The one hundred and fifteenth meeting of the
Joint Nature Conservation Committee to be held
at 08.45 on 7th June 2018
JNCC, Monkstone House, City Road,
Peterborough, PE1 1JY

This paper was provided to the Joint Committee for decision/discussion or information. Please refer to the minutes of the meeting for Committee's position on the paper.

To view other Joint Committee papers and minutes visit http://www.jncc.gov.uk/page-2671

To find out more about JNCC visit http://www.jncc.gov.uk/page-1729

JNCC 18 25
June 2018

Joint Nature Conservation Committee

Using eDNA and metabarcoding for nature conservation

Cover Note by Christine Maggs

Executive Summary

1. Action required

1.1. The Joint Committee is asked to:

i. **Note** the definitions of eDNA and metabarcoding, and some of their current applications in nature conservation.

ii. **Discuss** whether the opportunities provided by this new technology could be incorporated into some of JNCC's work, now and in the future.

iii. **Advise** on whether it would be helpful for JNCC to play a part in Defra's proposed new Centre of Excellence for environmental genomic applications and consider additional partnership with molecular laboratories.

2. Key issues

2.1. Over the last decade improvements in DNA technology, including relatively cheap high-throughput (next-generation) sequencing and better bioinformatics pipelines, have revolutionized the fields of ecology and evolution.

2.2. Some of JNCC's activities (e.g. terrestrial surveillance; analyses of anthropogenic pressures) could employ data obtained from relevant DNA-based studies but this will require intercalibration with current approaches for meaningful interpretation.

2.3. JNCC now has an opportunity to participate in a new Defra-led Centre of Excellence for environmental genomic applications, which would require commitment of some staff resource. It could also be the basis for enhanced involved with Defra group molecular laboratories.

Joint Nature Conservation Committee

Using eDNA and metabarcoding for nature conservation

Paper by Mike Nelson, Paul Woodcock and Christine Maggs

1. Introduction

1.1. Using DNA markers for ecology and evolution

Over the last decade improvements in DNA technology including relatively cheap high-throughput (next-generation) sequencing and a better bioinformatics pipeline have revolutionized the fields of ecology and evolution. This has resulted in an enhanced understanding of biodiversity, beyond the reach of traditional non-molecular investigations, and launched the new field of ecogenomics (Creer et al., 2016). A wide range of approaches is available with increasing levels of complexity from straightforward use of marker genes to metagenomics, metatranscriptomics, single-cell genomics and targeted genome sequencing for assessing the taxonomic and functional characteristics of biodiversity (Creer et al., 2016; Fig. 1).

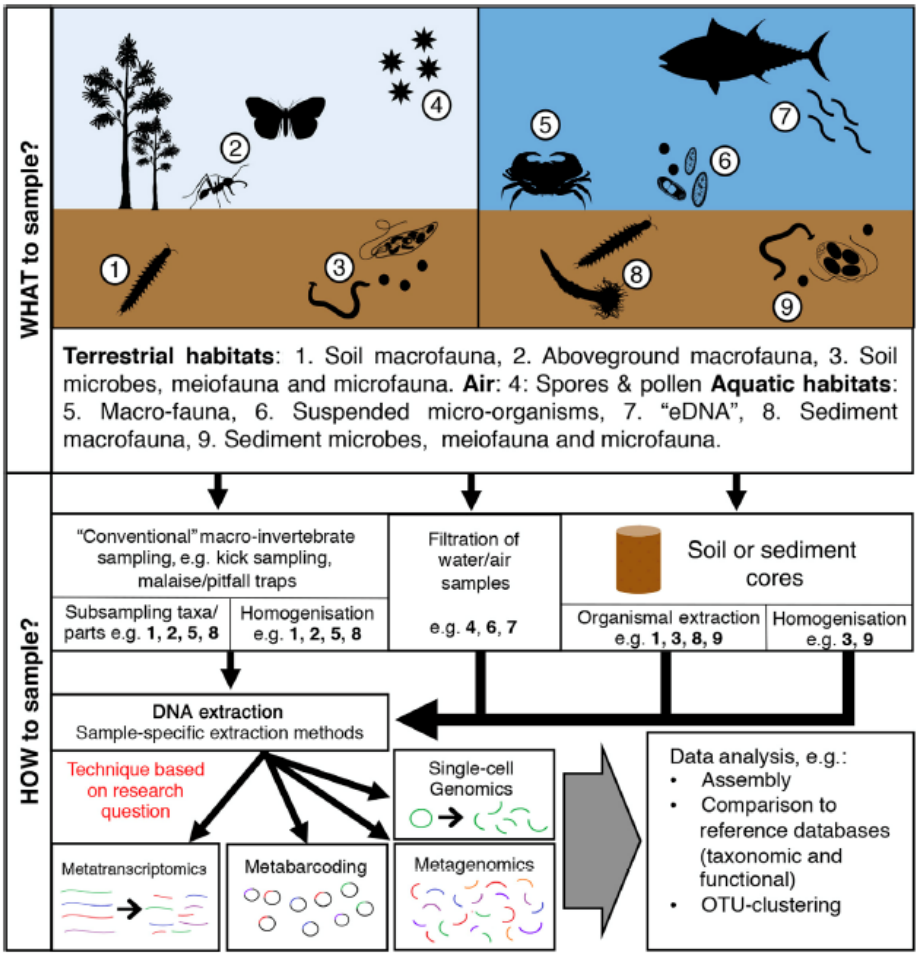

Fig. 1. Schematic showing the decisions involved in a molecular ecology workflow, reproduced from Creer et al. (2016); see Appendix 1 for terminology used here.

1.2. **Environmental DNA** (eDNA) has been used with varying success for the last 15 years to detect the presence of target species, particularly in aquatic habitats but also in some terrestrial environments (Nisbet & Bruce, 2018). DNA from living organisms is released into the environment in shed cells including gametes and in faeces and will be collected along with single-celled organisms. To identify the target species, field-collected samples, especially from aquatic habitats are subject to DNA extraction procedures and eDNA is amplified using PCR techniques with specific primers for an appropriate region (typically the mitochondrial barcoding gene COI or the 18S ribosomal gene for animals or the *rbc*L gene for plants) that only bind to the DNA of the target organism. Once species-specific primers and procedures have been optimized, quantitative (real-time) PCR machines allow the product to be visualized without sequencing, so that a positive presence/absence result can be obtained quickly.

1.3. **Metabarcoding** can be used on eDNA (particularly for fish) or from organismal DNA obtained from invertebrate samples such as collections from pitfall traps or light traps (Bruce, 2018). Universal primers for the barcoding gene of choice will amplify that gene from all the species present in the sample, followed by high-throughput (next-generation) sequencing which delivers thousands of sequences to the bioinformatics pipeline, with the output being a species-by-sample table that can be used for community analysis.

2. Case studies

2.1 Distribution of target species

2.1.1 The most successful eDNA project in the UK is for the Great Crested Newt (Triturus cristatus), sponsored by various agencies including Natural England from 2013 onwards (Biggs et al., 2015; Nisbet & Bruce, 2018). This species is difficult and expensive to survey using conventional methods, which require a license because the species is protected under European and UK legislation: disturbance or damage to the newts or their habitat requires a European protected species licence. Newts are widespread but occur at low density, and determining their absence using traditional surveys is very difficult. Knowledge about presence or absence is required for planning applications, and the species is the subject of a novel system that funds restoration and mitigation. As part of the project, great crested newt habitat is enhanced or created prior to any development taking place, saving developers time and money, and making newt populations more healthy and resilient. eDNA has been used for detecting this species in the UK for the last four newt seasons, by collecting and pooling multiple water samples from each pond, with the results carefully cross-checked by conventional surveys. The water samples can be collected by citizen scientists such as the Freshwater Habitats Trust, and transferred to the laboratory for processing (Biggs, 2018).

2.1.2 eDNA is particularly suitable for identification of freshwater fish, as there is an extensive and nearly complete barcode library for these fish. This sampling approach can replace expensive and potentially harmful methods such as gill-netting (http://www.dnaqua.net). Excellent results have been obtained, with some potential for quantitation of the fish populations. Detection of invasive alien fish species (e.g. chub) in new habitats and confirmation of eradication attempts have been particularly successful.

2.2 Community ecology and anthropogenic pressures

2.2.1 For freshwater invertebrates and phytobenthos, the main challenges for exploiting molecular methods are the modification of agreed indices and incomplete barcode libraries. In freshwater habitats in Europe, preliminary gap-analyses showed that on average around 50% of the species present lacked DNA barcodes, and only 4 groups out of 24 had more than 80% species coverage. This approach is nevertheless very promising, as current assessments of environmental quality status, such as those required by the Water Framework Directive, are based on several hundred different protocols, making them susceptible to issues with comparability, scale and resolution. eDNA metabarcoding may be a possible solution to these shortcomings. Such “next-generation biomonitoring” (Bohan et al., 2017), has many advantages over the traditional approach in terms of speed, comparability and costs. However, several major conceptual and technological challenges still hinder its implementation into legal and regulatory frameworks.

2.2.2 Ecological monitoring based on metabarcoding of protists has been tested as a complement to quality indices based on visual inspection of macrofaunal diversity for monitoring aquaculture sites (Pawlowski et al., 2014). This proof-of-concept study concluded that NGS metabarcoding using foraminifera and other protists has the potential to become a useful new tool for surveying the impact of aquaculture and other industrial activities in the marine environment. Pawlowski's research group in Geneva has recently used DNA metabarcoding to assess biodiversity in the deep sea and to explore the impacts of deep sea mining and offshore oil extraction.

3. The UK DNA Working Group and Technical Groups

3.1 UK DNA Working Group

The UK DNA Working Group was established in 2014 as an open forum for government agencies, academics and stakeholders to discuss priorities for DNA-based method development, informed by a group of end-users from the conservation agencies including JNCC, and to share results and opportunities. It holds an annual international conference, which is free to attend with costs covered by government agencies and/or the hosting organisation. The most recent conference in December 2017 updated attendees on the use of eDNA and metabarcoding in organisms from fish to fungi, in various habitats, at scales from micro to river catchment. The Working Group initially focussed on eDNA in aquatic environments but now covers all applications of this technology, including the direct use of organismal DNA, to monitor and understand diversity and ecosystems.

3.2 DNA End User and Technical Groups

To communicate end user priorities more clearly and foster collaboration, a smaller DNA End User group was established in 2017. This contains representatives from government (NE, SNH, NRW, EA, SEPA, JNCC) and meets several times a year. The End User Group jointly organises the annual Working Group conference, usually with an academic institution. Technical Groups have been established to address specific challenges with developing and using DNA methods. Some groups are system or issue-specific (e.g. river

macroinvertebrates, fish diversity, invasive species, and other marine and terrestrial applications) and some planned groups will consider more generic problems (e.g. developing standards, barcode reference libraries). The most relevant for JNCC are the Marine and the Terrestrial groups.

3.2.1 End Users have highlighted several factors limiting the use of molecular methods. Some of these are technical, e.g.

i. difficulty in quantifying species abundance;
ii. inability to assign species names to barcodes due to incomplete reference libraries;
iii. uncertainty over rates of eDNA release, degradation, and transport;
iv. capacity to process large volumes of data in-house

3.2.2 Others relate more to practical implementation and knowledge exchange, e.g.

i. uncertainty over appropriate field sampling methods;
ii. wide range of rapidly evolving lab and bioinformatics approaches, with limited guidance on suitability;
iii. lack of agreed standards for methods, and processes for validating results;
iv. lack of awareness of relevant research.

3.3 Current and recent JNCC involvement

3.3.1 JNCC participate in the DNA End User Group (PW), and the technical groups focusing on Terrestrial (PW), Invasive Species (PW), and Marine (MN) applications. The first full meeting of the Terrestrial Technical Group is being jointly organised for July by Natural England and JNCC. JNCC also help plan the annual Working Group conference, and intend to contribute £1k to the 2018 costs (with other government agencies providing similar amounts).

3.3.2 This involvement helps JNCC to (i) be aware of shared priorities and barriers for government organizations across the UK, (ii) contribute to end user discussion and dissemination of shared priorities, (iii) remain aware of emerging developments that may be relevant to JNCC (e.g. ongoing work, investment in lab or data infrastructure by different organizations, potential collaborations and opportunities), (iv) improve links with researchers, and (v) raise the profile of the organization.

3.3.3 JNCC terrestrial biological recording schemes are undergoing a review to determine fitness for purpose. This review is considering new technologies, and in particular whether there are key gaps in JNCC monitoring schemes that could be filled by using DNA/eDNA.

4. Proposed Defra-led Centre of Excellence for environmental genomic applications

4.1 Defra's consultation across the agencies, researchers and other institutions showed that there is interest across government conservation and environmental agencies in a range of potential applications of DNA sequencing technology, including (i) detecting rare and invasive species, (ii) evaluating the effectiveness of conservation interventions, (iii) monitoring status and trends for key

assemblages and taxa, and (iv) assessing ecosystem health, functioning, and resilience. It concluded that a virtual Centre of Excellence would provide UK scale added value, strengthen existing relationships, developing opportunities for partnership working, and enable improved funding. It would be modelled partly on the successful **Defra Earth Observation Centre of Excellence**, in which JNCC has played an important role. Forest Research, APHA, Cefas and the Environment Agency have laboratory capability; all are looking to further develop services and are interested in exploring the potential benefits from collaborating.

4.2 The consultation leaders (Andy Nisbet, Natural England & Vicki Rhodes, Environment Agency) requested time commitments from across the Defra Group to establish a virtual team, as well as dedicated resource to lead the activity. Longer term, the ambition is to secure funding to deliver shared, priority projects. The proposed Defra plan is:

i. Develop a roadmap for the Defra Group
ii. Work to secure funding to deliver the roadmap
iii. Examine the potential to make better use of laboratory facilities
iv. Engage with Devolved Authorities and other key partners and groups
v. Build capacity by support knowledge exchange, skills development and horizon scanning

Given JNCC's strengths in data analysis, initial involvement in the Centre of Excellence could lead to undertaking the bioinformatics aspects of DNA metabarcoding projects in partnership with a DNA laboratory in the Defra group.

4.3 Options for JNCC at this stage are

i. JNCC remains involved with the DNA End User Group but does not participate in the proposed Centre of Excellence.

ii. JNCC participates in the proposed Centre of Excellence, committing some staff resource.

iii. JNCC participates in the proposed Centre of Excellence and also actively explores the possibility of partnering with a molecular laboratory to advance its analyses of marine and terrestrial biodiversity.

References

Biggs, J. (2018) Finding newts - using environmental DNA to detect great crested newts. *In Practice* [CIEEM], 99: 18-22.

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R.A. 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.

Bohan, D.A., Vacher, C., Tamaddoni-Nezhad, A., Raybould, A., Dumbrell, A.J. & Woodward, G. (2017) Next-generation global biomonitoring: large-scale, automated reconstruction of ecological networks. *Trends in Ecology and Evolution* 2017 Jul;32(7): 477-487. doi: 10.1016/j.tree.2017.03.001.

Bruce, K. (2018) DNA metabarcoding of invertebrates to evaluate outcomes of ecological restoration. *In Practice* [CIEEM], 99: 13-17.

Creer, S., Deiner, K., Frey, S., Porazinska, D., Taberlet, P., Thomas, W.K., Potter, C. & Bik, H.M. (2016) The ecologist's field guide to sequence-based identification of biodiversity. *Methods in Ecology and Evolution*, 7: 1008-1018. DOI: 10.1111/2041-210X.12574

Nisbet, A. & Bruce, K. (2018) Developing DNA-based techniques in Natural England for surveying and monitoring biodiversity. *In Practice* [CIEEM], 99: 7-9.

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: 1129-1140.

Appendix 1. Glossary of terms (from Creer et al. 2017)

Community DNA. Defined here as the DNA derived from many individuals representing several species.

Cloning. The process of producing genetically identical copies of an organism, either naturally (e.g. as a result of asexual reproduction) or artificially. In the context of nucleic acid sequencing, cloning commonly refers to the insertion of DNA into a vector molecule (e.g. a plasmid) prior to selection for a gene of interest, DNA extraction and sequencing.

Degenerate primers. A mixture of similar, but not identical oligonucleotide sequences used for amplicon sequencing where the targeted gene(s) is typically similar, but not identical.

Environmental DNA (eDNA). DNA isolated directly from an environmentalsample (e.g. air, faeces, sediment, soil, water).

Genomic DNA. The DNA derived from a single individual or from a collection of individuals of the same species.

Locus. The specific location of a gene orDNA sequence on a chromosome.

Marker gene. A gene or DNA sequence targeted in amplicon sequencing to screen for a specific organism group or functional gene.

Metabarcoding. Uses gene-specific PCR primers to amplify DNA from a collection of organisms or from environmental DNA. Another term for amplicon sequencing.

Metagenomics. The random sequencing of gene fragments isolated from environmental samples, allowing sequencing of uncultivable organisms.

Metatranscriptomics. Shotgun sequencing of total RNA from environmental samples. Techniques such as poly-A amplification or rRNA depletion are often used to target messenger (mRNA) transcripts to assess gene expression patterns in complex communities.

Next generation sequencing (NGS). Recent advances in DNA sequencing that make it possible to rapidly and inexpensively sequence millions of DNA fragments in parallel. Also referred to as high-throughput sequencing (HTS).

Orthologs. Genes in different species that evolved from a common ancestor and normally retain the same function.

Polymerase chain reaction (PCR). Used to amplify a targeted piece of DNA, generating many copies of that particular DNA sequence.

Shotgun sequencing. DNA is fragmented into small segments which are individually sequenced and then reassembled into longer, continuous sequences using sequence assembly software.