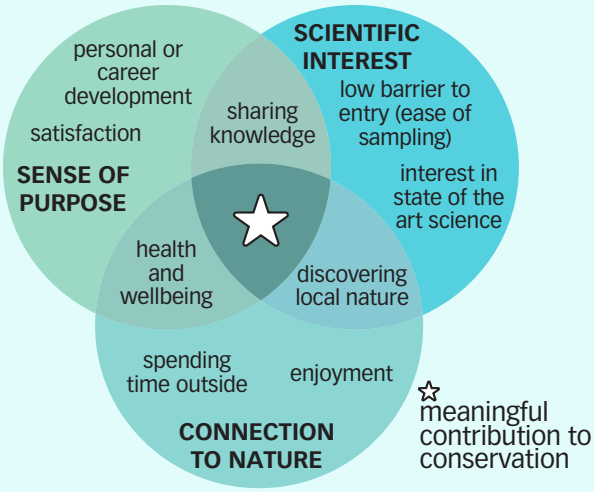
- the spatial and temporal scale of the project;
- the scope of the project's funding;
- the type and amount of data needed to obtain results;
- the level of expertise required to collect the data
- any training and coordination efforts needed;
- the stakeholder groups involved in the project.

Also consider the limitations of CS x DNA including volunteer bias, accuracy of CS datasets, health and safety, and the range of skills required in running the project.

2

Engaging with volunteers

What motivates volunteers?



Incorporate participant motivations through feedback and evaluation.

3

Engaging with volunteers (continued)

Tips for CS x DNA engagement

Writing: When working with volunteers of varying expertise, simplify complex subjects to a level understandable by a 9-year-old, as it enhances information absorption - even by the most skilled experts.

Introducing DNA: Provide context for DNA results, explaining what is typically found in environmental samples and how factors like UV exposure, pH, and temperature affect DNA persistence.

Managing expectations by ensuring participants understand the process and limitations of DNA analysis, including the role of primer choice in species detection.

Tell a story: Enhance stakeholder engagement by crafting contextually relevant narratives that ground findings into a coherent project 'story' – what is the beginning, middle and end?

Tailoring outputs: Enhance the accessibility and engagement of projects by incorporating participant-oriented outputs, including visual summaries, interactive web portals and tailored final reports.

Lasting impact: Consider project longevity beyond the funding timeline by creating lasting resources like videos and web portals.

3

CS x DNA project design

4

Main elements of CS x DNA project design

Data flow considerations

Project Management: Defining project objectives, specifying target species and geographic scope, and outlining how citizen scientists will contribute to data collection using DNA technologies.

Privacy and Ethics: Ensuring that data collection and sharing adhere to ethical guidelines and regulations, including obtaining informed consent from citizen scientists and handling personal data in compliance with GDPR.

Data Handling and Analysis: Consider the flow for DNA and participant data - including establishing a data management system, implementing data quality control measures, and considering data sharing, storage, access, and long-term sustainability.

Future horizons

5

Standardised biodiversity data platforms / DNA dashboards are being set up to increase synergy between different projects and biodiversity metrics.

Emerging global CS practitioner networks will start to address challenges in the field, assimilate lessons learned and foster collaboration and communication.

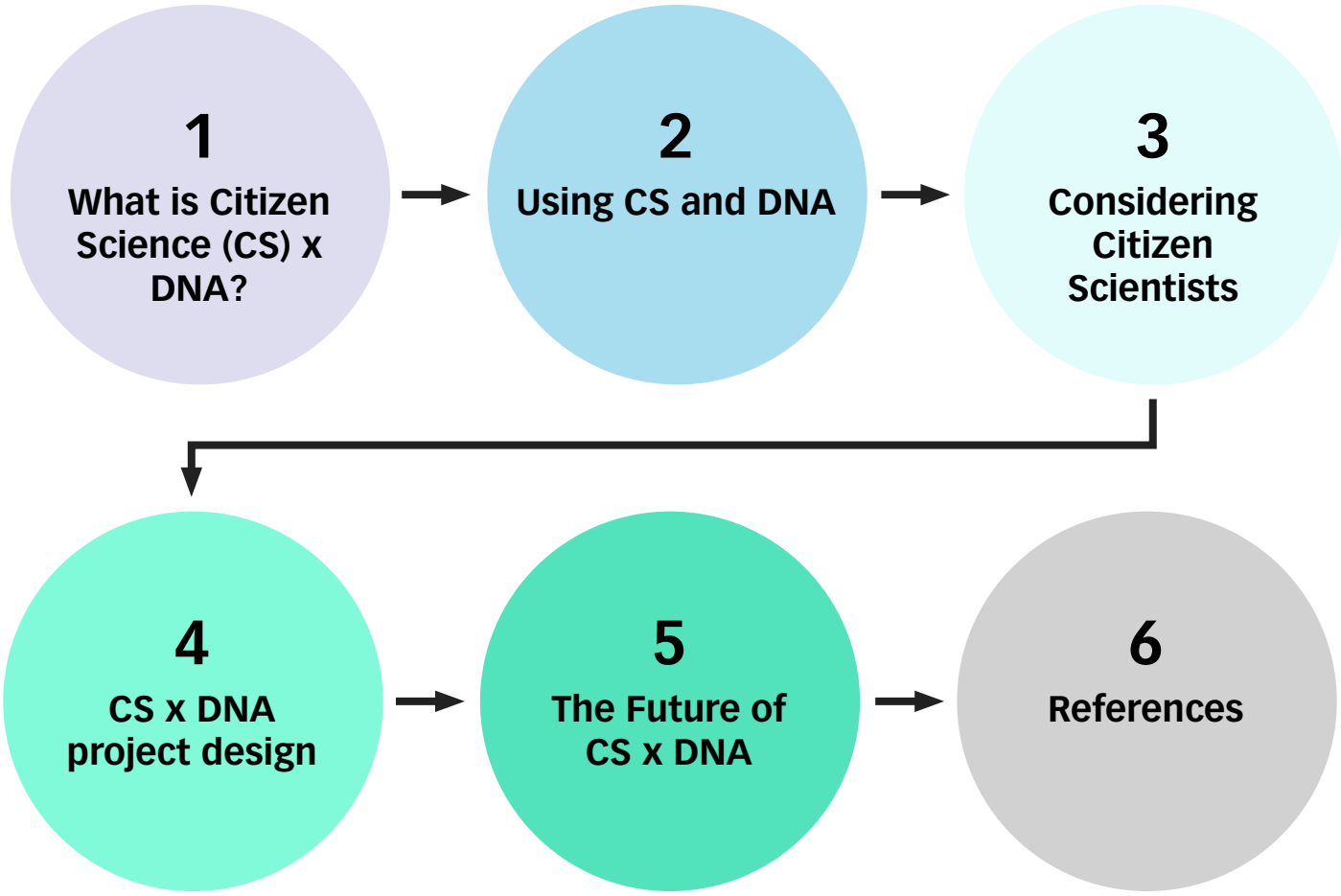
Portable DNA sequencers and mobile apps see volunteers able to carry out the whole DNA workflow in future.

Open Science - The transition towards 'open science' creates the opportunity to mainstream citizen science through the emphasis of public engagement, open access, FAIR data and open education.

Conservation genetics x CS projects through sampling organism derivatives and DNA with the development of sequencing technologies.

Document navigation

There are many excellent but separate resources on citizen science project design, DNA sample collection and analysis, and interpretation of resulting data. This document aims to facilitate crossover between these monitoring approaches and provides high-level advice for those wishing to use DNA-based technology in projects with volunteer involvement.



1. Introduction to Citizen Science (CS) and DNA

1.1. What is biodiversity monitoring?

Biodiversity is all the different kinds of life that make up the natural world, including animals, plants, fungi, and bacteria. **Taxonomy** is a branch of science that attempts to group biodiversity into hierarchical categories (**taxa**) based on shared characteristics and evolutionary relationships. A single taxon represents a division of life, for example, the common frog (*Rana temporaria*) is a species in the genus *Rana* (pond frogs), which belongs to the family Ranidae containing other species of true frogs such as pool frog (*Pelophylax lessonae*) and marsh frog (*Pelophylax ridibundus*). This family is one of many in the order Anura (frogs and toads) along with true toads (Bufonidae). Anurans belong to the class Amphibia, which includes all amphibians found on earth, including frogs, toads, salamanders and caecilians. This class is part of the phylum Chordata, a group of animals that share key features at some point in their development, including vertebrates which all have a vertebral column and a cranium or skull. The kingdom Animalia contains all animals that have or consist of many cells, eat organic material and can move for at least part of their life cycle. This kingdom is part of the domain Eukaryota, which includes all animals, plants, fungi, seaweeds, and other single-celled organisms.

Figure 1 overleaf illustrates the taxonomic classification of the common frog.

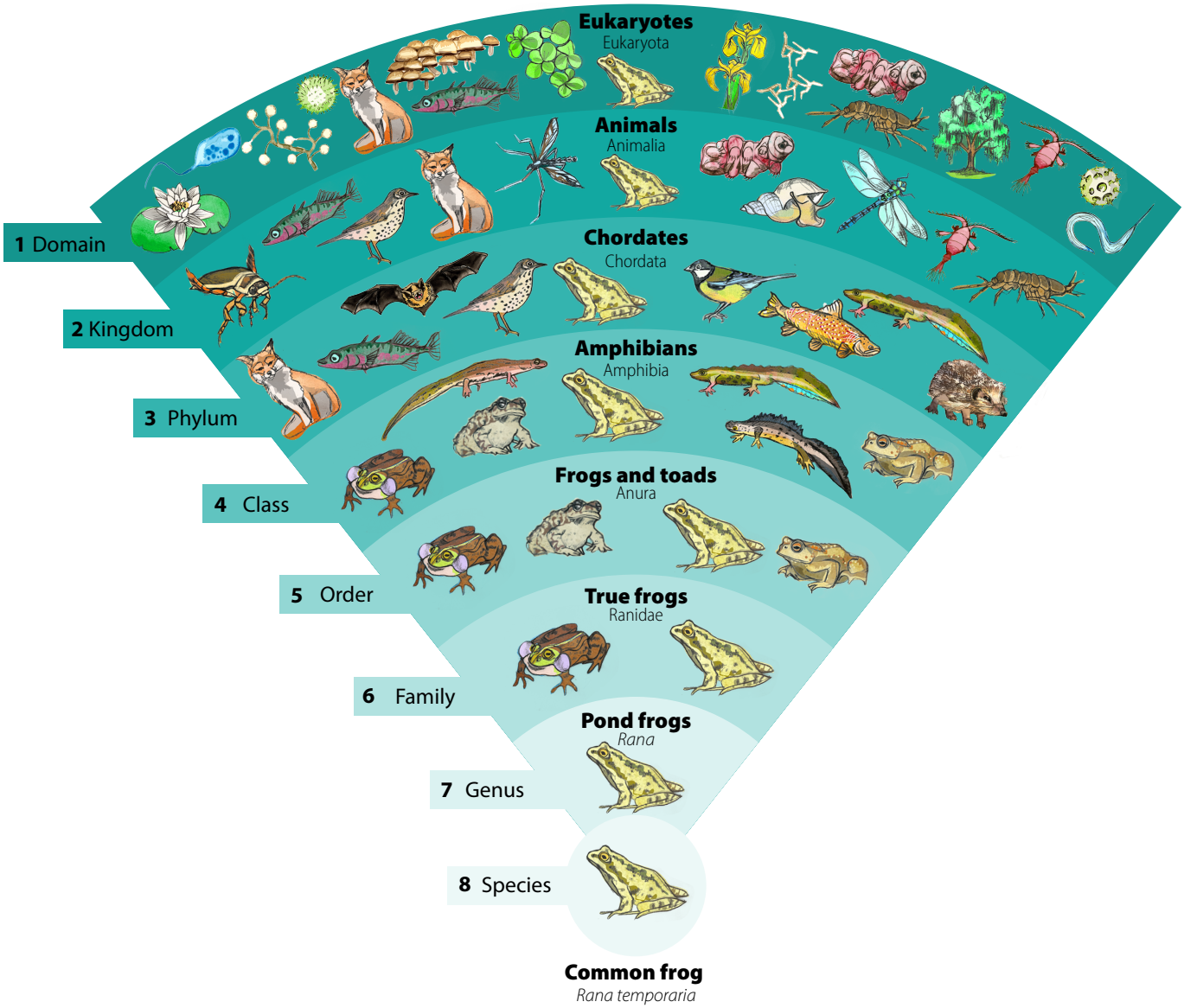


Illustration by Lauren Cook for GenePools 3 eDNA Pond App and modified for this document.

Figure 1. This illustration shows the tiered taxonomic classification of a common frog. Each level is illustrated with examples of species, included in that taxon, which you can find in UK ponds. Chordates are a group of animals that share key features at some point in their development, including vertebrates which all have a vertebral column and a cranium or skull.

Ultimately, taxonomy provides a naming system for life on earth, facilitating communication, research and conservation efforts as well as policy and decision-making.

A **biodiversity crisis** is unfolding, with biodiversity declining at unprecedented rates due to direct and indirect **human activity**, including climate change. Rising temperatures are accelerating habitat loss and degradation, natural disasters, weather extremes, food and water insecurity, and economic disruption. The climate-biodiversity crisis, coupled with rising awareness of health and wellbeing and the positive effects of nature connection, necessitate a thorough understanding of the interdependence of human and planetary health, and the benefits of activities that promote engagement with nature (Chawla, 2020; Lumber et al., 2017; Mackenzie et al., 2014; Ostfeld, 2017).

Monitoring is a term often used to mean continually tracking, or taking repeated measures, usually to look at trends over time, as opposed to a one-time experiment or looking at specific snapshots in time. Therefore, biodiversity monitoring is the **repeated measurement** of changes in biodiversity.

1.2. CS monitoring

1.2.1. What is CS?

Citizen science (CS), sometimes referred to as **community science**, is collaborative scientific research involving public participation. CS is a mutually advantageous collaboration between scientists and volunteers, where participants benefit from engaging with nature, contributing to conservation initiatives, and gaining insight into scientific practices, and on the other hand, facilitating cost-effective, large-scale research. When stakeholder priorities for a project include public engagement and high impact from research, CS will be especially beneficial.

The terminology for public participation in scientific research is evolving, with growing support for 'community science' as a more inclusive alternative to 'citizen science'. The latter can imply formal national membership, which may not suit all individuals due to factors like immigration status or political views. 'Community science' avoids these associations and is seen as more accessible (Lin Hunter et al., 2023). Despite this shift, 'citizen science' remains widely used and is the standard term in current practice and literature, so it will be used throughout this document.

CS research can encompass a **wide variety of activities and volunteer backgrounds** and is an increasingly acknowledged approach in many scientific domains, especially within the environmental and ecological sciences. It can range greatly in terms of **scale**, from smaller geographical areas (e.g. a park) over a short time, to larger areas (e.g. international waters) over many years. Large-scale, long-term monitoring (e.g. UK Butterfly Monitoring Scheme and Breeding Bird Survey) tends to involve networks of skilled volunteers, whereas many smaller or more time-limited projects often require little or no expertise or prior knowledge and may be more accessible to different groups of people (e.g. Bloomin' Algae and Nature's Calendar). More accessible large-scale projects requiring no prior expertise provide useful data and are equally important in engaging a wider portion of society (e.g. Big Garden Birdwatch and City Nature Challenge). If well-designed, any of these types of projects can be successful and collect data on a scale that would be prohibitive through paid surveyors. However, these projects still have costs and **volunteer engagement** is a key consideration (see section 3).

1.2.2. Practical value of CS monitoring

CS projects are particularly valuable for **collecting data for biodiversity monitoring trends** (e.g. for rare or threatened species), which are used to inform direct conservation actions, as well as informing national and international biodiversity indicators. CS data from different schemes (e.g. UK Butterfly Monitoring Scheme, Breeding Bird Survey, National Plant Monitoring Scheme) have contributed information to species trends reporting (e.g. UK Biodiversity Indicators, English Biodiversity Indicators, Scottish Biodiversity Indicators), international reporting (e.g. EU Habitats Directive, EU Birds Directive, Agreement on the Conservation of African-Eurasian Migratory Waterbirds) and natural capital assessments, e.g. the Office of National Statistics' natural capital accounts and Natural Capital and Ecosystem Assessment programme (August et al., 2019).

There has been past scepticism of CS related to **data reliability** (Cohn, 2008; Dickinson et al., 2012); however, CS projects addressing ecological and environmental questions are increasing globally (Conrad and Hilchey, 2011; Pocock et al., 2017), demonstrating that CS can produce **high-quality data** (Kosmala et al., 2016; McKinley et al., 2017). Existing CS programs are providing extensive data on species distribution, population abundance, species traits, habitat quality and ecosystem function (Hansen and Bonney, 2022; Poisson et al., 2019). In practical terms, CS projects add to **scientific and conservation advances** by producing peer-reviewed publications and grey literature publications such as government agency reports (e.g. Jackson, 2024; Rees et al., 2023). CS projects contribute evidence for direct conservation measures, such as advising official policy actions, identification of protected areas and control of invasive species (Chowdhury et al., 2023; Encarnação et al., 2021; Fontaine et al., 2022). CS programmes also raise the profile of rare or threatened species, contributing to global commitments on raising public awareness of the value of biodiversity, e.g. through the Kunming-Montreal Global Biodiversity Framework (Millard et al., 2021; Peter et al., 2021; Convention on Biological Diversity: Target 16).

The **scale** at which CS can collect data provides a means to respond to environmental threats, both local and global (Pocock et al., 2018). Despite this potential, much remains unrealised. Analysis by Fraisl et al. (2020) shows that CS could contribute to many more indicators for the United Nations Sustainable Development Goals than it currently does. To fully realise its potential, CS must address local needs for participants, communities and decision-makers, as well as global biodiversity monitoring goals in the **design and output of projects** (Johnston et al., 2023; Pocock et al., 2018). Furthermore, CS can contribute significantly to **knowledge generation, policy and conservation actions**, especially when programs are long-term, apply rigorous standards for data collection and management, and are the result of multidisciplinary collaborations (Fontaine et al., 2022).

1.2.3. Social and educational benefits of CS

Beyond its scientific contributions, CS offers profound **social and educational benefits** that further strengthen its value in environmental decision-making and conservation. Numerous studies have highlighted its potential to enhance science education, increase environmental awareness, and foster public engagement in local ecosystem management (Bell et al., 2009; Bonney et al., 2009; Dickinson et al., 2012; Feldman et al., 2021; Follett and Strezov, 2015; Pocock et al., 2019). CS empowers communities and can drive pro-environmental behaviour by democratising science, increasing awareness of local issues, building social capital (the potential ability to gain mutual benefit through informal or formal personal connections) and facilitating local stewardship and civic participation (Criscuolo et al., 2023; Jordan et al., 2012; McKinley et al., 2017; Peter et al., 2021).

These initiatives can help to **strengthen relationships between science, policy and society** by incorporating local knowledge, and fostering trust in decision-making processes by demonstrating the evidence-based nature of science, therefore enhancing adherence to policies related to critical issues, e.g. biodiversity, climate change and pandemics (Skarlatidou et al., 2024; Tan et al., 2022).

Pathways to **nature connection** (referring to an individual's emotional, cognitive and experiential relationship with the natural world; Martin et al., 2020) can be strengthened through purposeful activities involved in CS. CS activities can, for example, engage the senses, encourage

appreciation of the beauty of nature, and develop compassion through conservation actions (Coventry et al., 2019; Lumber et al., 2017; Pocock et al., 2023; Schuttler et al., 2018). Connecting with nature and time spent outdoors have many reported benefits for physical and mental health, including positive effects on self-esteem, stress reduction (Coventry et al., 2021), immune system functioning (Andersen et al., 2021), mood and general wellbeing (Cameron et al., 2020; Maas et al., 2006; Methorst et al., 2021; de Vries et al., 2003; White et al., 2019).

Thoughtfully designed projects can maximise these benefits while ensuring the delivery of high-quality data.

1.2.4. Considerations and limitations of CS and biomonitoring

CS can be prone to **volunteer bias** relating to population density; areas with larger populations may deliver a higher number of samples, and areas nearer to road networks are more easily accessed for sampling. This can be partially mitigated by a **structured survey** design imposed by project coordinators to ensure less biased coverage, or by employing analytical methods that account for bias. However, structured surveys require greater commitment from volunteers which can limit participation (Broughton and Pocock, 2022), especially if locations are limited and inaccessible by public transport (Gillings and Harris, 2022). **Opportunistic surveys** tend to have greater coverage across sites and taxa but can produce unstructured data that is more challenging to analyse (Broughton and Pocock, 2022).

CS may also only engage **certain parts of society**, and so potentially fail to include historically underrepresented groups such as individuals from certain socioeconomic, racial and ethnic groups. This raises questions on the relevance of CS to **diverse communities**. To mitigate this, it is important to understand **participant motivations** at the design stage and create tasks which appeal to different motivations, try to match participant expectations, and facilitate participant feedback and exchange throughout the project (see Section 4 for more information on engaging a volunteer group). However, in some cases, project objectives can only be partially aligned with volunteers' motivations and their capacity to participate, and the extent of this alignment will differ between projects.

CS is well-suited to projects that emphasise **community engagement** and / or **large-scale data collection**, but it is important to be aware of volunteers' level of interest in the research question(s) and how these will be explained. **Accessibility** of tasks and permissions required for volunteers to undertake tasks are also key, e.g. some volunteers may not be able to conduct physically demanding sampling and landowner permission will be required to sample on privately owned land. Volunteers will require training in tasks they are expected to perform and methods that are suitable for research studies may not be suitable for CS projects. For example, formalin and ethanol may be too hazardous for use by volunteers when sampling invertebrates, but are commonly used by professional surveyors and researchers (see section 2.3 for more information on these considerations).

There is also **taxonomic bias** in CS datasets due to the expertise needed and willingness to carry out survey methods; currently only birds, butterflies, moths and plants are monitored by CS on a large (e.g. national) scale (Chandler et al., 2017). This may have implications on how data can be used, e.g. for informing biodiversity indicators. To account for taxonomic biases, indicators using CS datasets can be **harmonised** with other methods to deliver comparable parameters,

such as population trends. This approach allows different methodologies to generate results that can be integrated for decision-making. For example, European bird (Pan-European Common Bird Monitoring Scheme, 2025) and butterfly (European Butterfly Monitoring Scheme, 2025) population trends are calculated using data from multiple monitoring techniques. These approaches allow results to be combined over larger spatial scales or across time periods (e.g. incorporating older datasets collected before the advent of newer techniques), and enable calibration between traditional and emerging methods (Schmidt and van Swaay, 2021).

Biological records from CS can be **verified** to ensure the accuracy of the identification, for example, through iRecord there is a network of expert volunteers to verify records, who often work closely with Local Environment Record Centres and National Biodiversity Network (NBN) data partners to verify data. Data can also be automatically **validated** by checking metadata, e.g. who took the record, where and when. Once records have been validated and verified, they will be held in **repositories** (e.g. Global Biodiversity Information Framework [GBIF] and NBN Atlas), and available for further use, e.g. policy evidence. For other record types, such as bioacoustic (Nature Overheard: Tune in to Your Streets) or DNA-derived data (Abarenkov et al., 2023), the observations need to be discoverable through biodiversity data platforms as well and adhere to the FAIR (Findability, Accessibility, Interoperability, Reusability) and CARE (Collective Benefit, Authority to Control, Responsibility, and Ethics) principles (Berry et al., 2020; Takahashi et al., 2025; Wilkinson et al., 2016).

Note: See 'Choosing and Using Citizen Science' by Pocock et al. (2014), including p. 15 for a useful decision tree for CS approaches.

1.3. DNA-based monitoring

1.3.1. What is DNA-based monitoring?

DNA carries all the instructions that a living organism needs to grow, reproduce and function, i.e. their genetic code. Regions of DNA which code for different functions, known as genes, can be shared across species or unique to individual species. Shorter sections of DNA within genes that vary across species can be targeted to genetically identify species. These short sections are called **DNA barcodes**; like every person has their own fingerprint, each species has its own DNA barcode. We can read the genetic fingerprint of living things by extracting DNA from samples, either directly from organisms (e.g. fur, blood or pollen) or from environmental samples (e.g. water, soil, ice or air), known as **environmental DNA (eDNA)** (Valentini et al., 2009).

DNA reference libraries or databases underpin species identification using DNA barcoding. These are collections of DNA barcodes for many different species that have been generated from specimens identified by taxonomic experts and given a permanent identification number. Scientists can compare unknown DNA sequences to these known DNA barcodes. High-quality reference libraries require substantial time and resource to build, but provide scientists with higher confidence in species identification (Taberlet et al., 2012).

DNA technologies are transforming biodiversity monitoring by enabling identification of species which are difficult to survey with traditional methods, and tracking of species distributions over time (Deiner et al., 2017). We can tell an **organism**, like an animal, plant or fungus, was there without having actually seen it. Hundreds of species can be surveyed at once by taking samples

of water, soil or air containing the DNA of everything (all the animals, plants, fungi, algae, viruses and bacteria etc.) which lives in or passes through that environment (Beng and Corlett, 2020; Bohmann et al., 2014).

Some species are highly suited to DNA detection, for example, those that are rare, endangered, hard-to-see, cryptic (indistinguishable from another species by their physical features alone) or some aquatic species which live in vast areas (Deiner et al., 2017). DNA surveys are especially powerful for revealing the hidden biodiversity that is not visible to the naked eye, like invertebrates, fungi and bacteria (Bohmann et al., 2014). For this reason, DNA technology has shown strong potential for monitoring disease, e.g. COVID-19 in wastewater samples (Farrell et al., 2021).

DNA technologies have become one of the fastest growing biodiversity monitoring tools in recent years, particularly due to the efficiency and sensitivity which they offer, as well as the option to use environmental samples as a less invasive method, suitable for surveying many habitat types and taxa across the tree-of-life (Beng and Corlett, 2020). DNA-based tools are already being used for routine biodiversity monitoring and in regulatory contexts. The great crested newt (*Triturus cristatus*), a legally protected species in the UK, was one of the first species to be monitored using eDNA analysis (Thomsen et al., 2012). After successful validation in the UK (Biggs et al., 2015), great crested newt eDNA surveys are now used in species licensing and inform new policies aimed at providing landscape-level species protection for the species (Rees et al., 2023). The Environment Agency has also recently deemed eDNA monitoring for white-clawed crayfish (*Austropotamobius pallipes*), signal crayfish (*Pacifastacus leniusculus*), and crayfish plague (*Aphanomyces astaci*) to be suitable for operational use (Environment Agency, 2024a). On the other side of the world, after development of analytical tests for invasive bigheaded carps, the United States Fish and Wildlife Service now collects about 10,000 water samples annually in Great Lakes tributaries as part of ongoing surveillance to support early warning detection and management of these invasive species (Lodge, 2024). Monitoring of whole communities has been more challenging to validate for operational use, but UK regulators are now using eDNA survey for lake fish communities and river microbial biofilm communities (Environment Agency, 2024b).

The ease with which DNA samples can be taken from the environment, for example, by scooping up a tube of soil or filtering a litre of water, makes them amenable to sampling by automated processes and citizen scientists, bringing DNA studies into the realm of 'big data' (Deiner et al., 2017). The data generated through DNA-based monitoring projects can help to generate robust and timely advice for managing species and habitats. Better and more readily available knowledge supports smarter decisions to protect nature now and in the future (Berry et al., 2020).

1.3.2. How does DNA-based monitoring work?

DNA analysis generally consists of sample collection, sample preservation, DNA extraction, and amplification via **Polymerase Chain Reaction (PCR)**, with workflows then diverging depending on the type of analysis. PCR makes millions of copies of a specific DNA sequence using an enzyme called DNA polymerase and primers in a series of heating and cooling steps to repeatedly break and replicate DNA. **Primers** are short, single-stranded DNA pieces which are complementary to a target DNA sequence. If a primer finds a sequence match in a DNA sample, it will bind, allowing the target DNA to be copied many times to a quantity which can be detected (De Brauwer et al., 2022). For example, primers binding to a target DNA sequence that is unique to fish will allow all fish DNA in the sample to be amplified.

For identification of single species (e.g. rare or invasive species), **quantitative PCR (qPCR)** or **digital PCR (dPCR)** are typically used as these technologies offer high sensitivity and accuracy for quantifying low concentrations of DNA. Depending on the length of the target DNA sequence, **Sanger sequencing** may be employed to verify species identity by determining the DNA sequence and comparing to reference sequences (Thalinger et al., 2021). For identification of multiple species, PCR followed by **High-Throughput Sequencing** is used, known as **metabarcoding** (De Brauwer et al., 2022). Each DNA sequence produced is known as a **sequencing read**. The sequencing reads must be **bioinformatically processed** to group DNA sequences according to samples (demultiplexing), combine forward and reverse sequence reads (merging), filter by quality, cluster into **Operational Taxonomic Units (OTUs)** or **Amplicon Sequence Variants (ASVs)** to remove redundancy, and assign taxonomy by comparing sequences to a reference database (Hakimzadeh et al., 2023). The taxonomically assigned data are then analysed to address the monitoring or research question (Beng and Corlett, 2020).

1.3.3. Considerations and limitations for DNA-based monitoring

The cost of processing a DNA sample can in some cases be less expensive than using traditional methods that involve physically observing or capturing species, and may save many hours, days or weeks of work by ecologists in collecting the samples and by taxonomists in identifying tricky organisms under the microscope (Beng and Corlett, 2020). It is especially cost-effective at larger scales as sampling consumables are often ordered in bulk and laboratory processing becomes progressively more cost-efficient as the number of samples increases (Bálint et al., 2018). However, there is a trade-off between sample size and the resources required for sample collection, processing and analysis. Greater sampling effort is needed for high spatial and / or temporal resolution, or the detection of low-density species (Erickson et al., 2019), and this will incur higher equipment, laboratory and personnel costs (Lyet et al., 2021). New projects may also face high set-up costs (e.g. purchase of equipment, development of analytical tests), or some sample types or analytical tests may require higher technical replication in the laboratory, both of which can influence cost-efficiency of DNA-based monitoring (Smart et al., 2016). Therefore, costs of sample collection (including equipment and personnel) must be balanced against those of laboratory processing and data analysis.

DNA-based methods can produce **false positives**, i.e. false detection of a species that is not present in the field (Beng and Corlett, 2020). Contaminants can be introduced by field surveyors or laboratory technicians if decontamination procedures, single-use equipment and / or unidirectional workflows are not in place. In the field, historical or ancient DNA can also be resuspended when sediment is disturbed. Environmental contamination can occur if DNA is introduced to a sampling site by the general public, domestic animals or other wildlife who have visited other sites. Similarly, DNA may be inadvertently transported on the body of another organism, or poo from predators will contain the DNA from other species they have eaten. For example, a bird might eat a fish from the sea, then deposit droppings containing DNA of that fish when flying over a lake (Furlan et al., 2020; Burian et al., 2021). Best practice to minimise contamination in DNA projects is well-described in the literature (Bruce et al., 2021; Goldberg et al., 2016). Controls at each stage of the workflow, especially at the earliest steps, are key to identify which contaminants are being introduced at each stage and for quality assurance (Hutchins et al., 2021).

False positives can be introduced by erroneous PCR amplification or sequencing resulting in false sequences (Furlan et al., 2020); these should be removed during bioinformatic processing but the efficiency of this will depend on the reference database used (Beng and Corlett, 2020) and the abundance of false sequences (Furlan et al., 2020). There may also be misidentifications where sequences are assigned to an incorrect species or higher taxonomic rank (also a source of non-detections) (Furlan et al., 2020). This could be due to lack of reference sequences, genetic similarity between species (e.g. domestic species and wild ancestors), or an incorrect record in the DNA reference library if a species had originally been misidentified based on appearance or sequence inaccuracy (Beng and Corlett, 2020; Furlan et al., 2020). This can happen when species are 'cryptic', i.e. they are almost impossible to distinguish by how they look but they are genetically distinct. For example, with the advent of DNA technologies, investigation has revealed that many earthworm 'species' are, in fact, groups of genetically distinct cryptic species (Marchán et al., 2018). In the case of genetically similar species, common examples are detecting wolf (*Canis lupus*) instead of dog (*Canis lupus familiaris*), Scottish wildcat (*Felis silvestris*) instead of cat (*Felis catus*), or green-winged teal (*Anas carolinensis*), a rare migrant, instead of mallard (*Anas platyrhynchos*) or common teal (*Anas crecca*) (Harper et al., 2019b).

DNA methods can also produce **false negatives**, i.e. failure to detect a species despite its presence in the field (Beng and Corlett, 2020). Species may not be detected when present if: the DNA is not captured in samples due to insufficient sampling effort and / or inappropriate timing of sampling; low DNA concentrations; fast DNA breakdown; and / or environmental conditions (Burian et al., 2021). Sampling only represents a portion of what lives in that environment. It is possible to miss some species living there by chance, i.e. if you know you have a frog in your pond and frog DNA does not come up in the results, that may just be because frog DNA was not collected in that particular sample (Brys et al., 2020).

Additionally, false negatives can occur if the sample is not preserved and stored appropriately, leading to breakdown of collected DNA. Samples typically have to be stabilised by freezing or addition of chemical solutions for storage at warmer temperatures. Samples should always be kept in the dark away from sunlight to avoid damage by ultraviolet radiation (Bruce et al., 2021). To analyse the collected DNA, it must be separated from the rest of the sample and purified. This process is known as DNA extraction and must be tailored to the type of sample and taxa of interest. Inefficient DNA extraction can lead to poor recovery of DNA or co-extraction of PCR inhibitors (i.e. substances that interfere with PCR amplification) and subsequently false negatives (Burian et al., 2021).

Similarly, suboptimal PCR conditions, reagents and replication can produce false negatives. During PCR, primer bias can occur, where primers bind more efficiently to DNA of some species than others (Beng and Corlett, 2020). The analytical test or **assay** may also have low sensitivity (Burian et al., 2021) Alternatively, there may be species masking, where DNA of larger and / or more abundant species is preferentially amplified over DNA of smaller and / or less abundant species (Kelly et al., 2014). Other technical factors that can increase the risk of false negatives are insufficient sequencing depth, suboptimal bioinformatic processing, and reference database coverage, i.e. the availability of reference sequences for taxa expected to be present in an area (Furlan et al., 2020). Additionally, species detected in past DNA projects that are still present may not be detected in new projects, or species detected by one laboratory may not be detected by another laboratory if different methods are used (Rodriguez et al., 2025).

There are key considerations at each stage of the workflow to minimise false positives and false negatives. When collecting DNA samples, the number of sampling locations, number of samples at each location, distribution of locations and samples, and sample quantity must be appropriate to the size and type of system being studied. DNA may be patchily distributed in still waterbodies due to lack of water movement (Harper et al., 2019a). DNA of different species will be spatially segregated in deeper lakes that experience thermal stratification in summer, but more evenly mixed in winter once layers dissipate (Lawson Handley et al., 2019). In flowing water, DNA could have a more homogenous distribution due to mixing of the water column, or a patchy distribution due to transport and dilution (Curtis et al., 2020). In large, fast-flowing rivers, DNA may be transported tens to thousands of kilometres from its source (Pont et al., 2018). Similarly, tidal currents can transport DNA long distances (Andruszkiewicz et al., 2019). As an example, at least 10 water samples (each 2 L) at equidistant intervals around the perimeter of a temperate lake in winter are required to detect the majority of fish species present. Greater sampling effort, including sampling at depth, is required in summer to ensure species detection, especially for rare species (Sellers et al., 2024).

Timing is crucial to ensure sampling is conducted when species are more active and likely to release more DNA, such as during breeding, spawning, rearing young, dispersal etc. (de Souza et al., 2016). Timing can make the difference between detection and non-detection of some species such as crayfish. Crayfish breed in the spring/summer so lots of DNA will be shed as a result of increased activity compared to winter (Troth et al., 2021). Environmental factors (e.g. temperature, pH, salinity) can also influence the rates at which DNA is released by organisms and breaks down in the environment, so it is important to take measurements of variables likely to influence DNA in different systems to aid modelling and interpretation of data (Harrison et al., 2019).

Laboratory protocols must be optimised to maximise DNA recovery and amplification, and minimise primer bias. Primer design and validation is key to target specific species or taxonomic groups and minimise non-target amplification (Collins et al., 2019; Langlois et al., 2020). Sequencing depth will depend on the expected diversity of samples and the purpose of the study, e.g. greater sequencing depth will be required to detect rare species in highly diverse samples (Shirazi et al., 2021). Bioinformatics parameters must be given careful consideration to ensure these are tailored to the target DNA sequence and taxonomic group (Bayer et al., 2025). Ultimately, a species cannot be identified by DNA analysis if it does not have a reference sequence so it is important to evaluate reference database coverage before undertaking a DNA study to understand what can and cannot be detected, and generate new reference sequences for priority species if none are available. There are potentially thousands of species which do not yet have reference sequences (Weigand et al., 2019). Work is ongoing around the world to fill in these gaps, such as the Darwin Tree of Life Project.

Metadata is key to understand the methods used to generate DNA data and ensure transparent reporting. It ensures reliable interpretation of results and enables a confidence judgement to be made for the data. Metadata standards / requirements are well-established in the literature for both single species (Thalinger et al., 2021) and metabarcoding (Klymus et al., 2024; Takahashi et al., 2025) projects.

1.4. Combining CS and DNA for biodiversity monitoring

A monitoring project combining CS and DNA (or "CS x DNA") can take many forms, the most common of which is involving volunteers in collecting DNA samples, which are analysed to monitor some aspect of biodiversity, such as surveying an area for endangered and elusive species (Biggs et al., 2015).

Combining DNA technologies with CS can bring many benefits, such as helping to address data gaps by improving under-sampled areas or taxa in a way that is cost-efficient, relatively quick and highly scalable. DNA analysis becomes more cost-efficient when many samples are processed at once, lending itself to the collective power of citizen scientists (Meyer et al., 2021). Involving volunteers means that science can glean local knowledge through participant input, and empower advocacy and action in the wider community (Pocock et al., 2018). CS x DNA can also engage volunteers who might find it difficult to participate in more time-intensive or physically demanding CS schemes. The combination of the two techniques therefore provides a potentially powerful tool for large scale monitoring across the UK and globally.

Citizen science is increasingly recognised for its valuable and often vital contributions in environmental monitoring, particularly in biodiversity assessments, and has tremendous potential to expand DNA survey efforts, thereby enhancing the reach and impact of both approaches (Hansen and Bonney, 2022; Pocock et al., 2018). Scaling up DNA analysis with CS could significantly contribute to Sustainable Development Goal indicators (Fraisl et al., 2020) and help to bridge taxonomic and geographic gaps in biodiversity data (Chandler et al., 2017; Johnston et al., 2023; Pocock et al., 2018). Successfully achieving this integration, however, requires substantial collaboration across molecular ecology, bioinformatics, data management, science communication and public engagement.

This guide aims to assist interested parties in effectively utilising DNA technologies alongside CS to monitor and understand the state of biodiversity and ecosystem health, and aims to highlight practical considerations for those deciding whether to use a combined CS x DNA approach.

2. CS x DNA projects

2.1. How volunteers can get involved

There are several stages in a CS x DNA project where volunteers can be involved. These include:

- **Sampling:** This is the most common stage where volunteers can add immense value to DNA surveys, as a cost-effective way for a project to collect a large number of samples across a large spatial scale (e.g. Agersnap et al., 2022), and to access locations otherwise unavailable to professional field surveyors such as private gardens (e.g. Rees et al., 2023). Sampling DNA from the environment (eDNA) is relatively straightforward and allows volunteers to spend time in nature. The particular considerations of sampling will vary depending on the habitat and taxa of interest, and there are many sampling options suitable for CS (see examples in Section 4);
- **At the design stage:** It is possible to involve volunteers in co-designing or participating in the initial design of the project. This will ensure participant needs can be addressed in the project aims, as well as the needs of the scientists and other stakeholders (e.g. Clarke et al., 2023). Factoring participant motivations into the design of the project could mean collecting additional metadata to address questions they are interested in, adding a test to analyse a particular taxon of interest, combining other techniques alongside DNA, or simply feeding back results in a way which will have the most impact;
- **Continual feedback opportunities:** Volunteers can share their thoughts throughout the project via formal or informal feedback, leading to the continual improvement of the project and shared learnings for future CS x DNA projects (e.g. Broadhurst et al., 2025);
- **Informed action:** Advice can be given to participants in light of the project results, increasing their understanding of the ecology and providing tips for managing their local environment, e.g. gardens or public green spaces;
- **Engaging in data analysis:** Citizen scientists may be motivated to contribute to data analysis or be involved in community-driven data interpretation efforts. To facilitate this, DNA sequence results and bioinformatics pipelines (code) can be shared on an open access platform as offered by Wilderlab, New Zealand. This offers participants (or anyone interested) the opportunity to work with the data for their own questions. However, this would require a relatively high level of expertise and would not be widely inclusive;
- **Lab processing:** It may be feasible to involve volunteers in the process of DNA extraction and PCR, which would require additional training, connection with a partner with lab facilities, and / or engagement with a skilled volunteer group, e.g. Tøttrup (2020), Urban Nature Project, DNA Sequencing by The British Lichen Society, and Knudsen et al. (2023). With the advent of portable DNA extraction, CRISPR-Cas and portable sequencing technologies, this option will become more viable in future.

Note: Acknowledging the efforts of citizen scientists ensures they receive appropriate recognition for their contributions to the project.

2.2. CS x DNA examples

CS and DNA have been combined for biomonitoring since 2015 (Biggs et al., 2015). In such projects, volunteers have shown strong motivation and commitment, with large numbers of volunteers and a high return rate of samples (Agersnap et al., 2022, Broadhurst et al., 2025). Successful projects combining the two methods span from monitoring a single endangered species (e.g. 'Can environmental DNA help find the kōkako?') and human disease vectors (Schneider et al., 2016), to monitoring entire communities of organisms to establish large-scale biodiversity baselines (Lin et al., 2021; Meyer et al., 2021). In some cases, volunteer engagement has enabled sampling strategies which would be otherwise logistically impossible for scientists, such as the simultaneous collection of samples from 100 sites, giving a view of fish diversity largely unaffected by temporal variation (Agersnap et al., 2022), or providing biodiversity data from private garden ponds which would otherwise be inaccessible (Rees et al., 2023). Further examples are outlined in Table 1.

Table 1. Examples of monitoring projects employing a CS x DNA approach.

Description	Reference/Link
Monitoring great crested newt (*Triturus cristatus*) in ponds - this was the first time volunteers were involved with an eDNA project in the UK	Biggs et al. (2015)
Monitoring populations of invasive mosquito vectors in Europe by sampling ponds and waterways (quantitative PCR and metabarcoding)	Schneider et al. (2016)
CaleDNA Project - monitoring entire communities of organisms for large-scale biodiversity baselines	Lin et al. (2021), Meyer et al. (2021)
Mapping coastal fish biodiversity on a national scale – sampling nearly simultaneously at 100 sites in Denmark	Agersnap et al. (2022)
Fish community monitoring along vast sub-Saharan coastlines	Burian et al. (2022)
Monitoring terrestrial mammals in Essex, England, with eDNA metabarcoding of river water	Lavin (2022), Broadhurst et al. (2025)
Creation of an eDNA toolkit for CS monitoring of urban wetland biodiversity in Nanjing, China, with a group of junior students and scientists	Zhang et al. (2023)
Fungus monitoring on mountaintops in the Scottish highlands	SEFARI
Environmental DNA Expeditions – global CS project measuring marine biodiversity (mainly fish) and impacts of climate change across UNESCO World Heritage marine sites with many school children involved in sampling campaigns	Environmental DNA Expeditions in UNESCO World Heritage Marine Sites

Continued overleaf

Table 1 continued

Description	Reference/Link
Using water eDNA samples with CS to find the South Island kokako (*Callaeas cinereus*), presumed extinct	The South Island kōkako Charitable Trust
The Great Australian Platypus Search – school children help scientists by collecting eDNA samples from their local waterways to map the distribution of platypuses, fish and other aquatic vertebrates across Victoria, Australia	EnviroDNA Case Study: The Great Australian Platypus Search
Detection of amphibian eDNA in Denmark - sample collection and quantitative PCR analysis carried out by high school students, producing 4 (of 2,250) unexpected test results that needed professional scrutiny and detecting 9 of 14 amphibian species	Knudsen et al. (2023)
eDNA samples collected by volunteers as part of Spot the Monk to be analysed for the endangered Mediterranean monk seal (*Monachus monachus*) to better understand distribution	Valsecchi et al. (2023)
Using water eDNA samples with CS to describe the distribution and abundance of groundwater amphipods	Couton et al. (2023a)
Six groups of volunteers recruited to collect samples for examination of coastal fish biodiversity with eDNA metabarcoding	Miya et al. (2023)
Citizen scientists designed and executed an eDNA-based survey of a small chalk stream catchment to explore questions of concern around vertebrate species	Clarke et al. (2023)
GenePools – monitoring the diversity in garden ponds across 3 UK cities in first year, then 6 UK cities in second year, with multi-marker metabarcoding targeting vertebrates, invertebrates, plants, bacteria and eukaryotes	Rees et al. (2023)
Unlocking the Severn – a CS x DNA approach was used to monitor the shad (*Alosa spp.*) migration and other fish species with metabarcoding	Griffiths et al. (2024)
800 lakes sampled worldwide by citizen scientists and scientific researchers on International Day for Biological Diversity 2024 to monitor terrestrial and freshwater biodiversity	LeDNA
DNA and life – high school students engaged in both fieldwork and advanced laboratory analyses by collecting and analyzing eDNA samples from marine environments across Denmark	Leerhøi et al. (2024)

2.3. Considerations for CS x DNA projects

Deciding whether CS x DNA is the right approach depends on several factors, each with specific considerations, pertaining to the research questions and scope of the project. Consider first if involving volunteers will help to achieve the desired results, while at the same time benefiting participants by addressing their needs or fostering new skills and expertise. Second, consider if DNA survey is the most effective method to provide results addressing the question being asked.

2.3.1. Type and amount of data needed

Consider the choice of **molecular methods** (e.g. DNA extraction methods, primers, bioinformatics pipelines), and **sample type** (e.g. water, soil, air, swabs etc.). Outline the **metadata** you will need to support the DNA output (more in Section 4).

Consider the **number of sampling locations** and **number of samples** at each location that might be needed. Sampling may need to happen at a **particular time of year** to maximise detection success. Consider if samples can be taken **individually or in teams**, and how **data will be recorded** – a smartphone app or data sheets may be needed. The time and effort required needs to be realistic for volunteer participation.

Consider how **comparable the dataset** might be to similar projects at local or national scales to maximise data use and validate results from citizen scientists, i.e. it will be beneficial if there is data from the same area / species which can be used to confirm / refute results from CS x DNA projects.

The **level of accuracy** required from the data should be considered. Studies have shown the quality of DNA data from volunteer-collected samples was comparable to those taken by professional researchers, and more species known to occur in the study area were detected by volunteers than by researchers (Broadhurst et al., 2025; Lavin, 2022). However, it is more challenging to train and coordinate volunteers to process samples in the laboratory with precision compared to collecting field samples. More time and established protocols will be needed for laboratory training, and additional precautions and controls will be required to minimise contamination and ensure quality. Detection thresholds for the resulting data will also require consideration. While involving volunteers in laboratory processing offers educational and training benefits and can motivate volunteers to engage with the research process on a deeper level, some volunteers may find laboratory processing to be too advanced and complicated (Leerhøi et al., 2024).

2.3.2. Cost

The **more DNA samples** you need to process, and any **additional analysis** on those samples will add cost. Projects that require expensive or **highly technical equipment** or demand a great deal of **time commitment**, such as collecting detailed measurements every day, may be unsuitable for CS.

Factor in **time for laboratory analysis** (you can usually expect this to take weeks for single species or months for metabarcoding). Consider including additional time as there is a high risk of analysis delays.

There should be some budget set aside for **providing curated and engaging feedback** to the volunteers (e.g. in the form of an interactive web app, portal or report) and responding to their

queries. Consider the time required for engagement as well as providing necessary training and disseminating results.

2.3.3. Level of expertise required

For most DNA sample types, sampling requires **no expertise** from the volunteers prior to basic training. This is important to stress to volunteers to promote diverse participation. However, there may be physical limitations that need to be considered, e.g. safe access for disabled vs. non-disabled individuals. **Advice on sampling** can be provided by research scientists and commercial companies.

Although volunteers can be trained in laboratory processing, molecular and bioinformatic analysis will typically be provided by an **external contractor or project partner.** It is also common to partner with **communications experts** to engage, motivate and look after volunteers.

2.3.4. Training and coordination

CS requires **training** in collecting samples as well as **coordination** in organising sampling trips for volunteers and sending and receiving DNA sampling kits via post. Resource development (such as web apps, instruction booklets) and training for volunteers in person, via video call, or in the form of a recorded video will be necessary. These resources may be provided by a DNA sampling kit contractor but may need to be tailored for CS.

Always check whether the sampling passes organisational **Health and Safety requirements**. **Safety conscious approaches** should be employed. If the project requires participants to take samples, the potential risks should be clearly communicated along with information on how to avoid or minimise these risks. **Biosecurity** must be considered and landowner permissions for sampling must be obtained which could limit location accessibility and CS involvement.

2.3.5. Accessibility

Some sites that may be useful to survey (e.g. ports and marinas, remote areas) may not be accessible to 'typical' citizen scientists. The degree of portability and safety required will be different for a group with lay knowledge to a skilled volunteer group (e.g. recreational divers / fishers). Sampling from bridges over rivers may be easier than sampling bankside, or sampling surface water will likely be more accessible than pelagic / benthic sampling.

To make it logistically possible to involve volunteers, a balance could be struck between reducing the size of the sample, which could become unwieldy for a volunteer, but increasing the number of samples to achieve an adequate representation, or considering another sampling method such as using passive samplers (Bessey et al., 2021; Maiello et al., 2022; Neave et al., 2023). The cost-benefits of involving citizen scientists would weigh into the decision.

2.3.6. Communicating results

Good communication must be maintained throughout a project, especially if it will take a long time for results. Consider managing expectations of DNA results and highlighting the quality assurance processes involved. DNA projects can have unexpected results, such as species which are present but have not been observed by volunteers or recorded by other monitoring schemes. Alternatively, these could be species which are false positives due to contamination or false negatives due to methods used (see section 1.3). The metadata required to understand and assess level of

confidence in DNA data must be communicated to all stakeholders (including volunteers) to avoid false conclusions being drawn, and avoid confusion or lack of trust in the data.

CS x DNA datasets must be **carefully scrutinised** by researchers to catch any unexpected or erroneous results from contamination or mismatches in the DNA reference library used (Knudsen et al., 2023). There are no standard procedures for scrutinising DNA results, and thus it must be subject to expert opinion / analysis. If a dubious result is found (e.g. finding tuna DNA in a garden pond), there is a choice whether to delete that datapoint, or keep it in but explain where it might have come from. It is of the utmost importance to **clearly communicate how the data can be interpreted** (see section 3.5) to volunteers and other stakeholders.

Sometimes the most valuable data may be the most difficult to interpret for participants. Finding the **balance between project needs and volunteer needs** (i.e. really considering the project aims, what you are willing to sacrifice, and allocating sufficient resources to volunteer engagement) can be critical to a successful CS x DNA project. For example, scientific names may not be that informative to volunteers, but can be key to describe microbial diversity as these taxa often lack common names.

2.3.7. Partner and stakeholder management

Consider that a CS x DNA approach requires a **wide range of skills** which may require substantial investment or partnerships with other teams and institutes. For example, support may be needed for planning and executing communication strategies, community building, participant management, and public engagement. This can be mitigated by partnering with another institution. Roles for all project partners should be outlined within the initial project specification / partnership agreements.

It is important to ensure the **output DNA data** (species distribution or community inventories) and **metadata** are fit for purpose for the original aims of the project, and consider the requirements of the different stakeholders (e.g. participants, policymakers, funders, scientific and practitioner communities) involved. Different stakeholders may be interested in different types of data and presentation.

3. Considering volunteers

3.1. What motivates volunteers to take part in a CS x DNA project?

To engage volunteers in the project and maximise the benefits to both parties, it is essential to consider what motivates citizens to take part and include activities to meet expectations in project design, whilst matching the objectives of the project funder(s). By taking part in ecology and conservation projects, participants may hope to gain the following (Broadhurst et al., 2025; Fraisl et al., 2022; White et al., 2019):

- Helping and finding out about wildlife in their local area;
- Wanting to help a specific site and / or supporting conservation efforts generally;
- Enjoyment or fun;
- Getting to know other people with similar interests;
- Spending time outside;
- Feeling of wanting to make a difference for the environment;
- Contributing to scientific knowledge;
- Sharing their own knowledge;
- Health and wellbeing;
- Feeling of accomplishment;
- Personal or career development;
- Corporate social responsibility initiatives.

Volunteers may be motivated to take part in a DNA monitoring project for many of the reasons stated above, and with DNA specifically, they may be motivated by these additional factors (Broadhurst et al., 2025):

- Discovering wildlife in their local area, i.e. 'solving the mystery' of what is going unseen in their daily lives;
- Involvement in exciting state-of-the-art science / gaining new subject matter knowledge and stronger scientific literacy.

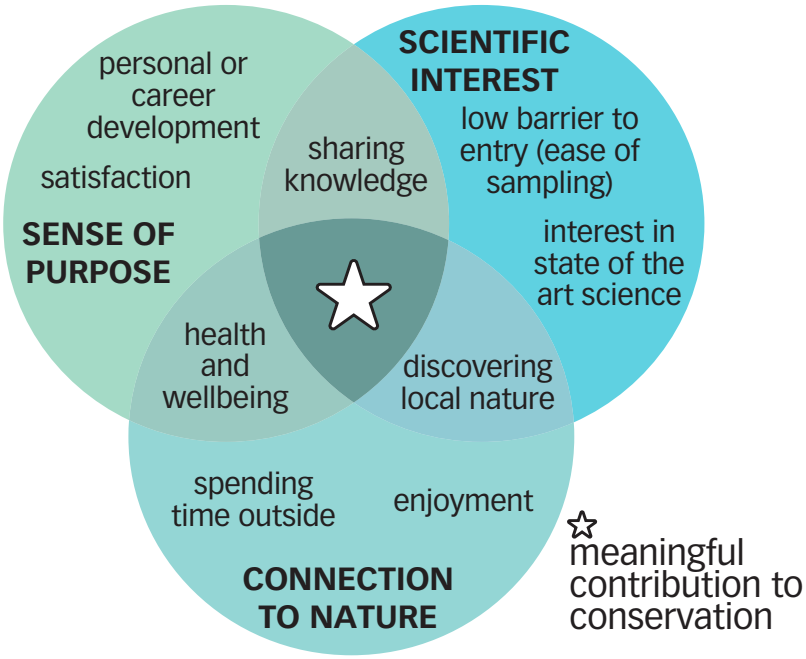


Figure 2. Some of the reasons why volunteers may be motivated to take part in DNA monitoring.

Volunteer motivations to take part in CS studies are well-understood (Geohagen et al., 2016), but understanding what motivates participants to initially, and continue to, take part in a CS x DNA study specifically is an area needing further research. Fulfilment of initial motivations to participate and good organisation / logistics is key to continued participation (Geohagen et al., 2016). It is unknown whether CS x DNA projects that only involve volunteers at the sampling stage will ensure fulfilment and continued participation, or whether involvement in design, laboratory processing and / or data analysis is required to sustain volunteer motivation. Evaluation is particularly important to fill this gap, and regular evaluation also helps accommodate diverse motivations specific to that project. For example, feedback from GenePools showed that many participants of the study already felt they had a strong connection to nature prior to taking part (Rees et al., 2023). Their motivations therefore may have been to further their scientific development, take part in meaningful contribution to conservation / science, understand more about their pond, or seek some ways to improve their pond for nature.

3.2. How are you factoring in the benefits to volunteers?

In a CS x DNA project, considering the benefits to volunteers is essential for **maintaining their participation and motivation**. Consider what will incentivise participants to sign up and continue to take part and ways to increase the benefits to volunteers.

As part of project design, strive to **encourage nature connectedness** and wellbeing activities to promote feelings of happiness, calmness and joy. Integrating enjoyable outdoor activities or "nature noticing activities" during sampling can deepen participants' connection with nature and increase enjoyment, e.g. by noting positive aspects of nature such as the 'Three Good Things in Nature' (3GTiN) activity (Pocock et al., 2023). A task as simple as this can have disproportionately positive effects on participants in terms of pro-nature conservation behaviours.

Offering opportunities for 'career' progression or skills development in CS, such as developing into a leadership role or pointing to similar projects, fosters long-term commitment and ensures their continued involvement and satisfaction. Providing sufficient training and ensuring the language of instructions, communications and results is accessible to non-specialists will increase the accessibility of DNA and biomonitoring science. Providing sufficient feedback and communication will increase participant satisfaction, making volunteers feel valued and that they are making a meaningful contribution.

Conversely, consider how you might **reduce feelings of frustration** which may arise from:

- Bad project management – lack of frequent updates, many changes and corrections, and coming across as generally not well organised;
- Tasks being too complex;
- Lack of success (e.g. not being able to take the sample due to weather, health and safety, faulty sampling kits, not sending kits back in time etc.);

- Excessive use of single-use plastics, which was shown to be an issue for volunteers taking part in eDNA projects (Broadhurst et al., 2025);
- Condescension / terminology – consider the care and attention to volunteers, being grateful for their contributions; wording can have a big impact on the feeling of 'us and them', for example, 'together we can' acknowledges volunteer input better than 'help us' (Eitzel et al., 2017).

3.3. Training for volunteers

Training requirements will differ depending on what volunteer roles involve (e.g. sampling vs. lab work), and any previous experience. Although volunteers have shown they can relatively easily take samples for a project, they will need plenty of training. Training may take various forms, as geographic spread of volunteers may limit the viability of training in-person, and in this case written instructions and video can provide sufficient training. Often, this training may be a collaborative effort between DNA specialists and CS / volunteer engagement specialists. Some options are outlined in Table 2.

Table 2. Volunteer training options for CS x DNA projects and links to resources.

Training	Description	Example / Link
Background to eDNA – animation	Animation outlining the process of eDNA sampling including barcoding and matching to species libraries	Animation created for the Urban Nature Project / Brilliant Butterflies: 'What is eDNA and why is it useful' by NHM
Sampling guide – booklet	Instructional booklet. Making instructions as visual and simple as possible and clearly set out will aid understanding	Environmental DNA expeditions in UNESCO World Heritage Marine Sites: field sampling booklet
Sampling guide – instructional video	A video clearly showing each part of the sampling kit and how to carry out sampling step-by-step	GenePools 2023: What's living in your pond?
Sampling training in person	When taking out volunteers in a group sampling session, it will be helpful to run through sampling step-by-step. Make sure to outline how contamination is reduced during the process and allow time for volunteer questions	Conducted in Unlocking the Severn (Freshwater Habitats Trust / Natural England)
Results walk-through	Walk through of how to navigate the results, such as a video with a screen-recording	Conducted in GenePools 2024

3.4. Communicating DNA science to diverse audiences

3.4.1. Accessible language

When working with a diverse audience of volunteers with varying levels of expertise, it is better to **explain complex subjects in simple terms.** Common advice is to aim the language used at a level understandable by a 9-year-old. By the age of 9, a person has learned a core set of around 5,000 words and although vocabulary will expand, these common words will remain into adulthood and will allow audiences to take in information as quickly as possible, even for experts in that field. It is okay to use scientific terms where necessary, but these should always be defined in simple terms (Government Digital Service, 2016).

3.4.2. Be personal

Learning from and engaging with people face-to-face, rather than being behind an organisational name is valuable to fostering connection with the CS project. **Put a face to the work** – who are the scientists, who is working on the project and what do they do? Have these people feature in the training resources, use their names in feedback etc.

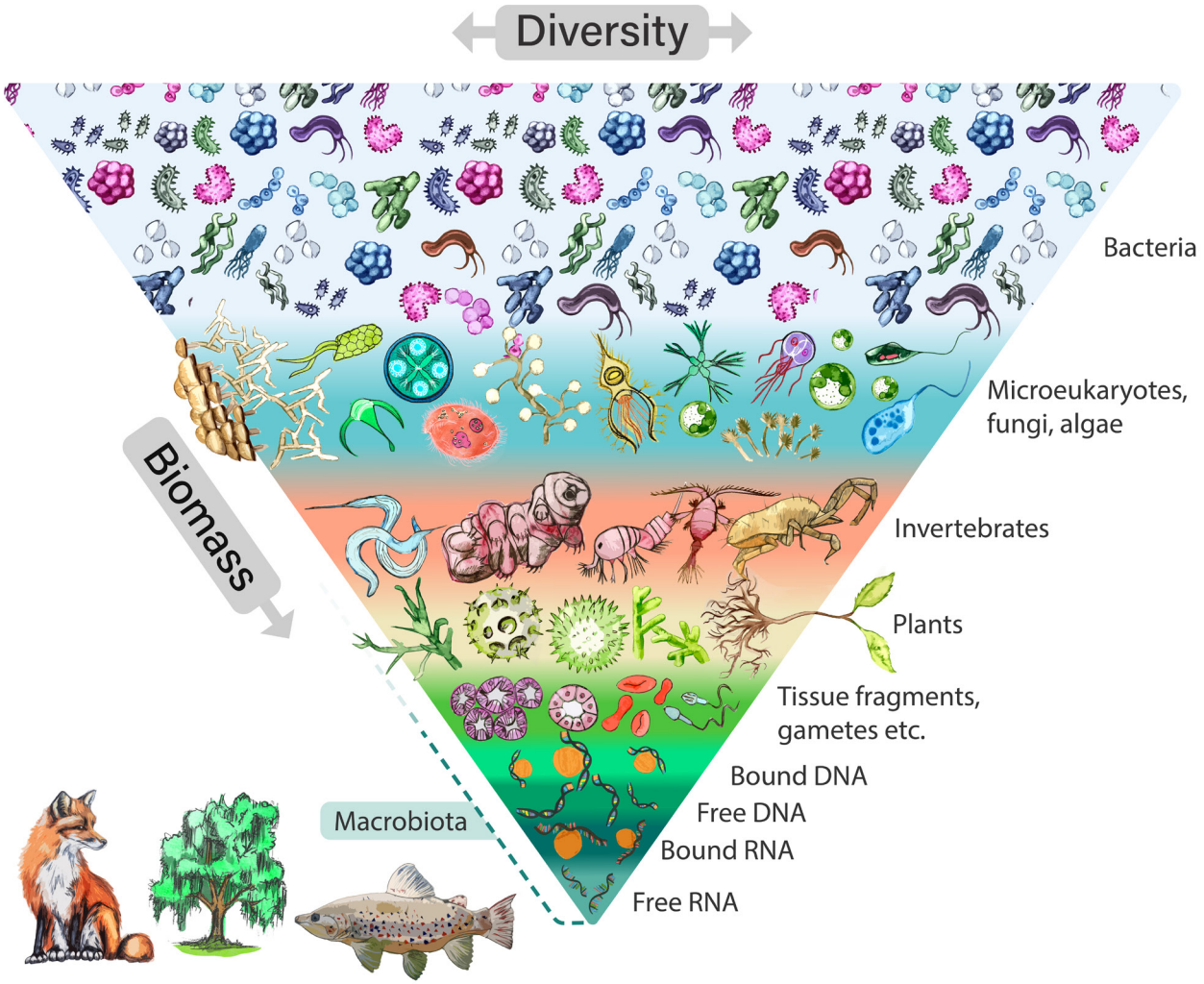
3.4.3. Level of detail

Think about how to communicate technical details to volunteers. Some volunteers may be mainly interested in results and the general approach, whereas others might want to understand a lot more of the detail.

3.4.4. Managing expectations through increasing understanding

Feedback from the GenePools project indicated that some participants were disappointed with the results, where some species they expected to find in their pond didn't show in the DNA results, e.g. birds or dragonflies (Rees et al., 2023). Conversely, species were detected which they could not explain. Samples may also fail entirely if not enough DNA was captured in the sample, preservation and storage conditions were suboptimal leading to degradation, or inhibitors were present in the sample and co-extracted with the DNA (see section 1.3). **Manage expectations by encouraging a more in-depth understanding** of the process and limitations.

To aid understanding of the DNA results, make sure to provide some context, e.g. what is found in an environmental sample and how those parts may relate to the organism detected (Figure 3 overleaf).



Reproduced from Cook et al. (2024) with permission. CC-BY 4.0

Figure 3. An illustration of biological diversity in a generalised hypothetical water, sediment, or soil sample. The biomass of organisms represented increases roughly logarithmically from top to bottom, but the actual material sampled (cells, gametes, organelles, free and bound nucleic acids, etc.) does not increase in size in the same way; in fact, the largest organisms are likely represented by the smallest particles. Viruses are not shown as they will be co-sampled with other organismal material, and the nature of their 'free' states is poorly known.

The length of time that DNA lasts in the environment depends on the type of sample, the type of organism, and factors that affect the breakdown of DNA, such as exposure to ultraviolet light, pH, and high temperatures, or presence of fine particles that will stabilise DNA molecules and increase the persistence in the environment (see section 1.3).

A species or group may also not be displayed in the results if primers for those species have not been selected for the project. Be clear about what the project is searching for – if you are doing a marine mammal survey with cetacean-specific primers, you will not see what fish live in that area in the DNA results.

3.4.5. Displaying DNA results

DNA results can be complex in comparison to other types of CS monitoring due to the large number of species detected – many of which may be invertebrates, fungi and bacteria that volunteers are less familiar with – and how results are formatted. Data from qPCR/dPCR is often conveyed as the number of repeated qPCR / dPCR reactions on a sample (i.e. technical replicates) that were positive, and data from metabarcoding may be expressed as ASVs, OTUs or sequence reads. eDNA data can also have different spatial and temporal resolution to data produced by

conventional monitoring. eDNA can persist in water for several days to weeks, and months to years in sediment (Harrison et al., 2019). Still waterbodies that receive inputs from rivers and streams are likely to contain DNA from taxa present in the wider landscape as well as taxa living in those waterbodies (Littlefair et al., 2023).

Consider **visual summaries** like graphics, graphs, and where possible with illustrations, icons, and photos. Common ways of presenting DNA results include plotting detections on a map, or using pie charts / sunburst plots showing the proportion of sequence reads for each taxa, e.g. interactive Krona charts.

Consider **accessibility guidelines** (e.g. colourblind-friendly colour schemes and descriptions of visuals for sight-impaired participants) and the **language** used, e.g. use common names of species where possible.

Interactive web portals, where a link to the sample taken by that individual (without including participant names or other information) is provided, would likely be highly valued by participants. eDNA data can be made available as an interactive map, e.g. from the New Zealand Government's Wai Tuwhera o te Taiao – Open Waters Aotearoa programme and the University of California Conservation Genomics Consortium's CALeDNA programme. Intermediate / final results can also be presented via an **online webinar or in person session**. 'Meet the scientist' sessions are appealing to participants where they have the opportunity to ask questions.

Consider a **final (short-term projects) or annual (long-term projects) report for participants**. Requirements for public facing summaries should be included in the project description and baked into any costings for project delivery. Reports should be as visual as possible and suitable for the level of the volunteer group, e.g. Marine Conservation Society Annual Wildlife Sightings Report. Participants should be surveyed to understand what they want to gain from being part of the research so that the results can be tailored to the audience, for example, school children, religious groups, or recreational divers etc.

A **scientific paper for the overall project** should be written for the scientific and monitoring community to learn from. It would be prudent to publish results in an open-access journal subject to scientific peer review (Gadermaier et al., 2018).

3.4.6. What's the story?

As well as the synthesis and presentation of evidence, an experience is generated among stakeholders by embedding and grounding the findings into a contextually relevant story (Sundin et al., 2018). The aim is to make the information more digestible for participants and other stakeholders, often employing personal perspectives and illustrative examples. Consider the 'story' of your project - what is the beginning, middle and end? (Figure 4).

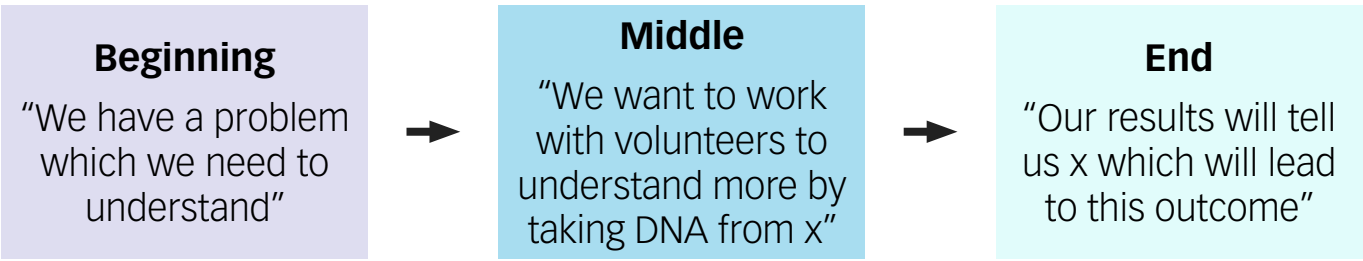


Figure 4. The 'story' of your project which should be included in communications outputs.

3.4.7. How frequently will you communicate?

Phased or continual communication will update the volunteers to any changes in the timeline, e.g. if there is a lag in the DNA analysis and feedback of the results. Transparency in the process will increase satisfaction and reduce frustration for volunteers.

Phases may include communication at the following points:

- Immediately after submission (thank you and basic details of their response);
- During the data collection period (details of goings-on such as how the processing happens, updates of stages, timelines, i.e. "where is your sample now?");
- Once the data collection season ends and all the results are in (e.g. total number of samples, number of co-researchers/participants, distribution of collected samples) and timeline for final results;
- Once the lab analysis has been completed and the species information is available.

Funded projects must take into consideration the funding timescales, deliverables and milestones. Although communications should be built into the project timeline, there may be limited time for follow-through with volunteers. It is important therefore to consider the project's longevity should funding cease, e.g. whether it is beneficial to have lasting resources like videos.

4. CS x DNA project design

The main elements of CS x DNA project design (Figure 5) are:

- Defining research questions and aims;
- Engaging a volunteer group;
- Sampling design;
- Molecular methods and data analysis;
- Creating and distributing outputs.

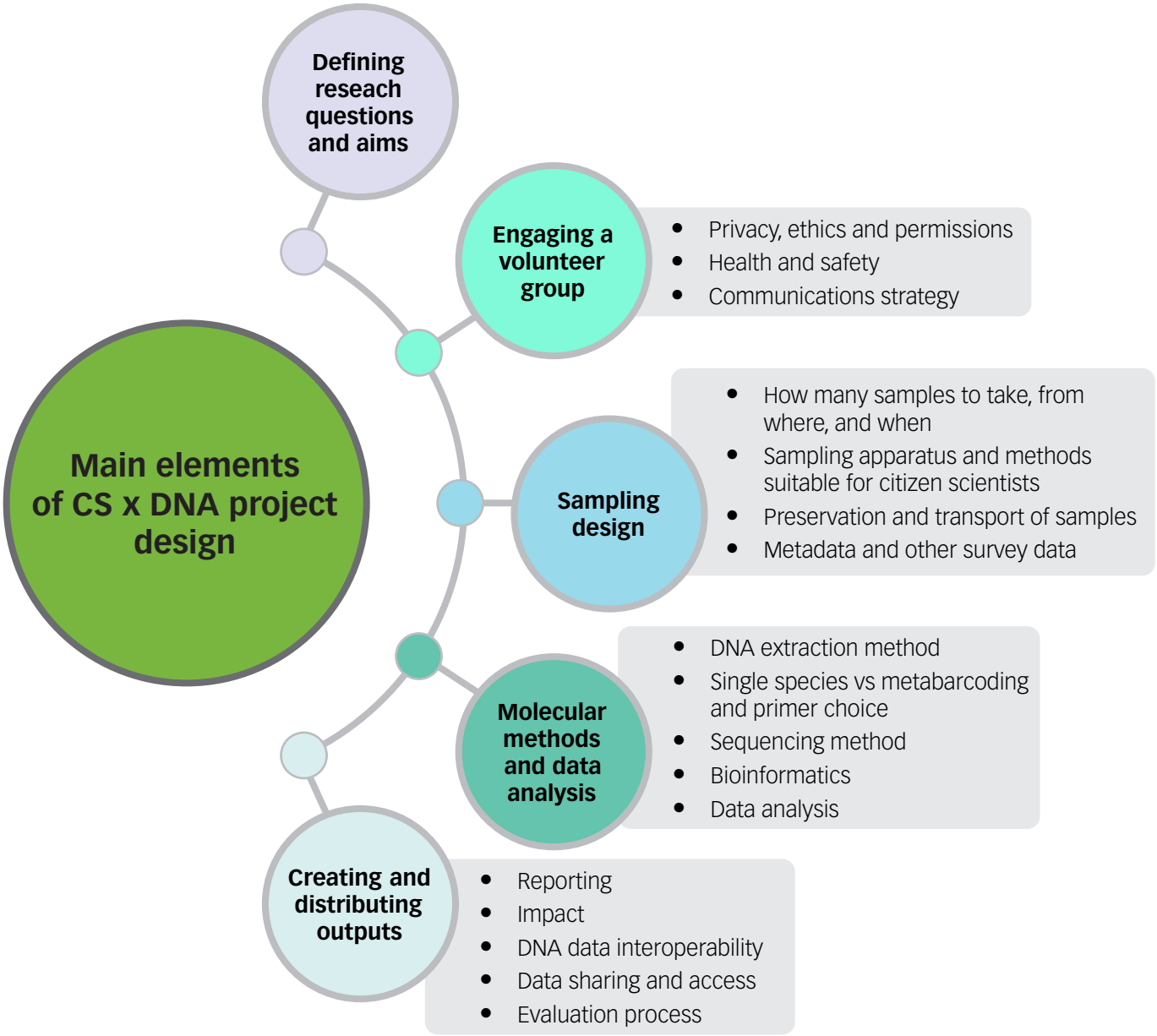


Figure 5. CS x DNA project design. Illustration by Lauren Cook.

Project design is illustrated above with specifics from the first year of the GenePools project (Rees et al., 2023) detailed in the boxes. Note that some of these elements are likely to happen in parallel, for example, the molecular methods will need to be established before data collection occurs, and these are likely to influence the design of the sampling strategy. The communications

strategy will also need to be at least partly established before engaging volunteers. For some projects, the design process will be more iterative or adaptive. Indeed, the methods and volunteer engagement for GenePools have changed in the second and third years. Genepools was funded by Defra's Natural Capital and Ecosystem Assessment programme. For further general advice on implementing citizen science for studying biodiversity and the environment in the UK, see Tweddle et al. (2012).

4.1. Defining research questions and aims

Work out what the question is that you need answering. The information gained through DNA analysis needs to serve a useful purpose towards this question, i.e. which taxa might be the most useful indicators of ecosystem health and is DNA a good way to survey these species?

Think about the suitability for citizen scientists. There may be a volunteer group with specific expertise which could carry out sampling.

Note: Co-design of the project means engaging the volunteers at the beginning of the process.

GenePools

The aim was to understand the ecology of urban garden ponds through eDNA samples. DNA present in water samples from organisms including bacteria, eukaryotes, plants, invertebrates, and vertebrates was sequenced.

It was important to involve volunteers that could send samples from ponds in private gardens that are inaccessible to professional surveyors and so represent a gap in scientific knowledge.

Recommendations on how to improve ponds for biodiversity in urban areas were made, based on the DNA findings and the other data on the ponds.

4.2. Engaging a volunteer group

The volunteer group engaged may depend on project factors such as the geographic location of sampling, any specific demographic group that should be targeted, and any prior expertise or skills required. The project may be suited to an already engaged network of volunteers, or it may be necessary to engage a new network as part of the project.

There are methods to increase the diversity of participants in CS by reaching out to members of the community which would be historically underrepresented in nature and the outdoors. Schools, education centres, community groups and initiatives such as the National Education Nature Park may be another avenue for engaging diverse communities.

GenePools

A collaboration was set up between Natural England, Cefas, Natural History Museum (NHM) and Joint Nature Conservation Committee. The volunteers were engaged through the NHM, where there is an existing network. Natural England and the NHM worked to widen participation of GenePools by targeting underrepresented religious groups through contacting places of worship throughout the cities being studied. The NHM also hosted a workshop with students from Imperial College London, who highlighted some intersectional groups to engage with.

4.2.1. Privacy, ethics and permissions

Permissions: Ensuring the appropriate permissions are in place is still required, e.g. access to the land, Site of Special Scientific Interest (SSSI) consent etc. If sampling on private land, land access / data storage / sharing permissions will need to be agreed before the project commences.

The project manager must be satisfied with the consents and licenses for any activity that takes place.

Privacy / Ethics: The use of personal data under UK General Data Protection Regulation (GDPR) must be agreed by participants.

GenePools

The Natural Capital and Ecosystem Assessment (NCEA) programme privacy notice was used for the project. It contains information on the collection of personal data, what personal data is collected, how it is used etc. Participants were also asked to confirm they had permission from the pond owner before sampling the pond.

4.2.2. Health and Safety

The potential risks to the participants should be clearly communicated to them along with information on how to avoid these risks. A risk assessment should be written, and appropriate steps taken to mitigate any risks, as well as discussed with the appropriate Health and Safety advisors within the lead organisation.

GenePools

Health and Safety guidance was outlined to volunteers prior to sampling via a risk assessment.

4.2.3. Communications strategy

A communications strategy depends on the project aim, the target audience(s), motivations of your participants, and your communication budget. Tactics for communication to promote continued participation can include offering a **fun experience** such as a social event or excursion, and use of **social media** to allow interaction between scientists and volunteers and volunteers to make connections with each other.

Use visuals, audio or text to tell a story, often including personal perspectives, as well as historical and educational contexts to the project. **Storytelling** can create a sense of belonging for the volunteers, especially if they are able to give their personal stories, and create interest and curiosity. Stories can be shared through the normal communication channels, e.g. blog, newsletter or social media etc.

Including aspects of **game playing** ('gamification') into the CS project can promote continued participation for volunteers who may feel motivated by taking on a challenge, tracking their improvements or winning a competition. This may include using points, badges and trophies, and awarding prizes for competition of a 'mission' or 'race against the clock' etc.

Employing **project 'ambassadors'**, such as a volunteer who has been involved since the beginning of the project, knows a lot about the research topic or with some prior experience in CS, can promote continued participation. They have a strong motivation to participate and can help with project logistics / administration / communication.

Note: Learn more by reading 'Tactics and Tools' from Communication in Citizen Science by Veeckman et al. (2019).

GenePools

Communications strategies included sharing the project with volunteer networks at the NHM, and on social media by the partner institutes. The NHM hosted a web page with information on the project. Frequent updates via email were sent to participants. The results were shared with participants via a written report and an interactive dashboard. The project was communicated with staff of partner organisations via intranet and newsletter articles. They project results were communicated with the wider scientific community via social media, presentations at conferences, and a Natural England commissioned report. A scientific journal publication is planned.

4.3. Sampling design

Consider whether to adopt a published sampling protocol or design a new sampling protocol. A new protocol should be validated against established protocols and / or conventional surveys to ensure quality and reliability of results.

4.3.1. How many samples to take from where and when?

The choice of sample matrix (e.g., water, air, soil, bulk samples) will depend on the ecology of the group(s) in question, for instance, where does that organism spend most of its time, and therefore where might DNA accumulate. Depending on the group(s) in question, it may be appropriate to collect bulk samples, e.g. invertebrates through pitfall or malaise traps.

GenePools

A broad baseline of biodiversity in and around ponds was required, so water samples from the pond were best.

The number of samples should reflect the spatial complexity and size of the system being sampled as well as the spatial and temporal resolution you wish to achieve, e.g. are you interested in biodiversity at local, regional or national scale, or in specific habitats, and at a single point in time or seasonally.

Note: Read more about factors to consider for aquatic sampling in p. 12 of 'A practical guide to DNA-based methods for biodiversity assessment' by Bruce et al. (2021).

GenePools

The sampling protocol was chosen to align with existing Natural England pond eDNA surveys to enable use of the data beyond this project. One water sample of 1 L is deemed sufficient for a pond balanced with the practicalities of volunteers taking samples themselves. Many small subsamples from around the pond were taken and combined to produce a mixed sample from throughout the pond.

4.3.2. Sampling apparatus and methods suitable for citizen scientists

Sampling kits need to be relatively **inexpensive** if sending out en masse to individuals. Some sampling apparatus may be less suitable for use by citizen scientists than others. The type of volunteer group will affect which apparatus may be suitable, i.e. if you are engaging a volunteer group with a particular skill which may have a higher level of fitness or capability.

Gloves should be provided with sampling kits but hand sizes can vary and some people may have allergies to the glove material. If gloves are not worn, there is a risk of human contamination and skin irritation / damage if the sampling kit contains any hazardous chemicals. You may want to consider the target for your survey and whether contamination may be an issue, i.e. whether you are analysing the samples for vertebrate or bacterial DNA where human or microbial contamination would impact results. You should also consider risk associated with any chemicals in sampling kits.

Citizen scientists previously involved in DNA projects have expressed concerns about **plastic waste** when sampling (Broadhurst et al., 2025). This would especially be an issue in countries where capacity for responsible waste disposal and recycling is limited. To minimise environmental impact by eliminating unnecessary plastic waste, provision of unwrapped kits (with unwrapped components such as the filter and syringe) could be considered depending on the target of the project, understanding the contamination risk with the primers chosen, and deciding whether to accept that risk.

Some options of sampling methods which may be suitable for citizen scientists include:

a. Water

In aquatic environments, **ladles** may be needed to access the water. These can be attached to a pole or to a rope to be lowered into the water from a height. **Collection bags / buckets** may suffice if a sampling location is sufficiently accessible and safe, and these can be lowered into water. **Sterile water bottles** can also be used for sampling (Broadhurst et al., 2025; Lavin, 2022).

After water has been collected, it is generally filtered in the field with a pump or syringe so only the filter with preservative needs to be sent back to the laboratory. For **manual filtering**,

apparatus which does not require a high level of physical strength will be necessary for a diverse volunteer group as pushing water through a filter by hand can be challenging, especially in more turbid water (Andreou et al., 2023). **Pumps** (e.g. Smith-Root) can be used where manual filtering of water would limit the accessibility of sampling, or where sample volumes are high (e.g. more than 1 L). However, pumps are expensive to purchase and could potentially be damaged when shipping to or being returned by volunteers, or during operation in the field.

Aquatic sampling kits suitable for CS need to be robust against contamination, for example, with filters enclosed inside a solid housing, e.g. Sterivex filters (Merck), Sylphium filters (Sylphium), or Whatman Polydisc filters (Camlab). The preservative solution should be pre-loaded into a syringe.

Alternatively, **passive samplers** (no filtering required) can be used to collect DNA, e.g. gauze inside a 3D printed container can be submerged following which the DNA is directly extracted from the gauze. Passive samplers could be attached to a diver during a normal recreational dive (Neave et al., 2023) or attached to a fishing line from a pier / boat / beach (Maiello et al., 2022). They can also be left in an aquatic area for a certain length of time while DNA accumulates, which is especially useful for capturing microorganisms (Sikorski and Levine, 2020).

b. Soil / mud / sediment

Soil or sediment can be collected by using a **corer**. More make-shift collection of soil and sediment can include using a sterilised spoon / spatula or tube to **scoop** material into a tube as used in the 'Sampling the Munros' project.

c. Swabs and Scrapes

Swabs or scrapes can be used to collect eDNA from surfaces such as rocks, plants, or other substrates where organisms have left genetic traces (Lyngaard et al., 2023).

d. Air

Similar to water, DNA in air can be captured using passive (e.g. dust traps, sticky surfaces) or active (e.g. fans or pumps drawing air in through a filter or over a microscope slide) sampling approaches for the detection of vertebrates, invertebrates, plants, fungi, bacteria, viruses etc. (Johnson and Barnes, 2024).

Note: Some of these sampling approaches are in the early stages of development, but new devices and increasing use are likely to be seen in the next few years, e.g. AirDNA Sampler designed by Harnpicharnchai et al. (2023).

e. Natural samplers

DNA can be concentrated naturally inside an organism or an organism derivative, e.g., bloodmeal, honey). Note that there are ethical considerations when working with live organisms and it may only be suitable for a volunteer group with some specific training. Some examples include:

- **Sponges** – sponges can be non-lethally sampled and concentrated DNA in their tissues from the surrounding biota can be screened (Mariani et al., 2019);
- **Haematophagous invertebrates** – sometimes referred to as invertebrate-derived DNA (iDNA), blood can be linked to animals that invertebrates have fed on, e.g., terrestrial mammals (Baker et al., 2021);
- **Honey** – honey contains DNA from plants foraged by pollinators, and microbiomes of bees, as collected by volunteer beekeepers, e.g. National Honey Monitoring Scheme.

f. Bulk sampling

Invertebrates can be collected in a preservative (e.g. propylene glycol) from various types of traps (e.g. malaise, pitfall) or collected using nets then added to a preservative. Invertebrates are killed on contact with preservative but specimens may be damaged in the process of collection. For DNA analysis, these bulk invertebrate samples are typically homogenised or a leg or piece of tissue taken from each specimen for DNA extraction. The resulting data can be useful for conducting a rapid biodiversity assessment in the area (Rees, 2022) or providing evidence for change in insect composition and abundance over time (Koperski, 2023). The suitability of this method for CS will depend on the volunteer group as the ethicality of sampling invertebrates is subjective.

Invertebrates do not have the same ethical procedures in place as vertebrates, which may result in the suffering or killing of many more individuals than necessary (Koperski, 2023). Where possible, efforts should be made to reduce suffering and mortality, through statistical power analysis, reducing bycatch, and making bycatch available for future use (Drinkwater, E. et al., 2019). New approaches, such as collecting aquatic macroinvertebrates and leaving them in a water sample for a set time period before releasing them and filtering the water (Sander et al., 2025) or collecting faeces that insects have come into contact with (Drinkwater, R. et al., 2021) may offer solutions. Where mortality is unavoidable, the reasons and need for this to fulfil project objectives should be carefully communicated to volunteers to avoid backlash and disengagement, e.g. UK Pollinator Monitoring Scheme pan trapping protocol. A BBC Radio 4 interview covered these issues in more detail.

GenePools

Enclosed Sylphium filters were used to minimise contamination. The filter pore size was changed from 0.22 µm to 0.8 µm as many volunteers complained that the manual filtering was too hard with the smaller pore size. A 100 mL syringe was included in the sampling kit, meaning a full syringe needed to be pushed through 10 times. Ladles were used to access the water and each scoop tipped into the large 2 L capacity sample collection bag, which was sealable to be able to shake and mix the sample.

4.3.3. Preservation and transport of samples

To ensure the quality of the DNA in a sample, freezing will be required or a preservative substance will need to be added. It is recommended to ensure a cold chain is in place for samples from field collection to laboratory processing, or purchase an appropriate preservative from the same company that will supply sampling equipment (e.g. filters) and / or process samples to ensure compatibility. The preservative must be in line with Health and Safety for CS, i.e. non-toxic and non-flammable to be used by volunteers.

GenePools

A preservative solution was injected into the filter housing after sampling from pre-loaded syringes. Using a preservative such as Longmire's buffer (Longmire et al., 1997) or Cetyltrimethylammonium bromide is not hazardous if spilled.

Filters loaded with preservative in a solid filter housing can be sealed and kept at ambient temperature until being received at the laboratory. Soils and sediments are often kept and transported cool without any preservative added, but can be fixed in a preservative solution and transported as a whole sample at ambient temperature.

GenePools

After preservative solution was added, samples were stored at ambient temperature until they were received at the laboratory where they were frozen.

When transporting eDNA samples from citizen scientists to a laboratory for analysis it is critical to ensure the samples remain viable and uncontaminated during transport. Consider the following guidelines:

- **Sample labelling:** Provide a label to clearly distinguish containers with unique identifiers, time, date, and location (GPS coordinates if possible). Using a pen or pencil appropriate for the containers and the preservative being used is also important;
- **Packaging:** Packaging should ideally be (i) insulated (samples should be kept cool to minimise DNA degradation), (ii) leak-proof, air-tight and in a secondary bag to prevent leaks, and (iii) properly cushioned to prevent physical damage;
- **Postage:** Use a reputable courier with experience in handling biological samples. Choose a shipping option that ensures samples reach the laboratory quickly and under appropriate conditions. Conditions of carriage need to be considered dependent on any preservative used. A sample tracking system to monitor the location of samples during transit will ensure samples can be traced in case of any issues;
- **Cost:** Consideration should also be given to the cost of posting, and ensuring that everyone is able to participate, i.e. ensuring in the contract with suppliers that the sample kits sent to participants must include a stamped return envelope;
- **Communication:** Establish clear communication channels, e.g. contact information for questions or concerns regarding sample transit. Clear deadlines should be included in the instructions indicating when the kits should be sent.

GenePools

Participants were sent their sampling kits via post with a pre-stamped and pre-labelled envelope included in the sampling kits.

4.3.4. Metadata and other survey data

Consider what additional data will be needed to describe the data which will provide valuable additional context for data analysis. Metadata also includes personal information from participants, which should be outlined in the privacy statement. Other survey data includes information about where the sample was taken, e.g. any geographical or biochemical information.

The use of standardised metadata templates will enhance the impact of the DNA results for wider use beyond the project. These descriptive data are required to enable the data to be understood and to provide confidence in the results. They enable the DNA records to be uploaded onto public repositories to be combined with other biodiversity data and accessible for other researchers to use. Templates are available from the Genomic Observatories Metadatabase (Deck et al., 2017), the Metabarcoding Data Toolkit (GBIF Secretariat, 2024), or the FAIR eDNA project (Takahashi et al., 2025). However, note that excessive metadata requirements can be off-putting to volunteers – the choice of what to collect should be carefully thought through and the rationale explained.

GenePools

Survey data collection (e.g. the size of the pond, vegetation cover, the age of the pond, the type of lining, the date and time of sample collection, the location of the pond etc.) was sent out to participants to fill in at the time of sampling.

Personal information of participants (e.g. locations and email addresses) were also collected and stored by the NHM on behalf of Natural England.

Participants also took measurements of pond chemistry (e.g. pH, nitrate levels etc.) with strip tests included in original sample kits.

4.4. Molecular methods and data analysis

Consider whether to adopt published molecular methods or design new protocols and / or primer sets for the target taxa. The former may save time and money if suitable for the study system, target taxa, and project aims, but the latter may be more efficient in the long-term if the project is on understudied systems or taxa. As with sampling, new protocols and / or primer sets should be validated against established protocols and / or conventional survey to ensure quality and reliability of results (Bayer et al., 2025; Biggs et al., 2015; Rodriguez et al., 2025; Thalinger et al., 2021; Vasselon et al., 2025).

4.4.1. DNA extraction method

The involvement of citizen scientists will not impact the DNA extraction method, although additional training may be required if volunteers are performing DNA extraction. This guide will not cover details of DNA extraction, but these are described in Bruce et al. (2021).

GenePools

This was agreed with Cefas. An extra homogenisation step after DNA lysis at 65°C overnight was included to break down the tough cell walls of plants and microeukaryotes.

4.4.2. Single species vs. metabarcoding and primer choice

Citizen scientists may have input into the target of investigation depending on the level of co-design of the project. Whether the project is targeting a single species (e.g. invasive or endangered species), a group of species (e.g. birds or mammals), or multiple groups will be dictated by the primers chosen for analysis. The specific gene region amplified by the primers will be compared to reference databases to identify the species present in the sample.

Primers from the literature will have different strengths and weaknesses, for example, a primer set targeting a broad group, such as eukaryotes, may not give as much detail on single species. Primer sets will be agreed between the project partners.

GenePools

Multiple primer sets were used to target vertebrates, invertebrates, eukaryotes, bacteria and plants.

4.4.3. Sequencing method

The choice of sequencing method depends on the specific research goals and the nature of the samples being analysed. There are several sequencing methods available, such as:

- **Sanger Sequencing:** The earliest sequencing method, less commonly used for large-scale biodiversity studies;
- **High-Throughput Sequencing (HTS):** Sometimes called Next Generation Sequencing, this is a contemporary sequencing method to analyse DNA fragments across many samples simultaneously. Often used in metabarcoding studies for biodiversity monitoring to sequence barcode regions across many species in each sample;
- **Shotgun Sequencing:** Involves sequencing all DNA present in an environmental sample, providing a comprehensive view of the genetic diversity in that sample. It is particularly useful for studying complex microbial communities but is not yet used widely in biodiversity monitoring due to the high costs and limited expertise in analysing shotgun data;
- **Nanopore Sequencing:** Sometimes referred to as third-generation sequencing, this involves DNA being sequenced as it is passed through tiny channels or nanopores. It allows real-time data acquisition, available on a portable hand-held device which can be valuable for in-field applications.

GenePools

Library preparation involved an index PCR step and HTS sequencing was performed on an Illumina MiSeq, NextSeq or NovaSeq.

4.4.4. Bioinformatics

Bioinformatics includes computational methods facilitating data analysis, interpretation and communication of DNA findings. It involves tasks such as quality control, data cleaning, data storage and matching sequences to species names. Sequencing methods, such as HTS and

nanopore sequencing, generate massive datasets which need to be organised and prepared for data analysis through bioinformatic methods. Bioinformatics can also include phylogenetic trees, to research the evolutionary relationships among species. Bioinformatics methods will be agreed between project partners who may have specific requirements, e.g. government regulators require open access software and reference libraries to be used. Results should also be scrutinised for unexpected results which could have occurred by contamination or mismatches in the reference library.

GenePools

Quality control reports generated by the sequencing service showed that returned sequences were of good quality. Sequences were converted into FASTA format before taxonomic assignment using the National Centre for Biotechnology Information (NCBI) reference library (see Rees et al. 2023 for detailed methodology).

4.4.5. Data analysis

A number of analytical approaches exist for DNA data as single species and community analyses tend to be transferable across conventional and molecular data sets. Single species DNA data has been used for distribution mapping and occupancy modelling. Metabarcoding data has been applied to alpha and beta diversity analyses, community structure, ecological and trophic networks, indicator species, prediction of ecological condition, impact assessment, and genetic diversity. We do not cover these approaches in detail as they are reviewed elsewhere (Deiner et al., 2017; Beng and Corlett, 2020; Environment Agency, 2024c).

GenePools

The total number of sequences and number of species detected with each primer set was reported, and the number of species per pond compared.

4.5. Creating and distributing outputs

4.5.1. Reporting

The frequency of reporting will vary depending on the length of the project, but report(s) should be accessible and contain visual summaries. Interactive web portals and webinars can be more engaging accompaniments to a written report. Publication of a scientific paper for the overall project will ensure wider visibility of findings and benefit the scientific and monitoring community. See section 3.4 for more information on reporting.

GenePools

There will be Natural England reports and a peer-reviewed paper for the scientific research community. There will be an interactive online dashboard (R Shiny app) and written report for participants with results of the study.

4.5.2. Impact

Consider how the results of the project can be shared and integrated into wider biodiversity monitoring and CS practice.

GenePools

Results will be feeding into the NCEA and creation of a pond health metric. Personalised results will be provided to participants, showing them what lives in their pond. They will also be given advice on how to improve their pond for wildlife and an in-depth explainer, which is aiming to increase scientific literacy of different life forms.

4.5.3. DNA data interoperability

The DNA community is beginning to work on a standardised approach to documenting DNA sequence data on biodiversity. This will enable analysis of DNA-derived biodiversity data from multiple projects and promote the creation of metrics for ecosystem health. In future, raw DNA sequence data will be shared with the community on open access sequence data repositories and DNA-derived species records will be shared on biodiversity platforms. Planning a data strategy for a DNA project involving citizen scientists requires careful consideration to ensure data quality, security, and that the data is working under the FAIR (Findability, Accessibility, Interoperability and Reuse) and CARE (Collective Benefit, Authority to Control, Responsibility, and Ethics) principles to maximise the effective use of the data generated (Berry et al., 2020; Takahashi et al., 2025; Wilkinson et al., 2016).

GenePools

Species lists will be available for participants to download from the dashboard. They will be published open access in a report for the wider scientific community. Raw DNA sequence data will be stored long term in Natural England, and shared when requested under an Open Government License.

4.5.4. Data sharing and access

All project data should be made accessible to the public, relevant scientific communities, and stakeholders while safeguarding sensitive and personal information, such as sensitive species information or landowner contact details. The FAIR eDNA project has established guidance for making eDNA data FAIR, including metadata, raw sequence data, raw and curated ASV / OTU tables, and raw and curated taxa / sequence tables (Takahashi et al., 2025). Participants must read the privacy notice before being asked for any personal data, as this sets out how their personal data will be used, stored and shared. There must also be secure data storage in place and compliance with GDPR.

4.5.5. Evaluation processes

Citizen science programs should be evaluated taking into account three important themes of participatory science: (i) scientific impact, (ii) learning and empowerment of participants and (iii) impact for wider society. Within this, resultant effects on wellbeing, nature connectedness and

pro-nature conservation behaviour could be evaluated. Evaluation and impact assessment should embrace the diversity and emerging nature of CS. Further information on how to evaluate CS projects is included in Kieslinger et al. (2018).

5. The future of CS x DNA

As DNA technologies continue to advance and CS initiatives become more widespread, exciting opportunities will arise for community involvement in biodiversity research and monitoring. Included below are some potential areas for expansion in the near future:

- **Standardised biodiversity data platforms:** Investigating platforms that can be a 'one-stop-shop' to input data and metadata from DNA projects, alongside conventional records such as the NBN Atlas and GBIF (Nilsson et al., 2022). Some eDNA-specific platforms have already been launched, such as the Aquatic eDNAtlas which is an open-access database developed through crowd-sourced eDNA surveys that provides precise spatial information on the occurrence locations of aquatic species in the United States. Integrated DNA data will be suitable for applying machine learning on a large scale, allowing predictive analyses for improved conservation and management (Cordier et al., 2017; Wilkinson et al., 2024). This data could also be sent one way for scientific analysis and another way for CS experts to interpret for volunteers;
- **Personalised CS Projects:** Tailoring CS projects to individual interests and skills will increase participation and foster a sense of ownership and commitment to long-term monitoring efforts (Clarke et al., 2023). GenePools has shown increased participant satisfaction where individuals can identify their direct contribution to the project by selecting the results from their sample on an online dashboard, i.e. R shiny app;
- **Newly emerging CS practitioner networks:** Groups of practitioners are collaborating at national, regional and global levels to exchange knowledge and skills, across disciplines and borders, such as the CitSci Africa Association, CitizenScience.Asia and UK Environmental Observation Framework Citizen Science Working Group. Where these associations continue to expand into under-represented countries, local CS practitioners can connect with the wider community, introducing insights from unique geographical contexts and diverse stakeholder groups (Fraisl et al., 2022). There is a lot of potential value in facilitating discussion and knowledge sharing between practitioners involved in CS and in DNA to address challenges in the field, assimilate lessons learned so far, and foster collaboration. This could be facilitated as workshops as part of wider events or focus / task-and-finish groups on more specific topics. Communication between research groups and organisations will also begin to progress some of the issues with interoperability of DNA datasets;
- **Open Science:** The transition of the global scientific community towards open science will increase the emphasis on public engagement, open access, FAIR data and open education (Berry et al., 2020; Wilkinson et al., 2016). This will be an opportunity to mainstream CS as these pillars become more fundamental in research communities (UNESCO, 2021);
- **Technological improvements:** DNA capabilities are constantly improving, for example, mobile apps and portable DNA sequencers are becoming increasingly accessible and user-friendly (Lu et al., 2024), expanding the scope of CS x DNA projects into new habitats and larger scales, revolutionising the speed and efficiency of biodiversity monitoring. However, these new technologies should be compared to existing approaches to understand any potential effects of changing methods on resulting data, especially in long-term projects;

- **Engagement in Population Genetics:** CS could play a role in conservation through population genetics, which aims to understand the dynamics of genes in populations to aid management of natural resources or prevent extinctions. Genes of individuals can be studied by sampling eDNA, or derivatives of organisms such as faecal samples and swabs (Couton et al., 2023b; Cunningham-Eurich et al., 2023). However, sampling requiring licenses (e.g. wing hole-punching for bats) would not be practical for CS.

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