Environmental DNA

A POLICY EXPLAINER

THE ROYAL SOCIETY

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Executive summary

What is environmental DNA?

Environmental DNA (eDNA) refers to DNA that can be obtained from an environmental sample, usually from water, soil or air. For larger species (including humans), eDNA is recovered from fragments of cellular material shed into the environment such as saliva, faeces, hair or skin cells. For microbes, whole organisms and their genomes may be captured. eDNA sampling techniques differ from more traditional DNA analysis where samples are taken directly from the organisms themselves, rather than the environment.

Why now?

Progress in eDNA research during the last ten years has been rapid and has led to important developments in biodiversity monitoring, pathogen detection and forensic science. As the volume of data and information that can be obtained using eDNA continues to increase, now is an important moment for the UK to consider how best to optimise the value of these technologies for both public and private sectors – and how to avoid harm. This document aims to show the breadth and strength of eDNA applications, and to discuss the opportunities and risks that they present.

The UK is already world-leading in using eDNA for regulatory purposes. For instance, this approach is used to detect the presence of great crested newts (a rare and protected UK species), which has greatly reduced the time and cost of monitoring. In the near future, the UK also has the potential to have a complete DNA reference genome library for all its native, multicellular, eukaryotic species[1], a vital foundation to support eDNA based research. For the private sector, the efficiency and volume of data that can be obtained using eDNA technologies make them an attractive addition to several growing markets, including environmental consulting, bioterror defence and agricultural pest control. In these sectors, eDNA technologies could be commercialised to make significant contributions to the UK economy.

Current and emerging eDNA applications could provide many important benefits to policymakers (see Figure 1). These include: biodiversity monitoring; water quality monitoring; tracking disease in wastewater (such as COVID-19); pre-emptively detecting agricultural pests, pathogens and invasive non-native species; linking subjects to crime scenes; and tracking the illegal wildlife trade.

1 Darwin Tree of Life Project. Wellcome Sanger Institute. See https://www.darwintreeoflife.org/ (accessed on 6 November 2024).

Future opportunities

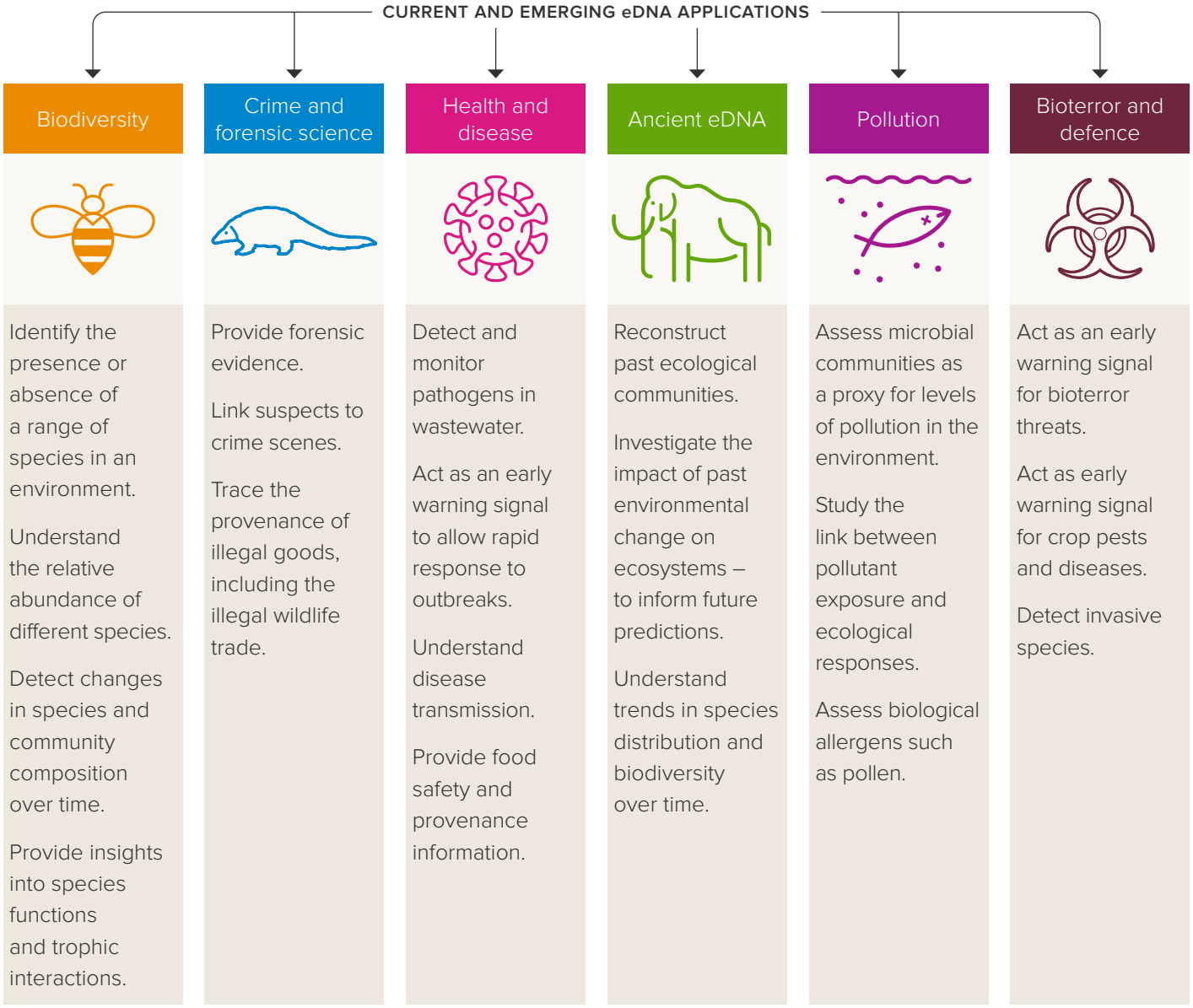
Future developments are expected to expand the range of eDNA applications, and will also help to mitigate the limitations associated with current applications. For example, increased knowledge of the persistence and transport of eDNA in the environment could allow more direct pinpointing of the time and place in which the DNA was shed. Not only would this improve the accuracy of existing insights but could lend itself to new applications, such as using eDNA to help detect missing people or identify victims of war or natural disasters. eDNA air capture technologies are also developing rapidly. It is likely to become possible in the near future to use these in defence to detect novel pathogens which could pose a bioterror threat. Air capture eDNA may also have use in forensic science, where eDNA shed from skin cells, hair and saliva, present in the air, may offer a thus-far untapped resource in criminal investigations.

Benefits

eDNA provides richer and more taxonomically complete data than has been available previously. It could therefore usefully complement or enhance existing routine monitoring, with relatively little additional effort or cost. In the future, once benchmarking is more complete, eDNA may also provide an alternative to existing environmental monitoring. Taking regular eDNA samples from the environment could provide data for several different applications at once (eg pathogen surveillance, biodiversity monitoring, pollution monitoring) – which might make these approaches particularly cost-effective if used in a joined-up way.

FIGURE 1

A summary of the current and emerging policy-relevant applications of eDNA



Challenges

Alongside new possibilities, there are legal and ethical challenges associated with eDNA, especially regarding the capture of human DNA sufficient to identify individuals. Consent, privacy, surveillance, data ownership and data storage will need to be considered and mitigated by policymakers, potentially in the form of guidelines or novel regulation.

One of the key current limiters of eDNA research is the availability of supporting research infrastructure such as reference libraries and sample repositories. Investing in these, alongside the development of standardisation or benchmarking tools, will be essential to support future developments. Contamination of eDNA samples also remains a key risk with both forensic and biodiversity applications, and further research is required to control and account for this.

Considerations

To ensure that society and the UK benefit from the potential that eDNA technologies have to offer, and avoid harm, policymakers should consider the following:

1. **How can we capture the potential value of eDNA research to benefit the UK economy?** eDNA holds potential value to a wide range of sectors. eDNA has the potential to be faster, more efficient and provide richer insights than many current monitoring methods. Now is an important moment to consider how the UK may best capture and capitalise on this potential value, both in terms of public and private benefits.

2. **How can eDNA sampling and monitoring be joined up to benefit multiple disciplines and applications at once?** The development of eDNA methodologies is often siloed into different academic disciplines. Greater join-up and collaboration would be beneficial to allow the cross-fertilisation of ideas, exchange of methodologies and sharing of best practice.

3. **What research infrastructure, benchmarking and guidance is required to support future eDNA research and applications?** So that eDNA research can benefit society as much as possible, investment in common foundations to support this research will be vital. This includes infrastructure such as DNA sample curation, reference libraries, data repositories and standardisation or benchmarking tools.

4. **How can we understand and mitigate the ethical, legal and regulatory concerns regarding human eDNA recovery?** eDNA technologies have the potential to either intentionally or unintentionally capture and sequence human DNA, sometimes sufficient to identify an individual. This presents important ethical dilemmas regarding consent, privacy, surveillance, and data ownership and storage.

Introduction

The technological advances in high-throughput sequencing and data analysis tools have facilitated the access to, and availability of, eDNA data.

All organisms, including humans, are continuously shedding DNA into the environment, for instance from skin cells, mucus, faeces, saliva and other sources. This environmental DNA (eDNA) can be collected and analysed (Box 1). eDNA can be extracted from many types of environmental samples[2, 3, 4] – but most usually water, soil or air. For larger species, DNA is recovered from fragments of cellular material, whereas for microbes, whole organisms and their genomes may be captured. eDNA may include samples of animal or plant material, such as organic material, dust, scales or hair. Similarly to eDNA, RNA is also found in environmental samples and is termed environmental RNA (eRNA)[5].

eDNA-based research has developed rapidly during the last ten years[6]. Although the basic concepts have not changed, the technological advances in high-throughput sequencing and data analysis tools have facilitated the access to, and availability of, eDNA data[7]. The volume of data that can be obtained using these methods continues to increase in terms of its accuracy, level of detail and the speed at which it can be analysed. Figure 2 illustrates the number of academic publications on eDNA over the past 10 years demonstrating the rapid growth in the field.

It has, therefore, become possible to monitor biodiversity with unprecedented precision and depth. Large environmental genomic datasets can rapidly be generated at relatively low cost[8]. The analysis of these datasets using machine learning and other AI approaches has made it possible to develop new groups of bio-indicators[9, 10, 11].

2 Taberlet, P., *et al.*, 2018. Environmental DNA: For biodiversity research and monitoring. Oxford University Press.

3 Pawlowski, J., *et al.* 2020. Environmental DNA: What's behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring. Molecular Ecology. 29, 4258–4264. (DOI 10.1111/mec.15643).

4 Rodriguez-Ezpeleta, N., *et al.,* 2021. Trade-offs between reducing complex terminology and producing accurate interpretations from environmental DNA: Comment on "Environmental DNA: What's behind the term?" by Pawlowski *et al*., 2020. Molecular Ecology. 30(19), 4601-4605.

5 Cristescu, M. E. 2019. Can environmental RNA revolutionize biodiversity science?. Trends in Ecology and Evolutionl. 34, 694–697. https://doi.org/10.1016/j.tree.2019.05.003

6 Taberlet, P., *et al.*, 2018. Environmental DNA: For biodiversity research and monitoring. Oxford University Press.

7 Pawlowski, J., *et al.*, 2021. Environmental DNA for biomonitoring. Molecular Ecology. 30(13) 2931.

8 *Ibid*.

9 Cordier, T., *et al.*, 2018. Supervised machine learning outperforms taxonomy-based environmental DNA metabarcoding applied to biomonitoring. Molecular Ecology Resources, 18(6), 1381–1391. (doi 10.1111/1755-0998.12926).

10 Cordier, T., *et al.*, 2019. Embracing environmental genomics and machine learning for routine biomonitoring. Trends in Microbiology, 27(5), 387–397. (doi 10.1016/j.tim.2018.10.012).

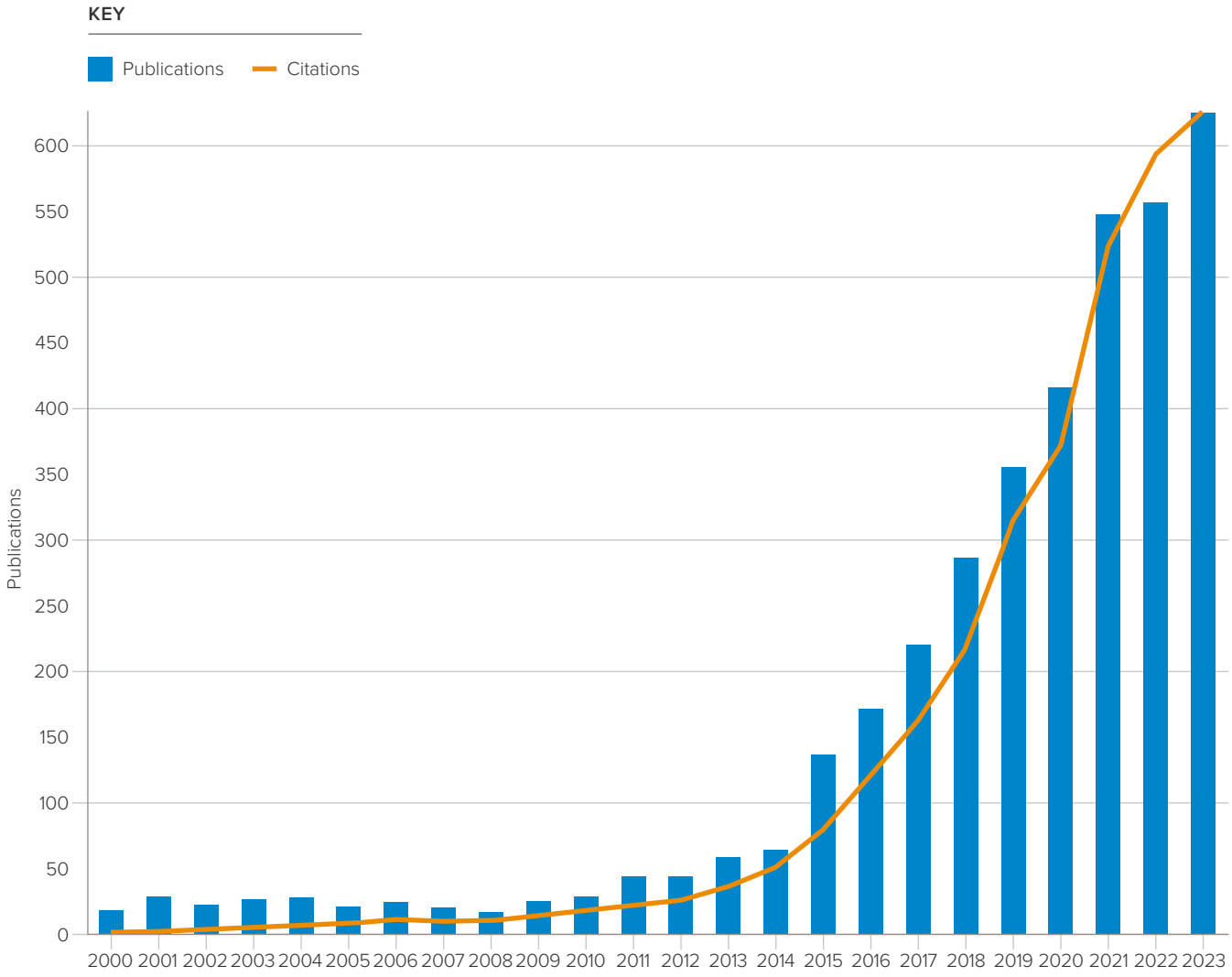
11 Pawlowski, J., *et al.*, 2018. The future of biotic indices in the ecogenomic era: Integrating (e)DNA metabarcoding in biological assessment of aquatic ecosystems. Science of The Total Environment, 637-638, 1295–1310

Alongside this, continual efforts to fill gaps in reference databases are increasing the effectiveness of taxonomic identification using eDNA data[12].

These developments have expanded the applications of eDNA across diverse fields and sectors, as this report will explore.

FIGURE 2

Number of eDNA publications per year from 2000 – 2023



Data from Clarivate's Web of Science.

12 Weigand, H., *et al.*, 2019. DNA barcode reference libraries for the monitoring of aquatic biota in Europe: gap-analysis and recommendations for future work. The Science of the Total Environment, 678, 499–524. (doi 10.1016/j.scitotenv.2019.04.247).

BOX 1

How is eDNA collected and analysed?

Figure 3 illustrates the common workflow for eDNA sampling, extraction and analysis. eDNA technologies rely on either DNA detection and amplification methods such as Polymerase Chain Reaction (PCR) or quantitative PCR (qPCR), or DNA sequencing approaches such as High Throughput Sequencing (HTS)[13].

Fragments of DNA (either mitochondrial DNA, nuclear DNA or extracellular DNA) are collected from the environment through sampling. Once in the laboratory, DNA is extracted from the environmental sample and for most current applications, specific regions of DNA known as 'DNA barcodes' are amplified using PCR, which generates millions of copies of the target region. PCR products are then sequenced using HTS technologies (eg Illumina or Oxford Nanopore Technologies platforms); a process known as 'metabarcoding'. Sequences are then compared to those in reference databases using bioinformatics pipelines to identify organisms detected in the environment from which the samples were taken.

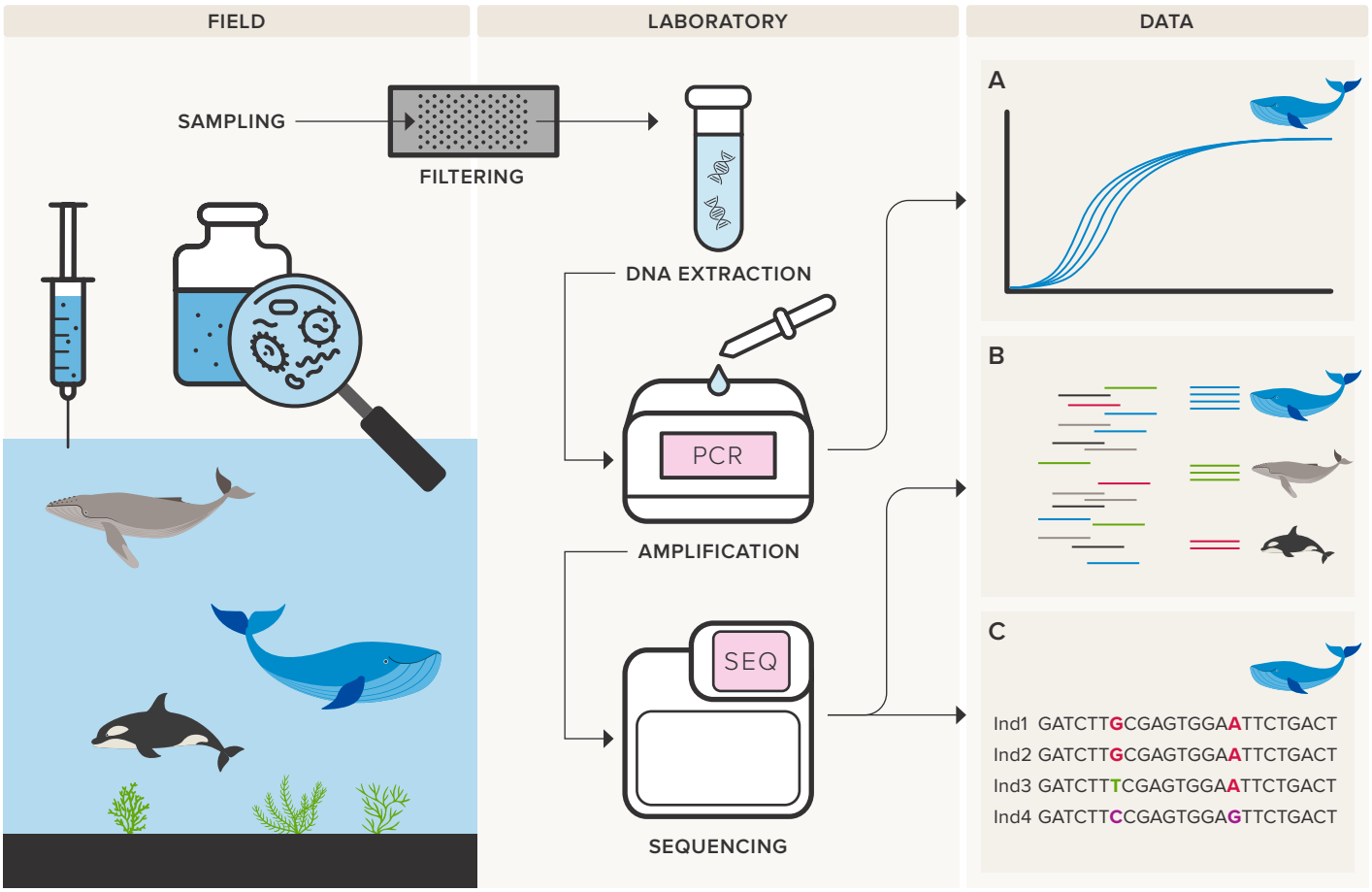
Whole genome shotgun sequencing (pooling together short fragments of DNA to obtain the entire genome) of environmental samples, without PCR amplification, is also used in certain circumstances but metabarcoding is currently the most widely applied approach for eDNA analysis. Depending on the quality of the DNA and completeness of the reference database, sometimes it may only be possible to identify organisms at a higher organisational level (eg genus or family), but often these methods can detect different species and subtypes, and increasingly, individuals.

Forensic applications focussed on the collection of human eDNA from samples usually rely on the recovery of nuclear DNA rather than mitochondrial DNA (except where bone analysis is used). This specific type of DNA is required to make comparisons to the national DNA database to identify potential suspects. However, to compare a DNA sequence to the national database the DNA needs to be relatively intact, and given complex (degraded) mixtures are very common in forensic application, special probabilistic software is required in these cases to help interpret the results.

13 Lawson Handley, L. (2015). How will the 'molecular revolution' contribute to biological recording? Biological Journal of the Linnean Society. Linnean Society of London, 115(3), 750–766. https://doi.org/10.1111/bij.12516

FIGURE 3

Workflow detailing eDNA sample collection, extraction and analysis



Pathway A = detection of single species of interest. Pathway B = assigning sequences to taxa. The number of red, green, and blue lines corresponds to separate sequence reads. Pathway C = population genetic inferences. There are four different DNA sequences (haplotypes) in the figure, so at least four different individuals have been sampled.

Now is an important moment to consider how recent advances in eDNA technologies can most effectively benefit society. eDNA has the potential to contribute to numerous government priority areas, including climate action, biodiversity conservation, food and water security, and economic resilience. It is important that policymakers understand both the relative strengths and weaknesses of eDNA so that it can be used in the right circumstances where it can add value, but not misused or the results wrongly interpreted. With such rapid developments it will also be essential that policymakers address the ethical concerns and dual use potential.

For example, the Convention on Biological Diversity's (CBD) Global Biodiversity Framework targets are fast approaching and there is a renewed interest in maintaining diversity within populations to build resilience to climate change and other stressors. eDNA could have a valuable role in associated ecosystem monitoring, regulation, biosecurity and prosecution. Closer to home, the Environment Act has set a statutory target in England to "halt the decline in species populations by 2030, and then increase populations by at least 10% to exceed current levels by 2042"[14].

This will require a range of integrated biodiversity monitoring methodologies, to which eDNA could valuably contribute. In terms of new and emerging applications, the improving accuracy of eDNA methodologies also means that they can increasingly be used to trace illegal trade, link individuals to the scene of a crime, and monitor pathogens and pollution. Air capture technology will also make eDNA a potentially valuable biosecurity and defence tool.

This report aims to raise awareness amongst policymakers of eDNA and its potential policy applications, current and future opportunities and limitations, and comment on the potential ethical and regulatory challenges.

14 25 Year Environment Plan. Defra. See https://www.gov.uk/government/publications/25-year-environment-plan (accessed on 20 September 2024).

Current and emerging eDNA applications

eDNA techniques originated in the field of biodiversity monitoring and therefore their use in this field is well-established. However, for other applications – such as disease monitoring, pollution monitoring, defence, biosecurity and forensic science – eDNA techniques are at varying levels of development and implementation. This section outlines the uses of eDNA technologies, within both established and developing sectors.

1.1 Biodiversity monitoring

eDNA applications related to biodiversity can be summarised into answering the following questions:

- **Is it there?** Detecting the presence or absence of individual species, for example conservation priority species or invasive non-native species.
- **What is the range of species present?** Describing whole communities of species within a certain environment. This can be used to inform biodiversity metrics such as species richness or community composition.
- **How many are there?*** Understanding the relative abundance of different species within environments over space and time.
- **How do they interact?** Providing insights into trophic interactions, for example the structure and complexity of food chains and food webs.
- **What do they do/what is their role in ecosystem processes?** Providing insights into the role of different species or combinations of species in delivering ecosystem services.
- **How are they changing?** Detecting trends and changes in species occurrence, relative abundance and community composition over time or changes in the expression of genes (using eRNA) as a result of environmental change or stressors.
- **How viable are they?** Using population genetics approaches (see Section 3.5) to monitor the genetic diversity of populations. This can be an indicator of their likely resilience in the face of stressors such as climate change or pollution.

*There is still some debate as to whether eDNA techniques can be used to measure abundance. It is possible to look at changes in the relative amounts of eDNA present from different species, in different environments, over time. However, the relative abundance of different species in relation to each other can be difficult to convert to absolute abundance. This is due to other variables such as DNA shedding rates, genome size, rate of degradation etc. Fundamentally, eDNA methods measure nucleic acid sequences and then use this as a basis for estimating either species presence or abundance, with predication of abundance subject to a greater level of uncertainty. The science here is still advancing, with researchers continually refining the accuracy and potential of these types of analysis, but there will be limits on how accurate this can eventually become (see Section 2.2 on limitations).

Current applications

During the past decade, eDNA approaches have been used for species detection in almost every type of aquatic[15, 16] and terrestrial ecosystem[17, 18, 19], including subterranean environments[20], Antarctic geothermal sites[21], coral reefs[22] and the deep ocean[23]. Across these habitats, eDNA will continue to have enormous potential for biodiversity conservation, monitoring and management by either complementing, enhancing or replacing traditional monitoring systems[24].

These approaches are proving particularly useful for understanding endangered, elusive or invasive species[25], or species within hard to access environments such as the deep ocean or dense rainforest. eDNA also enables the study of very small microbial and invertebrate communities, such as those in the soil, which were previously inaccessible using conventional visual survey methods[26].

Traditionally, eDNA has been used simply to identify the presence or absence of an individual species within a certain environment. Perhaps the most well-known example of this in the UK is the monitoring of the great crested newt. This is a protected species which needs to be considered as part of new infrastructure planning processes[27] (see Box 2).

15 Foote, A. D., *et al.*, 2012. Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals.

16 Yang, J., *et al.*, 2023. Small changes make big progress: A more efficient eDNA monitoring method for freshwater fish. Environmental DNA, 5(2), 363-374.

17 Allen, M. C., *et al.*, 2021. Terrestrial eDNA survey outperforms conventional approach for detecting an invasive pest insect within an agricultural ecosystem. Environmental DNA, 3(6), 1102-1112.

18 Beng, K. C., *et al.*, 2020. Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. Biodiversity and conservation, 29(7), 2089-2121.https://www.sciencedirect.com/science/article/pii/S0048969723009385

19 Van Der Heyde, M., *et al.*, 2020. Testing multiple substrates for terrestrial biodiversity monitoring using environmental DNA metabarcoding. Molecular Ecology Resources, 20(3), 732-745.

20 Saccò, M., *et al.*, 2022. eDNA in subterranean ecosystems: Applications, technical aspects, and future prospects. Science of the Total Environment, 820, 153223.

21 Takahashi, M., *et al.*, 2023. Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution. Science of the Total Environment, 873, 162322.

22 Richards, Z. T., *et al.*, 2022. Environmental DNA for biodiversity monitoring of coral reefs. In Coral Reef Conservation and Restoration in the Omics Age (pp. 203-224). Cham: Springer International Publishing.

23 McClenaghan, B., *et al.*, 2020. Harnessing the power of eDNA metabarcoding for the detection of deep-sea fishes. PloS one, 15(11), e0236540.

24 Pawlowski, J., *et al.*, 2021. Environmental DNA for biomonitoring. Molecular Ecology. 30(13) 2931.

25 Thomsen, P. F., *et al.*, 2019. Environmental DNA metabarcoding of wild flowers reveals diverse communities of terrestrial arthropods. Ecology and evolution, 9(4), 1665-1679.

26 Pawlowski, J., *et al.*, 2021. Environmental DNA for biomonitoring. Molecular Ecology. 30(13) 2931.

27 Great crested newts: advice for making planning decision. Natural England. See https://www.gov.uk/guidance/great-crested-newts-advice-for-making-planning-decisions (accessed on 20 September 2024).

BOX 2

Using eDNA to monitor the great crested newt

In 2014, the UK was the first country in the world to utilise eDNA for regulatory purposes – to demonstrate the presence or absence of the great crested newt. The rationale for this was that eDNA is more accurate in terms of detecting the presence of newts; per site eDNA monitoring costs 10 times less than traditional trapping; it only takes 0.5 hours per site to take a water sample for eDNA analysis as opposed to 20 hours to trap the newts[28]; and water samples can be taken in the daytime (as opposed to at night when newts are active). eDNA based methods also do not require personnel to enter the water, thereby also improving safety and practicality. Using eDNA has also enabled the District Level Licencing approach to be developed[29], which allows faster approval of planning proposals alongside more effective conservation of the newts[30].

Image
Once tracked using trapping and night-time surveys, the great crested newt is now monitored using eDNA.

28 Biggs, J., *et al.*, 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (Triturus cristatus). Biological Conservation, 183, 19-28.

29 District level licensing for great crested newts – by numbers! – Natural England (blog.gov.uk)

30 Natural England's Geoportal: England-wide data for great crested newts now available – Natural England (blog.gov.uk)

Emerging applications

eDNA techniques are increasingly being used in more complex ways to provide insights into the structure, interactions, wider functions and resilience of an ecosystem and the species within it. Methodological advances have rapidly evolved over the past few years to allow the detection of whole ecological communities and population genetic structures[31, 32], their trophic interactions (ie food chains and food webs)[33, 34], spatial and seasonal dynamics[35, 36, 37], and in some cases to quantify their relative abundance[38, 39]. These methods can also help with understanding changes in species distribution and community composition over time.

An example of this more complex analysis is the use of eDNA to understand animal-plant interactions. While traditional methods often limit investigations to pairwise species interactions[40], eDNA analysis offers the potential to explore intricate ecological networks. This includes the complex interconnections among diverse insect groups and the plants they pollinate (Box 3).

Consistent long-term monitoring based on the same metrics is valuable for understanding environmental trends over time. While eDNA-based approaches have often sought to replicate existing biodiversity indices, it is important to recognise that eDNA data offers a unique and often richer set of insights. By simply reproducing old indices with eDNA, we risk limiting its potential and retaining the constraints of traditional methods.

To fully leverage the power of eDNA data, researchers and policymakers should acknowledge its strengths, limitations, and differences from traditional techniques. This will enable an informed assessment of whether eDNA can replace or enhance routine monitoring on a case-by-case basis, focusing on the specific goals of the monitoring programme.

31 Deiner, K., *et al.*, 2021. The future of biodiversity monitoring and conservation utilizing environmental DNA. Environmental DNA, 3(1), 3-7.

32 Shum, P., *et al.*, 2021. Testing small-scale ecological gradients and intraspecific differentiation for hundreds of kelp forest species using haplotypes from metabarcoding. Molecular Ecology, 30(13), 3355-3373.

33 D'Alessandro, S., *et al.*, 2021. Sifting environmental DNA metabarcoding data sets for rapid reconstruction of marine food webs. Fish and Fisheries, 22(4), 822-833.

34 Thomsen, P. F., *et al.*, 2019. Environmental DNA metabarcoding of wild flowers reveals diverse communities of terrestrial arthropods. Ecology and evolution, 9(4), 1665-1679.

35 Carraro, L., *et al.*, 2023. Modelling environmental DNA transport in rivers reveals highly resolved spatio-temporal biodiversity patterns. Scientific Reports, 13(1), 8854.

36 Abrego, N., *et al.*, 2024. Airborne DNA reveals predictable spatial and seasonal dynamics of fungi. Nature, 1-8.

37 Gibson, T.I., *et al.*, 2024. Environmental DNA reveals ecologically relevant spatial and temporal variation in fish assemblages between estuaries and seasons. Ecological Indicators, 165, p.112215.

38 Bradley, D. L., *et al.*, 2022. Environmental DNA detection and abundance estimates comparable to conventional methods for three freshwater larval species at a power plant discharge. Environmental DNA, 4(3), 700-714.

39 Sard, N. M., *et al.*, 2019. Comparison of fish detections, community diversity, and relative abundance using environmental DNA metabarcoding and traditional gears. Environmental DNA, 1(4), 368-384.

40 Luna, P., *et al.*, 2021. Disentangling plant-animal interactions into complex networks: A multi-view approach and perspectives. In Plant-Animal Interactions: Source of Biodiversity (pp. 261-281). Cham: Springer International Publishing.

BOX 3

The National Honey Monitoring Scheme[41]

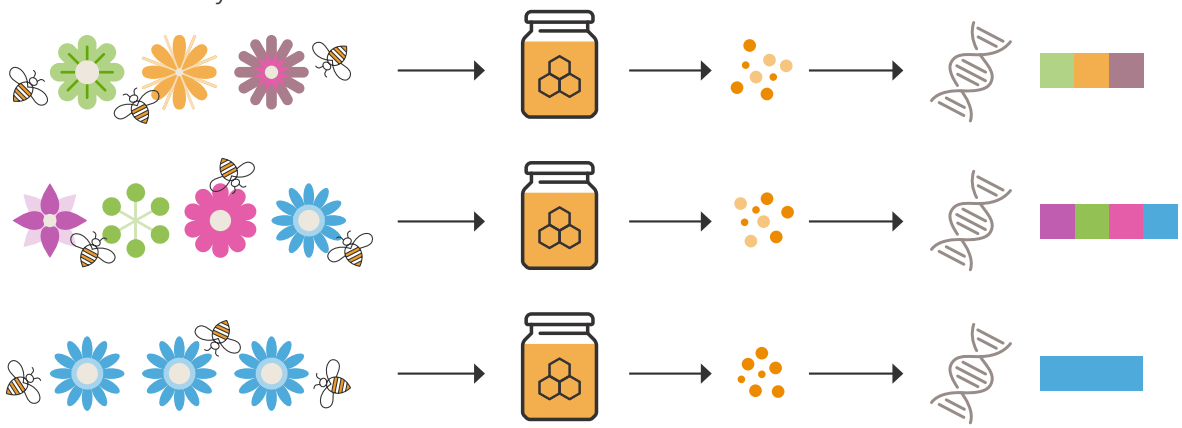
The National Honey Monitoring Scheme is a citizen science programme run by the UK Centre for Ecology & Hydrology (UKCEH). It uses eDNA from honey to understand pollinator foraging preferences and plant diversity in a range of locations across the UK (see Figure 4).

Due to the large distances over which honeybees forage, the honey collected by honeybees contains valuable eDNA information on the state of the landscape and wider ecology, the environmental pressures they are exposed to, as well as their foraging preferences and pollinator-plant relationships. This allows researchers to understand trends and long-term changes in the condition of the countryside, monitor honeybee population health, and understand potential future threats to honeybees' and wild pollinators' floral resources.

For example, using eDNA, researchers have found that as the area of arable crops surrounding honeybee hives increases, there is a decrease in the diversity of plant species that the bees feed on. This lessened diversity, as well as an increase in pollen taken from crop species such as oilseed rape, may negatively affect bee health in the long term. This is due to the reduced number of available micronutrients (gained from feeding on a range of pollen) and increased exposure to pesticides. There are hundreds of different plant species that bees forage on, and this variety is largest in the summer. In spring and autumn, when there are fewer floral resources, species such as brambles may be particularly important components of honeybee diet – highlighting the importance of land management characteristics such as hedgerows. Information such as this will be vital to inform government policies such as the UK Biological Security Strategy[42] and Environmental Land Management Schemes[43].

FIGURE 4

eDNA from honey is used to understand honeybee foraging preferences and gain insights into the wider health of the ecosystem



41 National Honey Monitoring Scheme. UK Centre for Ecology and Hydrology. See https://honey-monitoring.ac.uk/ (accessed on 20 September 2024).

42 UK Biological Security Strategy. Cabinet Office. See https://www.gov.uk/government/publications/uk-biological-security-strategy (accessed on 20 September 2024).

43 Environmental Land Management (ELM) update: how government will pay for land-based environment and climate goods and services. Defra. See https://www.gov.uk/government/publications/environmental-land-management-update-how-government-will-pay-for-land-based-environment-and-climate-goods-and-services/environmental-land-management-elm-update-how-government-will-pay-for-land-based-environment-and-climate-goods-and-services (accessed on 6 November 2024).

eDNA analysis offers a sensitive tool for monitoring changes in ecosystems and biodiversity over time, allowing an indirect estimation of the impact of aquatic pollution on ecosystems.

1.2 Environmental quality and pollution

Biomonitoring refers to the measurement of pollutants, such as chemicals or heavy metals, as contaminants in the environment. eDNA and eRNA can be used as biomarkers for biomonitoring, and researchers are using these to demonstrate which species and which genes are affected by aquatic pollution. These biomarkers are often used as a proxy to understand the presence of pollution in the environment and its likely effects on individuals, populations and ecosystems.

Current applications

Aquatic eDNA to monitor faecal pollution was one of the earliest applications of eDNA to environmental monitoring in the early-1990s[44] and these techniques are still used to detect and track the source of such pollution[45].

While eDNA can be used as direct evidence of pollution (eg detecting pathogenic organisms or allergens), its analysis also offers a sensitive tool for monitoring changes in ecosystems and biodiversity over time, allowing an indirect estimation of the impact of aquatic pollution. For example, eDNA and eRNA analysis of single-celled aquatic organisms and species such as nematodes and crustaceans have been used for monitoring the impact of pollution from fish farms[46, 47, 48], oil and gas drilling[49, 50], and mining activities[51, 52].

eRNA specifically allows an analysis of the expressed parts of the genome, which can change in response to polluted conditions. This can allow more of a 'real time' analysis of the effect of pollution on individual species.

44 Bej, A. K., *et al.*, 1990. Multiplex PCR amplification and immobilized capture probes for detection of bacterial pathogens and indicators in water. Molecular and cellular probes, 4(5), 353-365.

45 Staley, Z.R., *et al.*, 2018. Fecal source tracking and eDNA profiling in an urban creek following an extreme rain event. Scientific reports, 8(1), p.14390.

46 Pawlowski, *et al.*, 2014. Environmental monitoring through protist next-generation sequencing metabarcoding: assessing the impact of fish farming on benthic foraminifera communities. Molecular Ecology Resources. 14 (6), 1129–1140. (https://doi.org/10.1111/1755-0998.12261.)

47 Pawlowski, J., *et al.*, 2016. Protist metabarcoding and environmental biomonitoring: time for change. European Journal of Protistology. 55, 12–25. (https://doi.org/10.1016/j.ejop.2016.02.003.)

48 Pochon, X., *et al.*, 2015. Accurate assessment of the impact of salmon farming on benthic sediment enrichment using foraminiferal metabarcoding. Mar. Pollut. Bull. 100 (1), 370–382. (https://doi.org/10.1016/j.marpolbul.2015.08.022.)

49 Frontalini, *et al.*, 2020. Benthic foraminiferal metabarcoding and morphology-based assessment around three offshore gas platforms: congruence and complementarity. Environment International. 144, 106049. (https://doi.org/10.1016/j.envint.2020.106049.)

50 Cordier, *et al.*, 2019. Benthic foraminiferal DNA metabarcodes significantly vary along a gradient from abyssal to hadal depths and between each side of the kuril-kamchatka trench. Prog. Oceanogr. 178 (August), 102175. (https://doi.org/10.1016/j.pocean.2019.102175.)

51 Kavehei, A., *et al.*, 2021. Impact assessment of ephemeral discharge of contamination downstream of two legacy base metal mines using environmental DNA. Journal of Hazardous Materials, 419, 126483.

52 Bernardino, A. F., *et al.*, 2019. Chronic trace metals effects of mine tailings on estuarine assemblages revealed by environmental DNA. PeerJ, 7, e8042.

Emerging applications

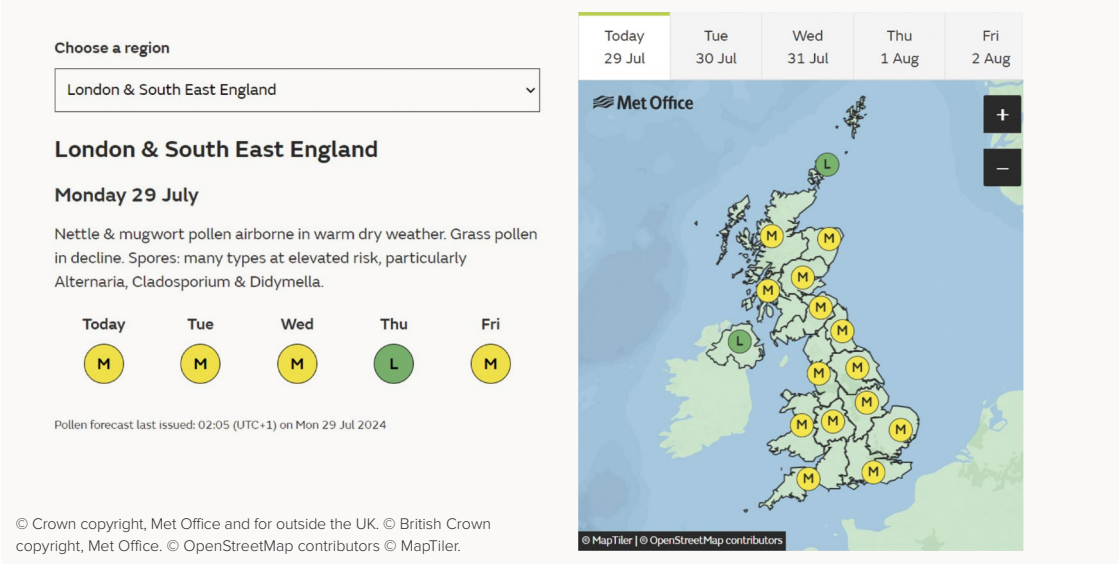
The microbiome of the air can be monitored using eDNA and used to inform public health decisions regarding allergens, pathogenic organisms, and indoor air pollution. Like the honey sampling described in Box 3, pollen dust can be captured and sequenced to inform seasonal pollen forecast data. Hay fever sufferers react differently to different types of pollen and by monitoring the relative abundance of varieties of pollen in the air, combined with meteorological data such as wind, rain and heat, it is possible to predict on any given day both the pollen count and the different types of pollen which make this up[53] (Figure 5). eDNA captured from the air can detect changes in certain types of pollen before they become clinically significant.

In addition, around 95% of hay fever sufferers are allergic to grass pollen[54]. Grasses often have extremely similar pollen grains that are difficult to distinguish using standard microscopy. However, these different types can be differentiated using eDNA, providing important new insights and allowing preventative public health applications.

Air capture sampling techniques for eDNA can also be used for monitoring indoor air pollution by identifying microbes, fungal spores and pathogens in the air which may be toxic, cause allergies or spread disease[55]. This may be particularly relevant in hospital or care home environments.

FIGURE 5

UK pollen forecasting from the Met Office on 29 July 2024, based on eDNA



53 Pollen Services and Research. University of Worcester. See https://www.worcester.ac.uk/about/academic-schools/school-of-science-and-the-environment/science-and-the-environment-research/national-pollen-and-aerobiology-research-unit/ (accessed on 20 September 2024).

54 National Pollen and Aerobiology Research Unit. University of Worcester. See https://www.worc.ac.uk/about/academic-schools/school-of-science-and-the-environment/science-and-the-environment-research/national-pollen-and-aerobiology-research-unit/what-is-pollen.aspx (accessed on 20 September 2024).

55 Ruppert *et al*., 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Global Ecology and Conservation. (https://doi.org/10.1016/j.gecco.2019.e00547)

eDNA sampling technologies in air, water and soil have been used to quantify the extent, spread and levels of contamination from biological agents.

1.3 Biosecurity and defence

Bioterrorism refers to the deliberate release of viruses, bacteria, toxins or other harmful agents to cause illness, disease or death in people, animals or plants[56]. These agents can quickly spread across wide geographical distances, making it difficult to monitor their distribution and impact. eDNA applications in this sector are relatively recent, but eDNA sampling technologies in air, water and soil have been used to quantify the extent, spread and levels of contamination from biological agents.

eDNA methods can also be used to detect non-deliberate biological threats such as invasive pests, and crop or animal diseases that pose a threat to biodiversity, food security, water resources, and human health[57].

Current applications

eDNA techniques already offer a valuable tool for monitoring and tracking the spread of invasive species in aquatic, terrestrial, and airborne environments (eg flying insects, pollen, or spores)[58, 59]. Climate change and related shifts in species ranges are expected to accelerate the pace of invasive species introductions[60, 61, 62].

Traditional monitoring methods such as capture-mark-recapture experiments, visual surveys, or net surveys, can be ineffective due to the low initial abundance of invasive species[63]. eDNA sampling and analysis provides an effective alternative, particularly for early detection[64]. This allows for preventive interventions and insights into the impacts of these invaders on native species diversity and distribution[65].

56 Biological weapons. The World Health Organisation. See https://www.who.int/health-topics/biological-weapons#tab=tab_1 (accessed on 20 September 2024).

57 IPBES (2023). Summary for Policymakers of the Thematic Assessment Report on Invasive Alien Species and their Control of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Roy, H. E., *et al.*, (eds.). IPBES secretariat, Bonn, Germany. https://doi.org/10.5281/zenodo.7430692

58 Blackman, R. C., *et al.*, 2018. The use of environmental DNA as an early warning tool in the detection of new freshwater invasive non-native species. CABI Reviews, 1-15.

59 Blackman, *et al.*, 2022. Monitoring invasive alien macroinvertebrate species with environmental DNA. River Research and Applications, 38(8), 1400–1412. (https://doi.org/10.1002/rra.3947)

60 Chen, I.C., *et al.*, 2011. Rapid range shifts of species associated with high levels of climate warming. Science, 333(6045), 1024-1026.

61 Seebens, H., *et al.*, 2021. Projecting the continental accumulation of alien species through to 2050. Global Change Biology, 27(5), pp.970-982.

62 IPBES (2023). Thematic Assessment Report on Invasive Alien Species and their Control of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Roy, H. E., *et al.*, (eds.). IPBES secretariat, Bonn, Germany. https://doi.org/10.5281/zenodo.7430682

63 Erickson, R.A., *et al.*, 2016. Detecting the movement and spawning activity of bigheaded carps with environmental DNA. Molecular Ecology Resources, 16(4), 957-965.

64 Rishan, S.T., *et al.*, 2023. Applications of environmental DNA (eDNA) to detect subterranean and aquatic invasive species: a critical review on the challenges and limitations of eDNA metabarcoding. Environmental Advances, 12.

65 Sepulveda, A. J., *et al.*, 2020. Are Environmental DNA Methods Ready for Aquatic Invasive Species Management? Trends in Ecology & Evolution, 35(8), 668–678. (https://doi.org/10.1016/j.tree.2020.03.011)

Emerging applications

Developments in air sampling methods for collecting eDNA (see Section 3.1) are leading to a range of notable new applications. Air capture of eDNA is being used as an early warning system to pre-emptively detect crop pests and diseases as well as to detect human pathogens and bioterror threats.

As gene editing and mRNA vaccine technologies become more straightforward and widespread[66], there is a potential increase in the threat posed by novel biological weapons. Early identification of a malicious pathogen involved in a biological threat is crucial for minimising the impact of its release.

Various technologies are currently available for the identification of pathogens. However, these are laboratory-based and typically rely on targeted detection approaches (ie one test looking for one threat). Although these methods can be quite fast, they also have disadvantages. They are not truly agnostic (ie can only find known targets), cannot always distinguish between viral strains or closely related species, and cannot always identify artificial or engineered bioagents. They also need to be calibrated with the target agent, or agents they must detect, before they can be employed.

To address these disadvantages, biodefence work is currently being undertaken[67] to design and manufacture autonomous air biosensors using eDNA technologies. eDNA technologies such as shotgun sequencing (the ability to pool together short fragments of DNA to obtain an entire genome), and detailed bioinformatics, offer advantages over traditional detection methods – in that they have shown promise in their ability to detect novel or artificial bioagents[68]. The idea is that sensors could be deployed in the field to continuously monitor the air for eDNA signatures of pathogens and provide a rapid report upon identification of any pathogen. These biosensors could be placed at high traffic areas, such as airports, or areas of strategic importance. Equally, biosensors could be placed near research facilities to detect an unintentional release of threats of concern and enable quick mitigation against any exposure or leak into the environment[69]. To support this, AI-based tools are currently being developed which can interrogate a series of eDNA reads and predict likely pandemic characteristics.

In the future, these combined techniques could provide ultra-rapid information, early-warning, and facilitate a quick response upon a bioterrorism attack.

Sensors could be deployed in the field to continuously monitor the air for eDNA signatures of pathogens and provide a rapid report upon identification of any pathogen.

66 CRISPR in the classroom. The New York Times. June 2022. See https://www.nytimes.com/interactive/2022/06/27/science/crispr-anniversary-classroom-explainer.html (accessed on 20 September 2024).

67 Kromek, as an extension of the DARPA Sigma+ program – see https://www.kromek.com/news/kromek-awarded-c-6m-contract-by-darpa-to-further-develop-its-bio-threat-detection-system/ (accessed on 20 September 2024).

68 Predicting Pathogenic Properties From Single Nanopore Reads. Kromek. See https://www.kromek.com/wp-content/uploads/2023/07/230517-London-Calling-A0-poster.pdf (accessed on 27 September 2024).

69 Foot and Mouth Disease 2007: A review and lessons learned. Defra. See https://www.gov.uk/government/publications/foot-and-mouth-disease-2007-a-review-and-lessons-learned (accessed on 20 September 2024).

Air capture of eDNA is also being developed as an early warning system to pre-emptively detect crop pests and diseases[70, 71]. As fungal spores arrive through the air, eDNA surveillance can detect them before they cause disease symptoms (Figure 6)[72]. Unlike livestock, which have adaptive immune systems, crops are either genetically resistant to disease or not. In addition, crop plants within fields and in adjoining fields usually share the same genetic resistance.

These monocultures make epidemics a risk to national food security. Using eDNA to pre-emptively detect threats may also have environmental benefits, allowing farmers to apply treatments such as pesticide sprays only when they are required.

Similarly, air capture eDNA methods have also been successfully used in museums and archives, including the Natural History Museum[73] to monitor threats to collections.

FIGURE 6

Innovaprep Cub air sampler which takes eDNA samples from the air to detect crop pests or diseases

Image courtesy of Earlham Institute, Norwich, taken by Richard Leggett (Earlham Institute).

70 Kestel, J. H., *et al.*, (2022). Applications of environmental DNA (eDNA) in agricultural systems: Current uses, limitations and future prospects. The Science of the Total Environment, 847(157556), 157556. https://doi.org/10.1016/j.scitotenv.2022.157556

71 Bass, D., *et al.*, 2023. Environmental DNA/RNA for pathogen and parasite detection, surveillance, and ecology. Trends in Parasitology, 39(4), 285–304. https://doi.org/10.1016/j.pt.2022.12.010

72 Giolai, M., *et al.*, 2024. Measuring air metagenomic diversity in an agricultural ecosystem. Current Biology, 34(16), 3778-3791.

73 Matthew D. Clark pers. comm.

1.4 Crime and forensic science

Human DNA has been used as forensic evidence since the mid-1980s, following the advent of PCR and DNA fingerprinting methods. This was followed closely by the application of forensic genetic analysis to non-human samples and trace evidence. More recently, improvements in sampling and sequencing technologies have fostered a range of newer applications and possibilities in this field.

Current applications

Within forensic science, eDNA can either refer to fragments of human DNA collected from the environment or to eDNA from soil, plants or animals which can provide evidence to link individuals or illegal goods to physical places. These human and non-human eDNA applications are very different and require different scientific approaches.

When collecting human DNA from the environment, for example by sampling a surface, the resultant sample often consists of complex mixtures of human DNA deposited at different times by different contributors. A contributor may or may not have deposited their DNA during an alleged criminal event, as eDNA may be transferred to where it was recovered by secondary or tertiary contact. DNA is highly mobile, so it can also be airborne or transferred through water. This means that it can be difficult to disentangle exactly when and how DNA from different individuals was deposited.

Statistical techniques are continually advancing to assess the weight of evidence supporting different hypotheses about the origin of human DNA samples. These techniques use Bayesian statistics and probabilistic reasoning to generate likelihood ratios to compare how well the observed evidence aligns with different hypotheses[74]. To fully utilise eDNA evidence within the justice system, new types of bioinformatic analysis will likely be required, and this will need to be validated prior to use in the Court.

The subsequent 'emerging applications' discussion relates to non-human eDNA only.

Emerging applications

The collection and analysis of non-human eDNA from dust, soil, plant or animal traces has an emerging role in forensic science. For example, a soil sample may be collected from the shoe of an individual to establish how likely it is that the shoe was at a crime scene. The bacterial, fungal, plant or animal eDNA profile from the soil of a given location may be sufficiently characteristic to allow the potential origin of an evidence sample to be evaluated or probabilistically assigned. Understanding the transfer, persistence and background abundance of eDNA (see Section 3.3) is essential to be able to interpret and assign weight to eDNA findings, and currently, very little of this data exists. This evidence gap remains a significant barrier to the use of eDNA analysis within the justice system.

The collection and analysis of non-human eDNA from dust, soil, plant or animal traces has an emerging role in forensic science.

74 Agudo, M.M., *et al.*, 2024. A comparison of likelihood ratios calculated from surface DNA mixtures using MPS and CE Technologies. Forensic Science International: Genetics, 73, p.103111.

The soil, plant and animal eDNA traces found in shipping containers or on illegally traded products can be used to predict the potential country of origin of the product and the trade route by which it may have travelled.

These same eDNA techniques have an application in tracking and tracing illegal trade or counterfeit goods. The soil, plant and animal eDNA traces found in shipping containers or on illegally traded products can be used to predict the potential country of origin of the product and the trade route by which it may have travelled.

It has been estimated that in low and middle-income countries, 10% of medicines are either substandard or falsified, representing a significant public health challenge[75]. As these drugs are often manufactured in non-sterile environments using local water sources, small particles of dust, soil, microbes, invertebrate, or vertebrate (including human) material may be present in the tablets, from which DNA can be sequenced. These falsified medicines can be tested for eDNA contaminants, which can give an indication of where these products may have originated[76].

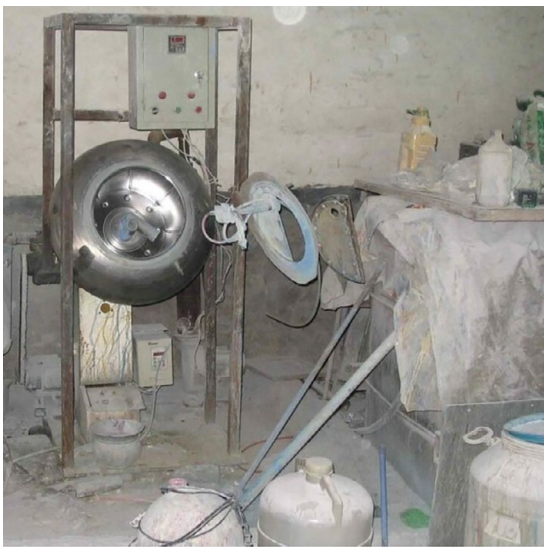


Image
Facility producing fraudulent drugs. Reprinted with the permission of Pfizer Global Security. © The American Society of Tropical Medicine and Hygiene.

75 World Health Organization. 2017. WHO Global Surveillance and Monitoring System for substandard and falsified medical products.

76 Young, J.M., *et al.*, 2022. Environmental DNA as an innovative technique to identify the origins of falsified antimalarial tablets – a pilot study of the pharmabiome. Scientific Reports, 12(1), p.21997.

The sealed nature of blister-packets may also capture and preserve genetic signals from the manufacturing processes[77], and importantly, prevent post-manufacture contamination of the samples. Similar to linking subjects to crime scenes, the specificity of flora, fauna, soil and/or the fungal eDNA found within a certain region or location may enable the presence, and to some extent the localisation, of samples to be inferred[78]. However, scientific research for these applications is in the very early stages and the precision and accuracy of such methods will need to be validated on a case-by-case basis.

Another important application of eDNA is its use for understanding illegal wildlife trade routes. Products such as pangolin scales and elephant tusks often pass through several different locations for sorting and repackaging before they end up at their destination. To add further complexity, pangolin scales from different individuals and species and tusks from the same elephant are often split up and mixed, making them harder to trace.

Alongside the genetic data that can be obtained directly from the illegally traded wildlife products themselves[79, 80, 81], eDNA traces from other species inadvertently transported within consignments of trafficked wildlife[82] (such as from dust, soil, hair or other biological debris) can give important insights into the likely trade route[83, 84] (Figure 7).

It is worth considering the dual use potential in this space – that is, that eDNA could be used by wildlife poachers and traffickers to detect the whereabouts of rare or valuable species. Such considerations should be borne in mind, especially as these methodologies become cheaper and more widely available.

77 Young, J.M., *et al.*, 2022. Environmental DNA as an innovative technique to identify the origins of falsified antimalarial tablets – a pilot study of the pharmabiome. Scientific Reports, 12(1), p.21997.

78 Lewis, M., *et al.*, 2024. The forensic potential of environmental DNA (eDNA) in freshwater wildlife crime investigations: From research to application. Science & Justice.

79 Wasser SK, *et al*. Combating the illegal trade in African elephant ivory with DNA forensics. Conservation Biology. 2008 Aug;22(4):1065-71.

80 Wasser, S.K., *et al.*, 2015. Genetic assignment of large seizures of elephant ivory reveals Africa's major poaching hotspots. Science, 349(6243), pp.84-87.

81 Wasser, S.K., *et al.*, 2022. Elephant genotypes reveal the size and connectivity of transnational ivory traffickers. Nature human behaviour, 6(3), pp.371-382.

82 Ewart, K.M., *et al.*, 2021. DNA analyses of large pangolin scale seizures: Species identification validation and case studies. Forensic Science International: Animals and Environments, 1, p.100014.

83 *Ibid*.

84 Tinsman, J.C., *et al.*, 2023. Genomic analyses reveal poaching hotspots and illegal trade in pangolins from Africa to Asia. Science, 382(6676), pp.1282-1286.

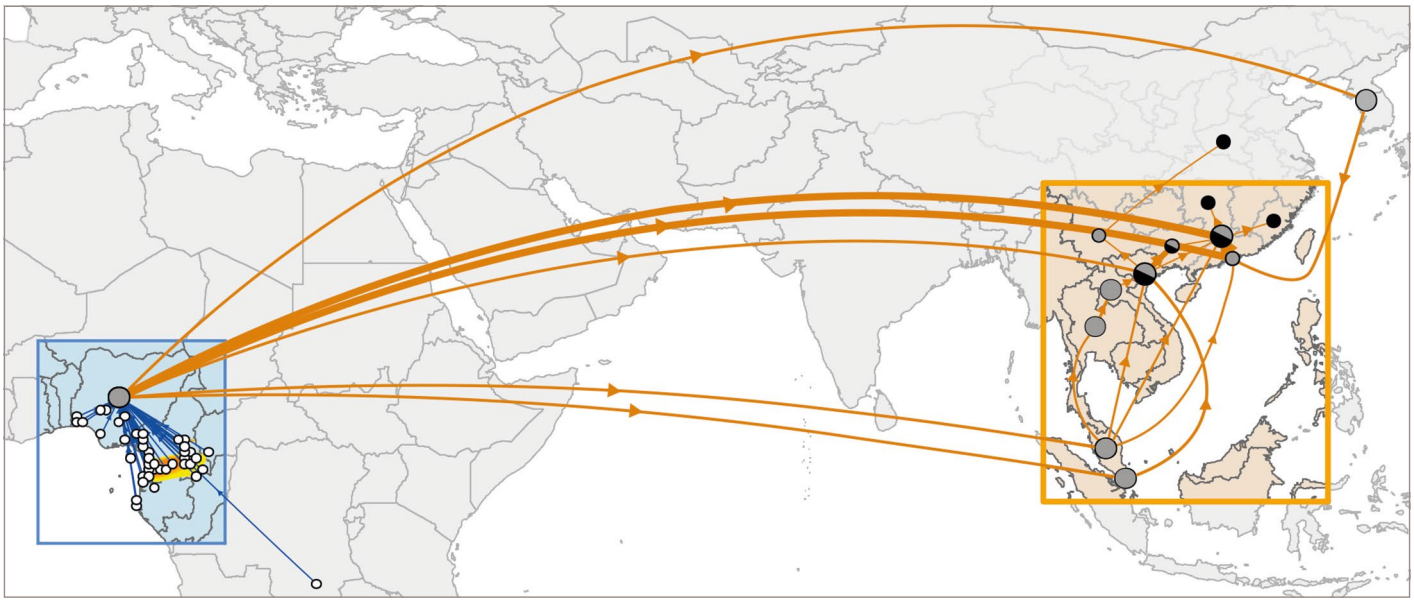
FIGURE 7

Global pangolin trafficking routes identified using eDNA analysis[85]

An origin-to-destination map of pangolin trafficking. A combination of genomics (blue lines) and publicly reported data on pangolin seizures (orange) reveal major trafficking routes. This map focuses on pangolins that transited through Nigeria. White dots represent estimated pangolin origins, transit locations are grey, and market or consumption locations are black. Line widths reflect the quantity of pangolins smuggled along a route. These lines represent possible routes between known stops, not the actual paths taken by traffiked pangolins and their scales.

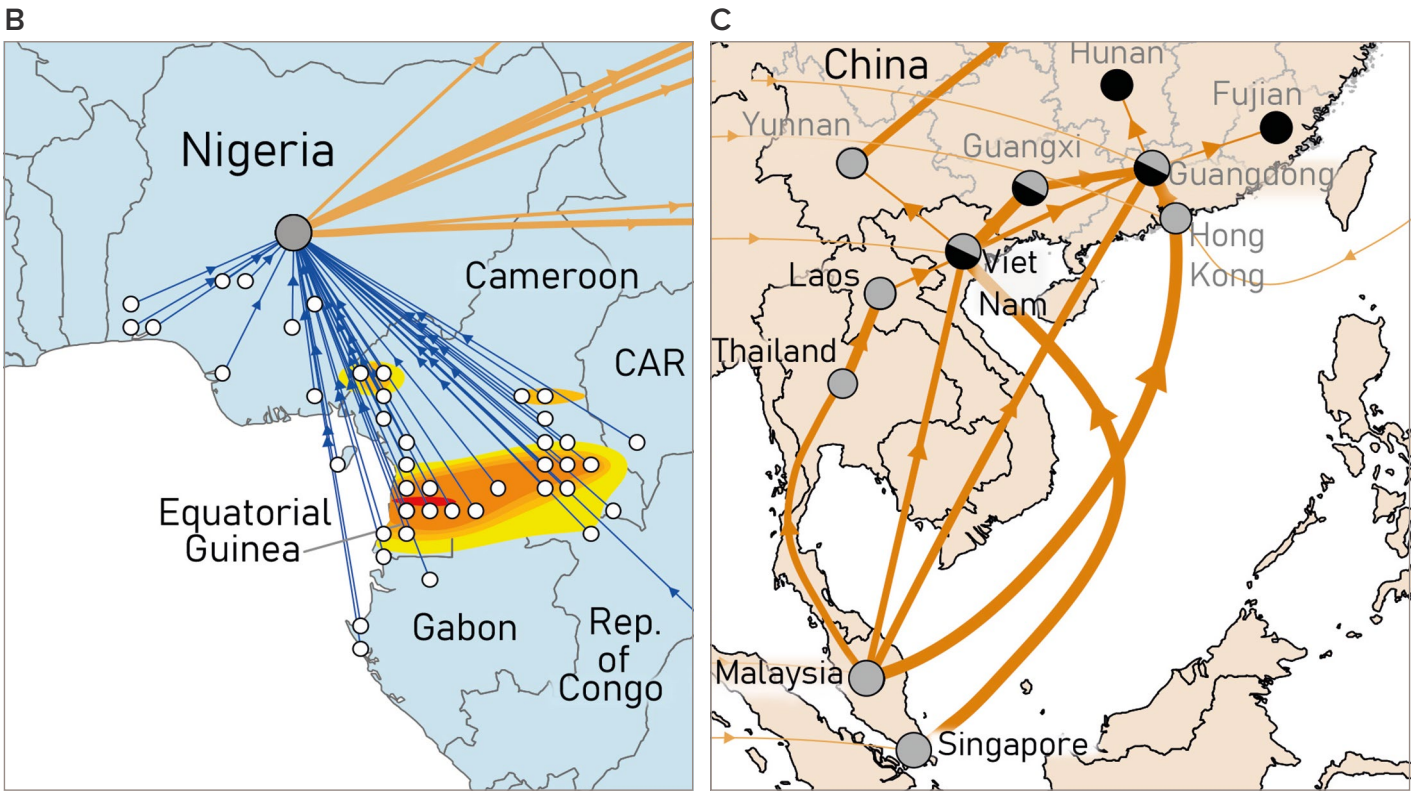
(A) Transcontinental routes for trafficking African pangolins to Asia.

A



85 Tinsman, J.C., *et al.*, 2023. Genomic analyses reveal poaching hotspots and illegal trade in pangolins from Africa to Asia. Science, 382(6676), pp.1282-1286.

(B) Source localities for African pangolins transited through Nigeria. We picked a central point in Nigeria for visualisation – most of these scales left the country via seaport. (C) Routes taken by African pangolins once they arrive in Southeast Asia.



CAR = Central African Republic.

Wastewater monitoring programmes based on eDNA and eRNA methodologies have aided the early detection of Polio, Monkeypox and Norovirus outbreaks during the past few years.

1.5 Health and disease monitoring

eDNA and eRNA techniques have shown to be an effective tool for disease discovery, monitoring and surveillance in both humans and animals[86]. To date, this has mostly been through sampling aquatic environments, including wastewater.

Current applications

The most prominent example of using eDNA for disease surveillance is for the COVID-19 pandemic. The spread of COVID and the emergence of different viral strains (Figure 8) was monitored by wastewater sampling[87, 88]. Box 4 describes this in detail. However, using eDNA and eRNA to monitor and detect pathogens in wastewater pre-dates the pandemic and offers important opportunities for preventative public health far more widely.

Wastewater monitoring programmes based on eDNA and eRNA methodologies have aided the early detection of Polio[89], Monkeypox[90] and Norovirus[91] outbreaks during the past few years.

Monitoring the wastewater entering and leaving treatment plants can provide useful and slightly different insights. Viruses such as enteroviruses and norovirus can be detected in wastewater entering treatment plants, allowing an estimate of the prevalence of these viruses circulating within the community. As many viruses are not completely removed during wastewater treatment[92] monitoring wastewater leaving the wastewater treatment plant allows an assessment of the level of contamination to the environment[93] and the subsequent risk of waterborne illnesses.

This kind of routine monitoring has important implications for bathing water quality, an issue currently high on public and political agendas. Bathing water quality is monitored through routine surveillance of freshwater samples and by analysing past freshwater-sample data to understand trends. However, monitoring freshwater directly relies on the detection of bacterial faecal indicators which significantly underestimates the risks associated with viruses, which are more resilient in the environment than bacteria and also harder to detect. Analysing the eRNA associated with viruses can provide a far more detailed assessment of freshwater quality.

86 Wade, M. J., *et al.*, 2022. Understanding and managing uncertainty and variability for wastewater monitoring beyond the pandemic: Lessons learned from the United Kingdom national COVID-19 surveillance programmes. Journal of hazardous materials, 424(Pt B). (https://doi.org/10.1016/j.jhazmat.2021.127456).

87 Brunner, F. S., *et al.*, COVID-19 Genomics UK (COG-UK) Consortium, Cairns, E., *et al.*, 2022. City-wide wastewater genomic surveillance through the successive emergence of SARS-CoV-2 Alpha and Delta variants. Water research, 226, 119306. (https://doi.org/10.1016/j.watres.2022.11930)

88 Hillary, L. S., *et al.*, 2021. Monitoring SARS-CoV-2 in municipal wastewater to evaluate the success of lockdown measures for controlling COVID-19 in the UK. Water research, 200, 117214. https://doi.org/10.1016/j.watres.2021.117214

89 Klapsa, D., *et al.*, 2022. Sustained detection of type 2 poliovirus in London sewage between February and July, 2022, by enhanced environmental surveillance. The Lancet, 400, 1531-1538.

90 de Jonge, E. F., *et al.*, 2022. The detection of monkeypox virus DNA in wastewater samples in the Netherlands. The Science of the total environment, 852. (https://doi.org/10.1016/j.scitotenv.2022.158265)

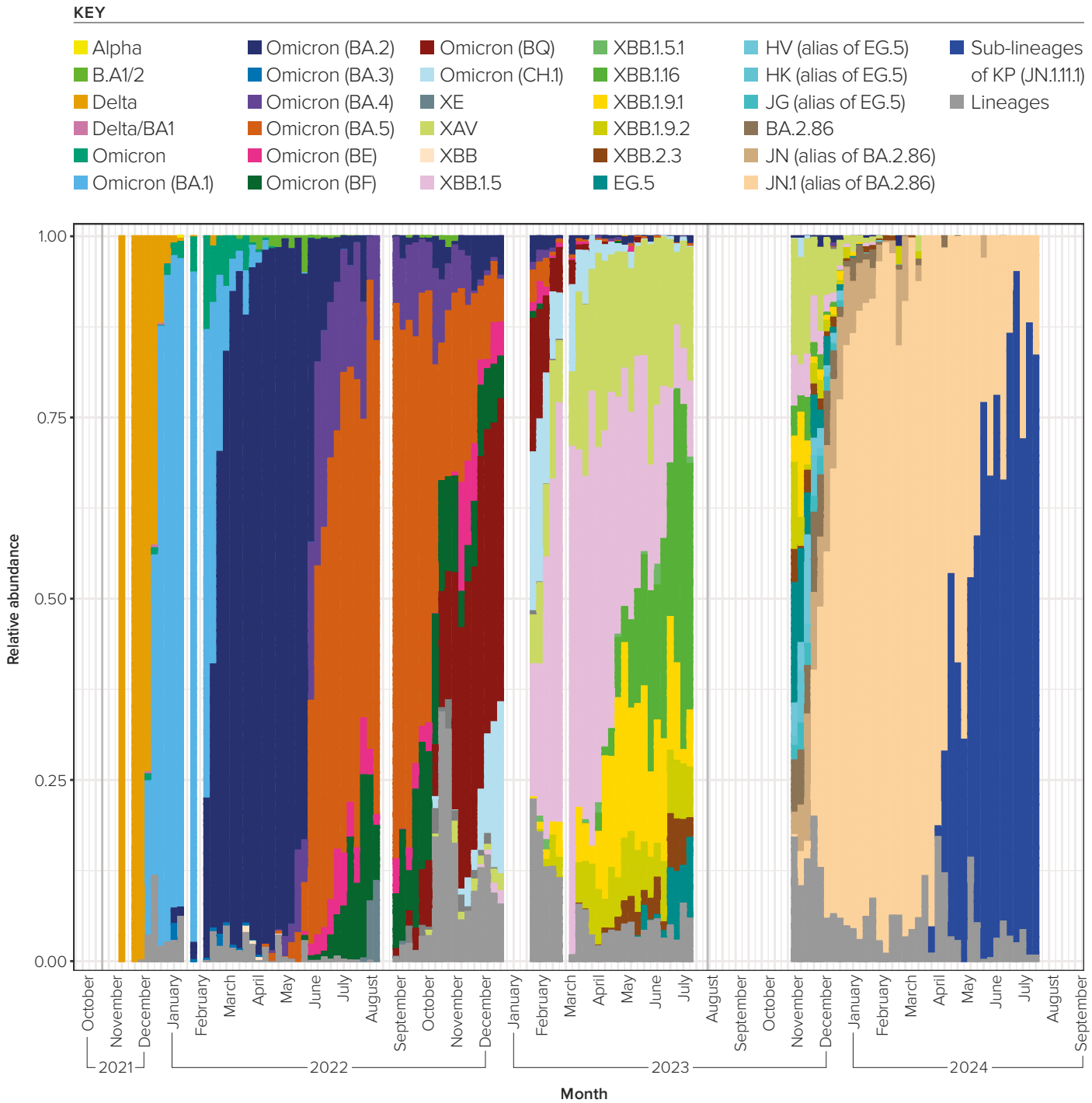
91 Guo, Y., *et al.*, 2022. Back-estimation of norovirus infections through wastewater-based epidemiology: A systematic review and parameter sensitivity. Water research, 219. (https://doi.org/10.1016/j.watres.2022.118610)

92 Kitajima, M., *et al.*, 2014. Relative abundance and treatment reduction of viruses during wastewater treatment processes – identification of potential viral indicators. Science of the Total Environment, 488, 290-296.

93 Sano, D., *et al.*, 2016. Risk management of viral infectious diseases in wastewater reclamation and reuse. Environment International, 91, 220-229.

FIGURE 8

Tracking the emergence of the COVID-19 Omicron variant using next-generation sequencing during the Welsh National Wastewater monitoring programme. September 2020 to March 2023. This programme was funded by the Welsh Government



Emerging applications

Increasingly, eDNA techniques can be used for tracking and monitoring Antimicrobial Resistance (AMR) in the community, environment and between species. Antibiotics and AMR-carrying organisms are regularly discharged into the environment from healthcare-derived and domestic wastewater. Effluent containing pharmaceuticals and runoff from their use in agriculture also enters freshwater. There are many genes that have been and are still being identified as associated with AMR in different bacterial and fungal species. These genes can be detected using eDNA from water or soil samples to estimate their prevalence and also to trace their origin[94]. Another emerging set of eDNA applications in this space is for international disease[95] and AMR surveillance (Box 5).

eDNA is also emerging as a promising tool for enhancing food safety testing procedures. eDNA-based monitoring can help to detect and classify ecologically beneficial and harmful organisms in food production systems. These techniques are already used to determine whether shellfish are safe to harvest (as shellfish are filter feeders they are particularly prone to picking up pathogens such as *E. coli*[96]), and also to trace the origins of *E. coli* or listeria outbreaks[97]. It is likely that these methods will be expanded in the future for a wider range of food products and pathogens.

Commercially, eDNA may also be used to confirm the provenance and constitution of food products, potentially avoiding incidents like the 'Horse Meat Scandal' when horsemeat was found in Findus 'beef' lasagne[98]. Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI) products require the product to possess a given quality, reputation or other characteristic attributable to a particular area[99]. For products where provenance is key, eDNA could be used to trace the product origin and therefore identify food or drink products that claim to be a certain product from a certain region (eg Manuka honey, Welsh lamb or regional wines such as Champagne). The techniques used for this are similar to the eDNA methods described for the illegal wildlife trade under Section 1.4.

94 Knight, M. E., *et al.*, 2024. National-scale antimicrobial resistance surveillance in wastewater: A comparative analysis of HT qPCR and metagenomic approaches. Water research, 262, 121989. Advance online publication. (https://doi.org/10.1016/j.watres.2024.121989)

95 Farkas, K., *et al.*, 2023. Wastewater-based monitoring of SARS-CoV-2 at UK airports and its potential role in international public health surveillance. PLOS global public health, 3(1).

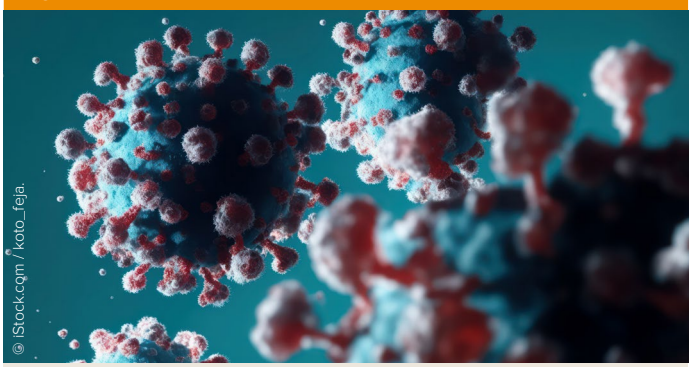
96 Shellfish Classification. Food Standards Agency. See https://www.food.gov.uk/business-guidance/shellfish-classification (accessed on 24 October 2024).

97 Zan, R., *et al.*, 2023. Environmental DNA clarifies impacts of combined sewer overflows on the bacteriology of an urban river and resulting risks to public health. Science of the Total Environment, 889, p.164282.

98 Findus beef lasagne contained up to 100% horsemeat, FSA says. BBC News. 7 February 2013. See https://www.bbc.co.uk/news/uk-21375594 (accessed on 24 October 2024).

99 Protect a geographical food or drink name. UK Government Guidance. See https://www.gov.uk/guidance/protect-a-geographical-food-or-drink-name-in-the-uk#pdo-or-pgi-how-to-apply (accessed on 24 October 2024).

BOX 4



© iStock.com / koto_feja.

Using eDNA to detect and track COVID-19 in wastewater

SARS-CoV-2 RNA can be detected in wastewater using eDNA several days before symptoms develop and detection is possible via clinical surveillance. It also captures individuals who are asymptomatic and would otherwise evade detection by conventional testing. This makes it a useful technique for providing early warnings of new clusters or outbreaks, and for monitoring trends over time. Monitoring wastewater also allows for rapid sampling of a much larger group of people compared to individual testing. This speed of data collection and analysis can help decision-makers to get ahead of a pandemic curve.

Quantitative data derived from PCR-based detection enables the tracking and prediction of case numbers and subsequently hospitalisation up to two weeks in advance, whereas the sequencing of the viral genome enables the identification of emerging variants that may be more harmful than the previous ones. This community-level data has been used in decision making on vaccine distribution, enhanced testing and lockdown measures, making it an invaluable tool for disease prevention in the UK and globally.In future, it may be possible to incorporate wastewater disease monitoring with routine water monitoring for other purposes (see Chapter 4, Consideration 2).

BOX 5

© iStock.com / william87.

Using eDNA for international pathogen and AMR surveillance

The Global Consortium for Wastewater and Environmental Surveillance for Public Health (GLOWACON)[100] is a collaboration between over 300 global partners including the European Union's Health Emergency Preparedness and Response and Joint Research Centre, the US Center for Disease Control and Prevention and the World Health Organisation. These organisations are working together to develop a surveillance system for the early detection, prevention, and real-time monitoring of epidemic threats and outbreaks. This will utilise eDNA technologies in transportation hubs, such as looking at wastewater at airport terminals and effluent from aircrafts, to understand the transmission routes of pathogens and AMR across the globe.

The combination of eDNA and AI analysis software means it is becoming increasingly possible to collect and analyse such large and complex international datasets. This information can provide valuable public health insights and bolster pandemic preparedness globally.

100 Launching GLOWACON: A global initiative for wastewater surveillance for public health. European Commission. 21 March 2024. See https://health.ec.europa.eu/latest-updates/launching-glowacon-global-initiative-wastewater-surveillance-public-health-2024-03-21_en (accessed on 23 September 2024).

Reconstructed ecosystems can be used to inform future conservation planning, backward testing of climate change models, invasive species emergence tracking, and the assessment of anthropogenic influences on biodiversity.

1.6 Ancient eDNA

Ancient eDNA (preserved specimens of eDNA obtained from soil, sediment, lakes, marine environments, caves, ice cores or permafrost) can be analysed to provide insights into ancient ecosystems and civilisations. The disciplines of anthropology, climatology, and palaeontology all utilise ancient eDNA techniques.

Current applications

One key application of ancient eDNA is for assessing the impact of environmental changes on ecosystems over time. This can provide important insights into the drivers of ecosystem change, species evolution and extinction. This application is termed paleo-environmental reconstruction[101]. These reconstructed ecosystems can be used to inform future conservation planning, the backward testing of climate change models, track the emergence of invasive species, and assess the impact of anthropogenic influences on biodiversity and the landscape[102, 103, 104].

A large proportion of ancient flora and fauna do not fossilise but leave extracellular eDNA traces in sediments or ice. Therefore, ancient eDNA can provide information regarding ancient ecosystems that would not be possible using other techniques. Ancient eDNA can either remain in, or be released from, cells and bind to inorganic particles that protect the DNA from microbial and spontaneous chemical degradation. This eDNA is then incorporated into layers of sediment (usefully corresponding to their age).

For example, ancient eDNA analysis has revealed a previously unknown ancient forest in Greenland[105], and been used as evidence to extend (by several thousand years) the survival dates for woolly mammoths[106]. More recently, eDNA was used to uncover the past 50,000 years of vegetation history in the Arctic, revealing massive vegetation turnover at the Pleistocene-Holocene transition, with important learnings and implications for the extinction of large mammals[107].

101 Pedersen, M.W., *et al.*, 2013. A comparative study of ancient environmental DNA to pollen and macrofossils from lake sediments reveals taxonomic overlap and additional plant taxa. Quaternary Science Reviews, 75, 161-168.

102 Sønstebø, J.H., *et al.*, 2010. Using next-generation sequencing for molecular reconstruction of past Arctic vegetation and climate. Molecular Ecology Resources, 10(6), 1009-1018.

103 Jørgensen, T., *et al.*, 2012. Islands in the ice: detecting past vegetation on Greenlandic nunataks using historical records and sedimentary ancient DNA Meta-barcoding. Molecular Ecology, 21(8), 1980-1988.

104 Giguet-Covex, C., *et al.*, 2014. Long livestock farming history and human landscape shaping revealed by lake sediment DNA. Nature communications, 5(1), 3211.

105 Willerslev, E., *et al.*, 2007. Ancient biomolecules from deep ice cores reveal a forested southern Greenland. Science, 317(5834),.111-114. (https://doi.org/10.1126/science.1141758)

106 Murchie, T.J., *et al.*, 2021. Collapse of the mammoth-steppe in central Yukon as revealed by ancient environmental DNA. Nature Communications, 12(1), 7120.6

107 Pedersen, M.W., *et al.*, 2015. Ancient and modern environmental DNA. Philosophical Transactions of the Royal Society B: Biological Sciences, 370(1660), p.20130383. (https://doi.org/10.1098/rstb.2013.0383)

The stomach contents of ancient mammals can also be analysed using eDNA which can give insights into their habitat choice, feeding behaviour, and their influence on plant communities[108].

Emerging applications

As eDNA sampling and analytical techniques develop, it is becoming possible to gain insights from even further back in history. For example, eDNA from frozen sediment samples taken from a polar desert in Greenland has been used to reconstruct a two-million-year-old ecosystem, showing an open boreal-forest inhabited by large animals such as mastodons and reindeer[109]. These findings also demonstrate that in rare and specific circumstances (ie in deep frozen environments) fragments of eDNA can survive for two million years in the environment, about twice as long as previously demonstrated.

eDNA analysis has also recently been used to provide insights into ancient human civilisations, such as how tools were used[110]. This provides a rich and largely untapped source of information for anthropologists. Recent developments in this field include a non-destructive method for the gradual release of eDNA trapped in ancient bone and tooth artefacts, which were used as tools or jewellery[111]. This is an important methodological breakthrough and has already allowed researchers to analyse a palaeolithic pendant and discover that the wearer was female, with strong genetic affinities to a group of ancient North Eurasians, previously found only further east in Siberia[112]. This data can provide insights about the ancestry and biological sex of the individuals who handled, used, carried or wore objects in the deep past.

eDNA from frozen sediment samples taken from a polar desert in Greenland has been used to reconstruct a two-million-year-old ecosystem.

108 Willerslev, E., *et al.*, 2014. Fifty thousand years of Arctic vegetation and megafaunal diet. Nature, 506(7486), 47-51.

109 Kjær, K.H., *et al.*, 2022. A 2-million-year-old ecosystem in Greenland uncovered by environmental DNA. Nature, 612(7939), 283-291.

110 Allentoft, M.E., *et al.*, 2024. 100 ancient genomes show repeated population turnovers in Neolithic Denmark. Nature, 625(7994), pp.329-337.

111 Essel, E., *et al.*, 2023. Ancient human DNA recovered from a Palaeolithic pendant. Nature, 618(7964), pp.328-332.

112 Essel, E., *et al.*, 2023. Ancient human DNA recovered from a Palaeolithic pendant. Nature, 618(7964), pp.328-332.

Benefits and limitations of eDNA methods

eDNA-led methods enable researchers to repeatedly sample a greater diversity of species across larger areas and longer timeframes than traditional approaches.

Whilst the developments in eDNA technologies are exciting, researchers and policymakers should be aware of the relative benefits and limitations of eDNA compared to other methodologies. This ensures realistic expectations of the possibilities and allows informed decisions about their use.

2.1 Benefits

eDNA makes it possible to do things at larger scales, more efficiently and achieve greater insights. Further detail on each of these is presented here.

These benefits have a number of policy applications. As well as helping to tackle the biodiversity crisis and delivering the country's international reporting commitments, the development of eDNA technologies will contribute to the UK government's priorities for economic growth, innovation, tackling crime, net zero and public health via some of the applications described in this report[113].

2.1.1 Scale

Larger volumes of data and information

eDNA-led methods enable researchers to repeatedly sample a greater diversity of species across larger areas and longer timeframes than traditional approaches.

2.1.2 Efficiency

Faster and more cost effective

Among the greatest benefit of eDNA is that it reduces the cost and time associated with conventional biological surveys, such as personnel hours, field-training, equipment, permits, and safety concerns[114,115].

Greater accuracy

Generally, eDNA methods have greater detection sensitivity than traditional methods as they do not rely on visual identification[116,117]. However, this is context-specific and will depend on the information required and the species and habitat in question[118]. Usefully, and unlike visual methods, eDNA can distinguish between taxonomically[119] or visually similar species, as well as those at immature life stages. Using eDNA as a complement to traditional approaches may help to enhance confidence in the taxonomic findings.

113 Labour Party Manifesto 2024. A Mission Driven Government. See https://labour.org.uk/change/mission-driven-government/ (accessed on 7 November 2024).

114 Fediajevaite, J., *et al.*, 2021. Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. Ecology and Evolution. 3. 7382. (https://doi.org/10.1002/ece3.7382)

115 Pedersen, M.W., *et al.*, 2015. Ancient and modern environmental DNA. Philosophical Transactions of the Royal Society B: Biological Sciences, 370(1660), p.20130383. (https://doi.org/10.1098/rstb.2013.0383)

116 Roger, F., *et al.*, 2022. Airborne environmental DNA metabarcoding for the monitoring of terrestrial insects – A proof of concept from the field. Environmental DNA. 4(4), 790-807.

117 Ruppert *et al.*, 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Global Ecology and Conservation. (https://doi.org/10.1016/j.gecco.2019.e00547)

118 Fediajevaite, J., *et al.*, 2021. Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. Ecology and Evolution. 3.7382. (https://doi.org/10.1002/ece3.7382)

119 Macgregor, C.J., *et al.*, 2019. Construction, validation, and application of nocturnal pollen transport networks in an agro-ecosystem: a comparison using light microscopy and DNA metabarcoding. Ecological Entomology, 44(1), 17-29.

2.1.3 Insight

Detect and monitor species without having to see or hear them

The ability of eDNA methods to detect species without having to see or hear them is particularly valuable for rare or elusive species and difficult to access habitats. eDNA methods also cause less disruption of species and habitats, as researchers do not necessarily have to be present at the same time as, or handle, the species they are studying.

Detect and monitor a wider range of taxonomic groups

eDNA techniques are able to detect a wider range of taxonomic groups than traditional monitoring methods (eg microbial communities), which can provide better insights into the health and resilience of whole ecosystems. eDNA techniques have the potential to fundamentally shift the nature of biodiversity study and monitoring, from a traditional focus on species that are visible or observable, to a broader and more inclusive assessment that includes very small organisms and communities.

2.2 Limitations

This section describes the current limitations of eDNA technologies. Some of these limitations will remain inherent, however some can be addressed with future research and investment.

It is worth noting that all scientific methods have their limitations and sources of error, and that those associated with eDNA are relatively well-understood and can be better quantified than for some other methods. This ability to quantify the likely accuracy of eDNA data makes it a potentially attractive monitoring tool.

Relies on good, open-access reference libraries

eDNA analysis relies on scientifically robust, open-access reference libraries. If a species does not have a genome sequence (or at a minimum, a DNA barcode) recorded within a reference library, then it cannot be identified using eDNA analysis. This challenge means that it is often not easy to detect poorly described or undocumented species using eDNA techniques. As these reference libraries improve, eDNA analysis will become more valuable and accurate. For more on this see Chapter 4.

eDNA techniques have the potential to fundamentally shift the nature of biodiversity study and monitoring, from a traditional focus on species that are visible or observable, to a broader and more inclusive assessment that includes very small organisms and communities.

It can be challenging to use eDNA methods to measure abundance, as the amount of DNA in a sample does not necessarily correlate to the number of individuals present in the environment.

Difficult to measure abundance

It can be challenging to use eDNA methods to measure abundance, as the amount of DNA in a sample does not necessarily correlate to the number of individuals present in the environment. Organisms shed DNA at different rates, and DNA can travel large distances, which is hard to control for. However, there are some recent examples of using eDNA to measure the relative abundance of species[120,121,122,123]. eDNA can also be used to monitor trends in the amount and location of eDNA over time, which can give an indication of population trends and be used as a proxy of abundance[124]. Nonetheless using eDNA to measure absolute abundance will remain inherently challenging.

Often not able to distinguish between individual animals of the same species

Related to measuring abundance, current eDNA approaches are not usually able to distinguish one individual from another (except within forensic genetics, where this is often the focus and therefore methodologies and analytics are more advanced). More detailed eDNA methodologies are being developed which may allow these applications across wider disciplines the future.

Difficult to determine the time and place that an organism was present

The exact time and place that the organism was present at the sampling site may be challenging to determine using eDNA[125]. This is especially true in fast changing environments such as water, where eDNA can quickly be transported away from where it was released. One of the key differences between eDNA sampling and visually or acoustically observing wildlife is that when eDNA is found, it is only possible to estimate the probability of whether and when the species was present in that location, rather than know for sure. However, there are increasingly advances in understanding a) how far eDNA is transported in aquatic environments and b) how recently the species that released the DNA was present (see Section 3.3).

Similarly, when using eDNA to identify individual humans within forensic science, eDNA cannot reliably provide data that can lead to evidence that suggests the presence or absence of specific individuals at a scene. This is because of the absence of current understanding relating to transfer, persistence and background abundance of eDNA. As noted in Section 1.4, this is an important evidence gap and current limitation.

120 Lacoursière-Roussel, A., *et al.*, 2016. Quantifying relative fish abundance with eDNA: a promising tool for fisheries management. The Journal of Applied Ecology, 53(4), 1148–1157. (https://doi.org/10.1111/1365-2664.12598).

121 Lawson Handley, L., *et al.*, 2019. Temporal and spatial variation in distribution of fish environmental DNA in England's largest lake. Environmental DNA, 1(1), 26–39. (https://doi.org/10.1002/edn3.5)

122 Rourke, M. L., *et al.*, 2022. Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. Environmental DNA, 4(1), 9–33. (https://doi.org/10.1002/edn3.185)

123 Di Muri, C., *et al.*, 2020. Read counts from environmental DNA (eDNA) metabarcoding reflect fish abundance and biomass in drained ponds. Metabarcoding and Metagenomics. 4, e56959. (https://mbmg.pensoft.net/article/56959/download/pdf/)

124 Sullivan, A.R., *et al.*, 2023. Airborne eDNA captures three decades of ecosystem biodiversity. bioRxiv, 2023-12.

125 Hinz, S., *et al.*, 2022. Evaluating eDNA for use within marine environmental impact assessments. Journal of Marine Science and Engineering, 10(3), 375.

Accuracy and risk of contamination

eDNA sampling methods are limited by their specificity and the risk of false positive results due to contamination. As described, DNA itself is highly mobile, being easily transferred via water, air or on moving vehicles or organisms. Therefore, it can be hard to determine if, how or when a sample may have been contaminated versus when it is a genuinely surprising result.

Contamination can occur when investigators themselves tthemselves to the item of interest. Risks of this can be mitigated by wearing protective clothing, changing gloves in between handling items, using appropriate eDNA recovery techniques, sealed sample packaging and clear documentation of processes. Key to this is understanding the background abundance and commonality of eDNA profiles. Currently databases of non-human eDNA profiles, such as soil surveys, which would help to understand background abundance are very limited. Investing in expanding reference databases for these microbial species would greatly aid the value of such samples.

Depending on the use, an acceptable probability of an eDNA result being correct will vary. False positives or non-specific results can occur because much eDNA analysis relies on fragmented, degraded DNA. Depending on the quality of the eDNA, the sections of genome present and the completeness of the reference library, the analysis may only be able to determine the genus or family level of a sample (ie that it is one of a group of closely related species). eDNA analysis can therefore present random results if the section of genome sequenced happens to match that of a completely different species. This can increasingly be dealt with by recent developments in bioinformatics, which use a range of different algorithms and compare the results across these. Such developments must be robust, transparent and openly available in order to be used in forensic science applications.

For use in defence, forensic science or to trace the illegal wildlife trade, the accuracy of this probability estimate is crucial, as there is a chance that false positives could result in false bioterror alarms or miscarriages of justice[126]. For all applications, the likelihood of false positive results occurring needs to be well understood and any opportunities for contamination minimised. There are already published guidelines in place to avoid contamination at different stages of the eDNA sampling and analysis process. This quality control is, and should remain, an inherent aspect of eDNA research.

It can be hard to determine if, how or when a sample may have been contaminated versus when it is a genuinely surprising result.

126 Gill, P., 2014. Misleading DNA evidence: reasons for miscarriages of justice. Elsevier.

Recent developments and future opportunities

Air capture eDNA techniques may eventually be accurate enough to allow researchers or investigators to understand exactly which and how many people, pathogens, or species have recently been in a room or enclosed space.

As this report has outlined, the use and application of eDNA techniques have increased rapidly within the last 10 years and continues to advance. This section summarises some of the latest developments and describes some of the opportunities that could be afforded by them in the future. These future opportunities are mostly speculative but not outside of the realms of possibility given the trajectory of current research.

3.1 Air sampling technologies

Recent developments

Air capture eDNA sampling technologies have the potential to revolutionise the application of eDNA to a number of sectors. Indeed, recent proof-of-concept studies have shown that human DNA can be collected from indoor air samples in offices, homes and hospitals[127,128,129].

Risks of contamination would need to be factored in for air capture eDNA to provide meaningful information to aid criminal investigations or to monitor the number of unique individuals using a space.

The extension of air eDNA collection to other mammalian species remains in its infancy. However, there have been some recent proof-of-concept studies demonstrating that these techniques can be used to detect mammalian DNA in the air in enclosed spaces[130,131,132] and in outdoor enclosures[133,134]. These developments are an important precursor to potentially using air capture eDNA techniques for border security or to detect pests or invasive species.

Air capture technologies are also a core component of emerging eDNA applications in the biosecurity and defence sectors (Section 1.3).

Future opportunities

Air capture eDNA techniques may eventually be accurate enough to allow researchers or investigators to understand exactly which and how many people, pathogens, or species have recently been in a room or enclosed space[135]. Many of the filters used in these studies are similar to high efficiency particulate air filters, which are common to most houses for heating, ventilation, and air conditioning. Similar to the outdoor air filters, these could have utility for eDNA sampling.

127 Fantinato, C., *et al.*, 2023. The invisible witness: air and dust as DNA evidence of human occupancy in indoor premises. Scientific Reports, 13(1), p.19059.

128 Fantinato, C., *et al.*, Detection of human DNA in the air. Forensic Science International: Genetics Supplement Series. Volume 8, 282 – 284.

129 Fantinato C., *et al.*, 2023. The invisible witness: air and dust as DNA evidence of human occupancy in indoor premises. Sci Rep. 13(1): 19059–19059.

130 Clare, E.L., *et al.*, 2021. eDNAir: proof of concept that animal DNA can be collected from air sampling. PeerJ, 9.

131 Serrao, N.R., *et al.*, 2021. Molecular genetic analysis of air, water, and soil to detect big brown bats in North America. Biological Conservation, 261.

132 Whitmore, L., *et al.*, 2023. Inadvertent human genomic bycatch and intentional capture raise beneficial applications and ethical concerns with environmental DNA. Nature Ecology & Evolution, 7(6), 873-888.

133 Clare, E.L., *et al.*, 2021. eDNAir: proof of concept that animal DNA can be collected from air sampling. PeerJ, 9.

134 Lynggaard, C., *et al.*, 2024. Airborne environmental DNA captures terrestrial vertebrate diversity in nature. Molecular Ecology Resources, 24(1).

135 Whitmore, L., *et al.*, 2023. Inadvertent human genomic bycatch and intentional capture raise beneficial applications and ethical concerns with environmental DNA. Nature Ecology & Evolution, 7(6), 873-888.

For example, sampling these filters retrospectively for hair, skin cells, or saliva may hold potential for detecting a person of interest and/or linking them to locations where criminal activity is alleged to have occurred[136,137,138]. The relative amount of eDNA present in the air may also allow additional insights, such as how much time different individuals spent at this location. This may be useful not only for crime or justice applications, but also for routinely monitoring the number of people using public transport such as trains or railway stations, or other public spaces such as libraries, and so help to inform infrastructure and planning. eDNA monitoring of the air in places, such as hospitals or care homes, may also help to provide early-warning of any potentially transmissible illnesses or pathogens, which could help to protect vulnerable people.

There are ethical concerns associated with using eDNA in such a way, these are discussed in detail in Chapter 4.

3.2 Portable and autonomous collection and analysis

Recent developments

In recent years portable eDNA collection and analysis has opened up the field of eDNA research to a far broader audience, due to less reliance on specialist labs. An example of this is the Oxford Nanopore Technologies MinION device[139] or the Smith Root eDNA sampler, which can be carried as a backpack[140] (Figure 9).

This portability is valuable, not only in terms of accessibility, time, and resources, but also to allow sampling and analysis to take place in hard-to-access environments.

eDNA sampling technologies are also becoming more autonomous[141]. Autonomous sampling methods can facilitate persistent monitoring and/or monitoring of remote and otherwise inaccessible or dangerous sites, whilst also reducing the number of person-hours required to collect the data.

Future opportunities

Advances such as these will likely have important policy applications in a number of sectors, in terms of their potential to improve the safety of personnel as well as reduce the time and cost of monitoring the environment. Alongside the use of these technologies for biodiversity monitoring, autonomous sampling could also be used to more safely detect bioterror threats, for routine water quality assessment, or for the routine monitoring of disease, invasive species, or pathogens. It may be possible to mostly automate eDNA sampling in the future.

Autonomous sampling methods can facilitate the monitoring of remote and otherwise inaccessible or dangerous sites.

136 Goray, M., *et al.*, 2024. Up in the air: Presence and collection of DNA from air and air conditioner units. Electrophoresis, 45(9-10), 933-947.

137 Fantinato, C., *et al.*, 2023. The invisible witness: air and dust as DNA evidence of human occupancy in indoor premises. Scientific Reports, 13(1).

138 Goray, M., *et al.*, 2024. Emerging use of air eDNA and its application to forensic investigations – A review. Electrophoresis, 45(9-10), 916-932.

139 MinION. Oxford Nanopore Technologies. See https://nanoporetech.com/products/sequence/minion (accessed on 23 September 2024).

140 eDNA sampler. Smith-Root. See https://www.smith-root.com/edna/edna-sampler (accessed on 23 September 2024).

141 Hendricks, A., *et al.*, 2023. Compact and automated eDNA sampler for in situ monitoring of marine environments. Scientific Reports, 13(1).

FIGURE 9

Examples of portable eDNA technologies

Oxford Nanopore MinION

All Oxford Nanopore sequencing devices use flow cells which contain an array of tiny holes – nanopores – embedded in an electro-resistant membrane. Each nanopore corresponds to its own electrode connected to a channel and sensor chip, which measures the electric current that flows through the nanopore. When a molecule passes through a nanopore, the current is disrupted to produce a characteristic 'squiggle'. The squiggle is then decoded using basecalling algorithms to determine the DNA or RNA sequence in real time.

Image: © Oxford Nanopore Technologies, 2025.

Smith-Root eDNA sampler

A Smith-Root eDNA sampler is a portable, backpack-mounted system designed to collect eDNA from water. Using a computer-controlled pump to draw water through a specialized filter cartridge, this captures trace amounts of DNA shed by organisms present in the water, allowing for analysis of species composition in a given area without directly capturing the organisms themselves. It features onboard data logging, GPS geotagging, and customizable settings for sample volume and flow rate, ensuring consistent and accurate eDNA collection in field settings.

Image: © Smith-Root.

3.3 Understanding persistence

Recent developments

Understanding the persistence of DNA in the environment from different organisms and under different conditions is vital for many of the applications that this report has presented, and essential for making spatial or temporal inferences from eDNA findings[142,143,144,145]. Therefore, there are efforts underway to characterise the longevity, decay rate, and distribution of eDNA and eRNA, particularly in water.

Research into the likely spatial and temporal distribution of species within the environment[146,147,148] is allowing for a more accurate and more detailed understanding of species and community ecology. This in turn allows for an assessment of any changes in these natural patterns due to stressors, such as pollution or environmental change. For example, in the marine environment efforts are underway to develop predictive probability maps of eDNA distribution[149]. These maps combine high-resolution data on the biological characteristics of eDNA (decay, shedding rates) and oceanographic and meteorological data (on tides, currents, and weather) to build biophysical models upon which the maps are based[150,151,152].

142 Andruszkiewicz, E. A., *et al.*, 2019. Modeling environmental DNA transport in the Coastal Ocean using Lagrangian particle tracking. Frontiers in Marine Science., 6, 477. (https://doi.org/10.3389/fmars.2019.00477)

143 Andruszkiewicz, E. A., *et al.*, 2017. Persistence of marine fish environmental DNA and the influence of sunlight. PLoS One, 12(9). (https://doi.org/10.1371/journal.pone.0185043)

144 Harrison, J. B., *et al.*, 2019. Predicting the fate of eDNA in the environment and implications for studying biodiversity. Proceedings of the Royal Society B: Biological Sciences, 286(1915). (https://doi.org/10.1098/rspb.2019.1409)

145 Goray, M., *et al.*, 2024. Emerging use of air eDNA and its application to forensic investigations – A review. Electrophoresis, 45(9-10), 916-932.

146 Perry, W.B., *et al.*, 2024. An integrated spatio-temporal view of riverine biodiversity using environmental DNA metabarcoding. Nature Communications, 15(1).

147 Carraro, L., *et al.*, 2023. Modelling environmental DNA transport in rivers reveals highly resolved spatio-temporal biodiversity patterns. Scientific Reports, 13(1).

148 Gibson, T.I., *et al.*, 2024. Environmental DNA reveals ecologically relevant spatial and temporal variation in fish assemblages between estuaries and seasons. Ecological Indicators, 165.

149 Scriver, M., *et al.*, 2023. Harnessing decay rates for coastal marine biosecurity applications: A review of environmental DNA and RNA fate. Environmental DNA, 5(5), 960-972.

150 Andruszkiewicz, E. A., *et al.*, 2019. Modeling environmental DNA transport in the Coastal Ocean using Lagrangian particle tracking. Frontiers in Marine Science., 6, 477. (https://doi.org/10.3389/fmars.2019.00477)

151 Ellis, M. R., *et al.*, 2022. Detecting marine pests using environmental DNA and biophysical models. Science of the Total Environment, 816, 151666. (https://doi.org/10.1016/j.scitotenv.2021.15166)

152 Fukaya, K., *et al.*, 2021. Estimating fish population abundance by integrating quantitative data on environmental DNA and hydrodynamic modelling. Molecular Ecology, 30(13), 3057–3067. (https://doi.org/10.1111/mec.15530).

Future opportunities

A greater understanding of eDNA decay and distribution may eventually lend itself to applications such as using eDNA methods in water to detect missing people. Currently, this takes considerable specialist human resource and is time-consuming. Analysing water samples for eDNA could be a simpler way to determine whether a person was or is in freshwater, but the accuracy of this needs to be well understood. Early research has found that the amount of human eDNA from blood in water declines significantly over time; the amount of DNA reduced by 41% – 90% in the first 24 hours and 99% by 840 hours[153]. eDNA detection of a missing person in freshwater after 24 hours therefore seems likely to be challenging if relying on blood. However, eDNA from the body itself may persist much longer and is more promising. Other research has explored the possibility of using wastewater to detect the DNA of missing persons in densely populated areas[154].

Whilst at very early stages, some of this proof-of-concept work suggests that in the future using eDNA for this type of investigation is not completely outside the realms of possibility[155]. It has also been suggested that similar eDNA related techniques could also be used to help identify victims of war or natural disasters, although further research is required before these approaches could be reliably used in the justice system.

Predictive models based on eDNA or eRNA monitoring data, combined with meteorological data, could feasibly be used to assess the distribution of human and animal pathogens in the aquatic environment, and to foresee any health risks associated with bathing or the consumption of seafood[156]. These predictive capabilities are likely to become increasingly important as more frequent heavy rainfall and extreme weather events increase the concentrations of pathogens in water[157]. Predictive models could not only inform public health interventions during risky periods but can also identify safe regions where health risks are negligible.

153 Dass MA, *et al.*, 2022. Assessing the use of environmental DNA (eDNA) as a tool in the detection of human DNA in water. J Forensic Sci. 67: 2299–2307.

154 Boger N, *et al.*, 2023. Monitoring sewer systems to detect the eDNA of missing persons and persons of interest. Forensic Science International. 349:111744.

155 Boger N, *et al.*, 2023. Monitoring sewer systems to detect the eDNA of missing persons and persons of interest. Forensic Science International. 349:111744.

156 Robins, P.E., *et al.*, 2019. Viral dispersal in the coastal zone: a method to quantify water quality risk. Environment international, 126,.430-442.

157 Robins, P.E., *et al.*, 2019. Viral dispersal in the coastal zone: a method to quantify water quality risk. Environment international, 126,.430-442.

3.4 eRNA-based approaches

Recent developments

eRNA based approaches are already routinely used for monitoring RNA viruses, such as SARS-CoV-2 and poliovirus. However, for other applications eRNA represents a relatively new but potentially useful technique[158]. Whereas eDNA represents fragmented genomes of cellular organisms present in the environment. eRNA represents only the expressed parts of those genomes (the transcriptome), or in the case of RNA viruses such as SARS, the entire genome. eRNA analysis gives slightly different, but complementary information, to eDNA analysis, because gene expression is influenced by external factors. An understanding of gene expression can allow inferences on the response of different organisms to environmental stressors, and enable understanding of the age structure of populations based on the presence of different life-history stages, determine the sexes of organisms, and generally understand the expression of specific genes within a species.

eRNA also has another useful feature when compared with eDNA – it generally degrades faster and so persists for less time in the environment than eDNA. This rapid degradation can be exploited to allow researchers to quantify the RNA only from species or individuals that were recently present and metabolically active[159,160,161] (see Section 3.4). Comparing the ratio of eDNA to eRNA can give an indication of how recently the organism was present[162]. eRNA should therefore hypothetically reflect how the genome is being expressed in that environment at that particular time[163].

Future opportunities

Given these advantages, eRNA is expected to have great potential as a future biomonitoring tool. For example, it may allow the detection of AMR genes in the environment in real-time, or be used to measure how species and ecosystems are responding to environmental stressors such as climate change or pollution.

An understanding of gene expression can allow inferences on the response of different organisms to environmental stressors.

158 Yates, M. C., *et al.*, 2021. Environmental RNA: A Revolution in Ecological Resolution? Trends in Ecology and Evolution. 36, 601–609. (doi: 10.1016/j.tree.2021.03.001)

159 Yates, M. C., *et al.*, 2021. Environmental RNA: A Revolution in Ecological Resolution? Trends in Ecology and Evolution. 36, 601–609. (doi: 10.1016/j.tree.2021.03.001)

160 Cristescu, M.E., 2019. Can environmental RNA revolutionize biodiversity science?. Trends in Ecology and Evolution. 34(8), 694-697.

161 Wood, S.A., *et al.*, 2020. Release and degradation of environmental DNA and RNA in a marine system. Science of the Total Environment, 704.

162 Marshall, N.T., *et al.*, 2021. Environmental (e) RNA advances the reliability of eDNA by predicting its age. Scientific reports, 11(1), p.2769.

163 Takahashi, M., *et al.*, 2023. Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution. Science of the Total Environment, 873.

New techniques using mitochondrial eDNA and nuclear eDNA markers, provide a means for obtaining population level genetic information from environmental samples.

3.5 eDNA as a population genetics tool

Recent developments

The study of population genetics describes changes in the genetic diversity of populations through evolutionary mechanisms such as natural selection, genetic drift, mutation, and gene flow. This can provide an understanding of the past distributions[164], present health status[165], and the likely future resilience of species. New techniques using mitochondrial eDNA and nuclear eDNA markers[166,167,168], provide a means for obtaining population level genetic information from environmental samples. These markers do not aim to identify different species, rather they are used to distinguish between genetic variants within a single species. This may be particularly useful when collecting DNA samples directly from specimens is challenging, such as for endangered, invasive, or morphologically indistinguishable species. Advances in shotgun sequencing (the ability to pool together short fragments of DNA to obtain an entire genome) are also providing important new insights in this space.

As an example, eDNA from seawater has been used to examine mitochondrial DNA variation in whale sharks[169]. eDNA sampling in areas of known whale shark presence was able to detect the same genetic variation as directly sampled tissues, and indicate that a recently identified Qatar population of whale sharks was more closely related to the whale sharks found in the Indo-Pacific than those in the Atlantic[170]. eDNA has also been used to uncover previously unknown genetic diversity within other elusive marine species.[171].

Future opportunities

eDNA has a promising future as a population genetics tool, allowing researchers to assess genetic changes associated with AMR resistance, pathogen virulence, and comment upon species and ecosystem resilience in the face of environmental stressors. eDNA methodologies are likely to become almost comparable to sampling tissues direct from a subject[172].

164 Drummond, A.J., *et al.*, 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. Molecular Biology and Evolution. 22, 1185–1192

165 Dussex, N., *et al.*, 2016. Low spatial genetic differentiation associated with rapid recolonization in the New Zealand fur seal Arctocephalus forsteri. J. Hered. 107, 581–592

166 Aylward, M.L., *et al.*, 2018. An environmental DNA sampling method for aye-ayes from their feeding traces. Ecol. Evol. 8, 9229–9240.

167 Sigsgaard, E.E., *et al.*, 2016. Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. Nature, Ecology and Evolution. 1, 0004

168 Parsons, K.M., *et al.*, 2018. Water, water everywhere: environmental DNA can unlock population structure in elusive marine species. Royal Society Open Science. 5, 180537

169 Sigsgaard, E.E., *et al.*, 2016. Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. Nature, Ecology and Evolution. 1, 0004

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171 Parsons, K.M., *et al.*, 2018. Water, water everywhere: environmental DNA can unlock population structure in elusive marine species. Royal Society Open Science. 5, 180537

172 Schlötterer, C., *et al.*, 2014. Sequencing pools of individuals – mining genome-wide polymorphism data without big funding. Nature Reviews Genetics, 15(11), 749-763.

There is no reason why these advancements could not be applied to human eDNA collected from wastewater and used to assess human population health through genetic markers. However, such an application would necessitate a detailed evaluation and consideration of the ethical implications (see Chapter 4).

3.6 New analytical capabilities – eDNA in combination with other approaches

Recent developments

Recent developments in AI and computing power have expanded the range of possibilities and insights that eDNA data can offer. One of the key limitations of eDNA analysis has been (and remains) the sheer volume and complexity of the data. It takes a large amount of statistical and computing power to compare thousands of fragments of DNA to reference databases that include thousands of genomes. The number of comparisons that need to be made will only increase as sampling becomes more efficient and reference databases become more complete. The co-evolution of AI techniques based on machine learning will allow researchers to fully exploit the richness and complexity of eDNA datasets.

New analytical and statistical models[173] are already providing increasingly rich eDNA insights[174] and levels of accuracy[175]. Machine learning algorithms are particularly promising as a compliment to eDNA methodologies within the biodiversity monitoring space, as they can capture complex relationships between multiple environmental pressures and the diversity of biological communities[176,177]. Such possibilities have led to the advent of new data driven tools, termed the 'de novo approach'[178], that can assemble DNA sequences from scratch and aims to directly associate biological profiles obtained from eDNA with known ecological states or stressors/disturbances. Analysis based on machine learning can hence make fuller use of novel types of data to allow new insights, as well as making these insights available more quickly and with fewer personnel.

Future opportunities

AI techniques can increasingly combine remote sensing data with eDNA data. In the future, this could potentially be used to identify patterns between species occurrences and various environmental variables, and to build and validate models that extrapolate predictions across wider areas[179].

Developments in AI and computing power have expanded the range of possibilities and insights that eDNA data can offer.

173 Gill, P., *et al.*, 2021. A review of probabilistic genotyping systems: EuroForMix, DNAStatistX and STRmix™. Genes, 12(10), p.1559.

174 Fonneløp, A.E., *et al.*, 2021. Who packed the drugs? application of Bayesian networks to address questions of Dna transfer, persistence, and recovery from plastic bags and tape. Genes, 13(1).

175 Bleka, Ø., *et al.*, 2022. MPSproto: An extension of EuroForMix to evaluate MPS-STR mixtures. Forensic Science International: Genetics, 61.

176 Li, X., *et al.*, 2023. Embracing eDNA and machine learning for taxonomy-free microorganisms biomonitoring to assess the river ecological status. Ecological Indicators, 155, p.110948.

177 Keck, F., *et al.*, 2023. A combination of machine-learning and eDNA reveals the genetic signature of environmental change at the landscape levels. Molecular Ecology, 32(17).

178 Cordier, T., *et al.*, (2021). Ecosystems monitoring powered by environmental genomics: A review of current strategies with an implementation roadmap. Molecular Ecology., 30, 2937–2958. (https://doi.org/10.1111/mec.15472)

179 Winkowski, J.J., *et al.*, 2024. Integrating spatial stream network models and environmental DNA to estimate current and future distributions of nonnative Smallmouth Bass. Transactions of the American Fisheries Society, 153(2), pp.180-199. (https://doi.org/10.1002/tafs.10454)

eDNA methods may be most effective in combination, yielding more accurate and richer insights.

Similarly, in the future, eDNA methods could also be beneficially combined with other survey methods such as distance sampling (using transects to estimate abundance), acoustic detection, or camera traps. eDNA methods may be most effective in combination, yielding more accurate and richer insights. However, analytical and statistical methodologies and frameworks would need further development to support this. Based on the recent developments described, such complex analysis seems likely to become increasingly possible.

Predictive models that extrapolate eDNA findings across larger geographical areas would need to be supported and refined, by taking regular and strategically distributed eDNA samples from the ground. The exact methods used will also need to be made transparent, and the reference sequence data openly available, with the assumptions and limitations clearly articulated. This will be particularly important if the data is to inform public policymaking.

Considerations and conclusions

To ensure that society and the UK benefit from the potential that environmental DNA technologies have to offer, and avoid associated harm, eDNA research would benefit from the following considerations:

CONSIDERATION 1
MAXIMISING ECONOMIC BENEFITS

How can we capture the potential value of eDNA research to benefit the UK economy?

Environmental DNA holds potential value to a wide range of sectors. It can be faster, more efficient and provide much richer insights than many current monitoring methods. Now is an important moment to consider how the UK may best capture and capitalise on this, both in terms of public and private benefits.

In 2014, the UK became the first country in the world to utilise eDNA for regulatory purposes. This costs the government ten times less than traditional trapping-based monitoring[180]. There is a real opportunity for the UK to continue to be a global leader in using eDNA-based-approaches to assist policymaking and regulation. This could not only lead to improved monitoring efficiencies, but also new insights and public health benefits.

For the private sector, the efficiency and volume of data that can be obtained using eDNA technologies make them an attractive addition to many growing global markets. Examples include: the environmental consulting market which is forecast to reach $50 billion by 2028 with a 5.4% annual growth rate[181]; the forensic technologies market which was worth $4.88 billion in 2022 and is predicted to grow by 9.9% annually from 2023 to 2030[182]; the bioterror defence market which was worth $15.2 billion in 2022 and is predicted to grow annually 5.0% from 2023 to 2030[183]; and the global agricultural pest control market which was valued at $5.5 billion in 2023 and is predicted to grow annually by 6.4% from 2024 to 2030[184]. All of these represent sectors which eDNA technologies could be commercialised to make significant contributions.

Only recently have companies based solely on offering eDNA services emerged (more usually these are offered in combination with other biomonitoring services). The UK has a strong research base in eDNA and there are already examples of eDNA specific companies that originate from university labs. There are still a relatively small number of corporate and academic labs in the UK (and globally) that offer these services representing an important market opportunity.

180 Biggs, J., *et al.*, 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (Triturus cristatus). Biological Conservation, 183, 19-28.

181 Environmental Consulting Services Market Size. The Insight Partners. See https://www.globenewswire.com/en/news-release/2022/12/20/2577207/0/en/Environmental-Consulting-Services-Market-Size-to-Gain-50-97-Bn-Globally-by-2028-with-5-4-CAGR-Exclusive-Report-by-The-Insight-Partners.html (accessed on 23 September 2024).

182 Forensic Technology Market Size & Trends. Grand View Research. See https://www.grandviewresearch.com/industry-analysis/forensic-technology-market (accessed on 23 September 2024).

183 Biodefence Market Size & Trends. Grand View Research. See https://www.grandviewresearch.com/industry-analysis/biodefense-market (accessed on 23 September 2024).

184 Pest Control Service Market Size & Trends. Grand View Research. See https://www.grandviewresearch.com/industry-analysis/pest-control-services-market (accessed on 23 September 2024).

These factors combined – a growth in the relevant markets, increased awareness and acceptance of eDNA methodologies and their possibilities among policymakers and increased corporate and consumer demand (particularly relating to environmental sustainability) – could act to create a market value for eDNA methodologies which the UK would be well placed to leverage.

CONSIDERATION 2
FOSTERING INTERDISCIPLINARY COLLABORATION

How could eDNA sampling and research be better joined up to benefit multiple disciplines and applications at once?

Currently, the development of eDNA methodologies is siloed into different academic disciplines and applications. Greater join-up and collaboration between these different disciplines would be beneficial to allow the cross-fertilisation of ideas, exchange of methodologies and sharing of best practice. At a national level, current environmental monitoring could relatively easily be further expanded to provide eDNA samples or data for multiple applications at once.

This would mean that new applications could more quickly be realised and remove the risk of re-inventing the wheel. In addition, joined up eDNA sampling could represent a far more cost-effective use of research funding and resources.

National monitoring programmes regularly take air and water samples. These samples could be analysed for eDNA and the findings used to inform biodiversity monitoring, infectious disease monitoring, to detect invasive species or bioterror threats and as an indicator of pollution or environmental quality more broadly. Such information would therefore act to provide metrics for a number of policy areas simultaneously. This wealth of information from a single sample could make eDNA a very cost-effective national or international policy tool. The COVID-19 pandemic demonstrated how quickly and straightforwardly a routine sampling network could be established if this were the objective.

Air filter technologies which are commonplace in many towns and cities to monitor pollution have also been shown to be a valuable source of eDNA data for pollen or biodiversity monitoring[185]. In some regions, air quality samples are stored for decades, presenting the potential for high resolution biodiversity time series – with minimal additional effort. Similarly, historical water samples are also stored and could also be analysed to indicate changes in species composition over time.

Creating integrated and open-access databases that can be shared between government departments will be critical to support these ambitions. As will a joined-up, standardised approach to eDNA monitoring and analysis between disciplines. The establishment of such a platform would require collaboration between different research communities, government departments and agencies, and businesses.

185 Littlefair, J.E., *et al.*, 2023. Air-quality networks collect environmental DNA with the potential to measure biodiversity at continental scales. Current Biology, 33(11).

At a UK scale, interdisciplinary, cross research council funding and/or an eDNA cross-departmental Task Force, which had a co-ordination function and data portal – could be one way of achieving this. A good example of co-ordination already happening is the UK through the UK Environmental Observation Framework's (UKEOF) UK DNA Working Group[186] and the Defra DNA Centre of Excellence[187], but these currently focus mostly on biodiversity monitoring. It is likely that initially, resource-sharing could build on this existing infrastructure, perhaps being only partially integrated at the start, but with a more comprehensive exchange and data sharing between disciplines/applications promoted and developed over time.

Internationally, there are already coordination and data-sharing efforts underway focused on disease surveillance (including COVID-19)[188] and to track the global spread of AMR (see Section 1.5). Whilst it will undoubtedly be logistically complex, it should soon hypothetically be possible to run global surveillance efforts based on eDNA that benefit public health and the environment. For example, to track the global spread of infectious diseases, invasive species and antimicrobial resistance – or to monitor the state of biodiversity to track progress against global framework goals. Nagoya Protocol[189] implications would have to be considered with regards to sharing genetic data at an international level.

CONSIDERATION 3
INVESTING IN SUPPORTING RESEARCH INFRASTRUCTURE

What research infrastructure, benchmarking and guidance is required to support future eDNA research and applications?

So that eDNA research can benefit society as fully as possible, investment in research infrastructure such as DNA reference libraries, data repositories and benchmarking tools, will be vital.

eDNA analysis will only ever be as good as the reference library upon which it is based. Currently, only 52% of eukaryotic (multicellular) species in the UK have reference database sequences, which is the biggest constraint on eDNA based research. Open-access reference libraries need to be expanded to include identifiable sequences for all target species. With continued funding and the right initiatives, the UK has the potential to be one of the first countries in the world to have publicly available genome sequence data for all of its eukaryotic species. The Wellcome-Sanger Darwin Tree of Life programme[190], and the UK Barcode of Life consortium[191] are currently working on providing a comprehensive lists of reference genomes and barcodes for UK species.

186 UKEOF UK DNA Working Group. See https://ukeof.org.uk/our-work/ukdna (accessed on 23 September 2024).

187 Defra DNA Centre of Excellence. See https://www.gov.uk/government/groups/defra-dna-centre-of-excellence (accessed on 23 September 2024).

188 Farkas, K., *et al.*, 2023. Wastewater-based monitoring of SARS-CoV-2 at UK airports and its potential role in international public health surveillance. PLOS global public health, 3(1).

189 The Nagoya Protocol on Access and Benefit-sharing. Convention on Biological Diversity. See https://www.cbd.int/ABS (accessed on 24 October 2024).

190 Tree of Life. Wellcome Sanger Institute. See https://www.sanger.ac.uk/programme/tree-of-life/ (accessed on 23 September 2024).

191 UK Barcode of Life. See https://www.ukbol.org/ (accessed on 23 September 2024).

There is already a complete barcode library for UK flowering plants and conifers[192]. Furthermore, for many of the species for which there are currently gaps, there are specimens housed in UK collections (such as Kew Gardens and the Natural History Museum). Efforts utilising rapid DNA sequencing technologies could be expanded to fill these gaps with relatively little additional investment and indeed are already underway (see Biodiversity Genomics Europe programme[193]). In his 2023 Research, Innovation and review for the UK Government, Sir Paul Nurse recommended: "Support for research undertaken by galleries, libraries, archives, museums, and the heritage and cultural sectors should be increased, and support for long-neglected collections-based research put in place."

To handle the shifts required to utilise the full potential of eDNA technologies, open-access reference libraries, data and sample repositories and advanced data platform facilities (with user friendly interfaces) will need creating, to house the growing data libraries. This is both for the DNA sequences themselves, but also for the methodological and analytical tools. This is in line with the UK's National Data Strategy[194]. Standards for this data should include ensuring that data are Q-FAIR (quality-assured, findable, accessible, interoperable and reusable).

Widespread implementation would also benefit from optimised and standardised collection, extraction, analysis and bioinformatics pipelines. However, there is also legitimate concern that standardisation can limit progress if outdated methods are locked in, so these tools will have to be flexible and iterative from the outset so that innovation is not stifled.

Benchmarking between different methodologies as opposed to strict standardisation may allow a more flexible approach.

The standardisation or benchmarking of eDNA methodologies and protocols both within and between disciplines remains at a very early stage. This is a fundamental component of some eDNA applications, such as those within forensic science – and some form of benchmarking would greatly aid others, such as biodiversity or pollution monitoring. Standardisation of techniques and the development of open-access benchmarking datasets, primers and protocols would help to ensure that eDNA data are reproducible and comparable across both time and space – and between disciplines. By benchmarking the genetic marker/s and the testing protocols used for different applications, eDNA data could more easily be shared and co-analysed between different researchers, institutions and between different countries (which relates closely to Consideration 2, above). These efforts would also act to ensure the quality of, and to build confidence in, eDNA analyses.

Alongside benchmarking or standardisation of methodologies, comprehensive guidance on how to implement, analyse and report eDNA-based findings, targeted at end-users, would ensure that users across the research and policy landscape are able to accurately and confidently apply these techniques and interpret the results. This end-user guidance and best practice would also help to ensure that eDNA techniques are used for beneficial applications as and when appropriate, and that this data is not misused or wrongly interpreted. With good guidelines, end users should also be able to critically assess the suitability, quality, accuracy and limitations of eDNA results.

192 Jones, L., *et al.*, 2021. Barcode UK: A complete DNA barcoding resource for the flowering plants and conifers of the United Kingdom. Molecular Ecology Resources, 21(6), pp.2050-2062.

193 Biodiversity Genomics Europe. See https://biodiversitygenomics.eu/ (accessed on 23 September 2024).

194 National Data Strategy. UK Government. See https://www.gov.uk/guidance/national-data-strategy (accessed on 30 September 2024).

CONSIDERATION 4
ADDRESSING ETHICAL AND LEGAL CONCERNS

How can we better understand and mitigate the ethical, legal and regulatory concerns regarding human eDNA recovery?

eDNA technologies already have the potential to either intentionally or unintentionally capture human DNA, possibly sufficient to identify an individual. This presents important ethical dilemmas, relating to consent, privacy, surveillance, and data ownership and storage.

Human genetic bycatch refers to the inadvertent collection of human eDNA when collecting eDNA samples for other purposes[195]. Many eDNA samples are likely to have some background human DNA present, particularly in anthropogenically impacted environments, such as urban areas or downstream of wastewater treatment plants.

As this report has outlined, there are many potential benefits of collecting human genetic information from the environment, including population-based disease risk studies, health and personalised medicine, as well as potential uses in criminal investigations.

However, there are concerns that human eDNA data could potentially be used for the wrong reasons, such as involuntary genetic surveillance, genome harvesting, covert accumulation of genetic data for malicious commercial purposes, and inadvertent location tracking or population surveillance.

As humans cannot 'consent' to eDNA data being collected and processed, the legal status of this is currently a grey area. There are UK laws that prohibit the collection, or 'theft', of human DNA without consent (Human Tissue Act 2004[196]). However, the increasing accuracy of untargeted eDNA techniques will require a shift in regulation towards the storage, analysis and use of such data – especially if this is used in conjunction with other data, such as linking a DNA sequence found in a certain place to the profile of an individual stored in a national database.

Identifying individual humans from their DNA data has been possible in forensic science for over 40 years using targeted and specialist approaches. However, using untargeted eDNA data for such purposes has not been possible previously as the level of analytical detail that was possible could only identify eDNA fragments as 'human' rather than identify a particular individual. However, more recently, increasingly long human eDNA reads have been successfully sequenced from relatively untargeted analysis, including detailed information such as ethnicity, ancestry and disease susceptibility[197].

195 Goray, M., *et al.*, 2024. Emerging use of air eDNA and its application to forensic investigations – A review. Electrophoresis, 45(9-10), 916-932.

196 Human Tissue Act 2004. See https://www.legislation.gov.uk/ukpga/2004/30/contents (accessed on 1 October 2024).

197 Whitmore, L., *et al.*, 2023. Inadvertent human genomic bycatch and intentional capture raise beneficial applications and ethical concerns with environmental DNA. Nature Ecology & Evolution, 7(6), 873-888.

This means that in the future, individual humans could potentially be identified from a much larger range of eDNA samples. It should be noted that it is possible to remove human DNA sequences from eDNA readouts computationally – and this is likely to be an important component of upholding the ethical standards of such research in the future.

Mitigating concerns and developing appropriate regulation will require input from all stakeholders, including scientists from across eDNA disciplines, end users such as policymakers or public health authorities, legal and ethical experts and the general public. The aim should be to set acceptable guidelines and standards to safeguard the public whilst allowing eDNA to be used beneficially across the range of applications that we have outlined.

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