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Genetic Techniques and Technologies

In this issue

Genetic Techniques and Technologies

Back in 2012, when I was a PhD student at the University of East Anglia, groundbreaking molecular methods for ecology were regularly being published in scientific journals. Researchers claimed these methods would solve age-old problems faced by environmental managers in species detection and identification - yet the studies were never disseminated in an accessible way, nor the methods made available for routine use. I remember giving a talk at the BTO and explaining that you could use high-throughput DNA sequencing to identify all the insects in a trap sample in a single reaction. Someone said they had thought such a thing might be possible in twenty years' time but had no idea it could already be done.

Likewise, the use of environmental DNA (eDNA) for species monitoring was first demonstrated in the scientific literature as early as 2008, when a study showed that American bullfrogs could be detected by analysing samples of pond water. When a researcher at Central Michigan University read this and proposed using the same approach for monitoring the invasion of Asian carp, he was nearly laughed out of academia. Thankfully for him, their subsequent study showed that eDNA was the most sensitive tool in the box for detecting the presence of the fish at low population levels and it has now become deeply embedded in the US Fish and Wildlife Service's ongoing monitoring programme as they try to limit the ecological damage caused by these disruptive species.

In the UK, eDNA first gained prominence in 2014 with the publication of the DEFRA-funded trial for great crested newt (GCN) monitoring. Four years on and thousands of eDNA samples are now analysed every spring. A generation of ecologists are becoming familiar with DNA as a powerful tool for monitoring species, and the UK is leading the way in adoption and standardisation of cutting-edge molecular methods.

The use of DNA monitoring comes with a set of limitations, which vary among environments and sample types. Ecologists should seek to develop an understanding of the methods (hopefully the articles in this issue should help!), including their limitations and the factors that influence their performance. This will allow informed decision-making about whether DNA is the right tool for addressing any specific question, together with robust interpretation of results. At the same time, it is important to remember that *all* survey methods are subject to inherent limitations and biases - this is not something that is unique to DNA!

As with any new technology, some teething problems are inevitable as we all find our feet and explore how to integrate new tools with more familiar ones. But DNA monitoring has the potential to dramatically increase the scale at which we can monitor species in the environment, providing data on groups that have been extremely difficult to survey in the past. This will help us to better understand the impacts of our actions on biodiversity, and ultimately to achieve better outcomes.

The publication of this issue of *In Practice* is a real marker of how far we have come in just a few years, but I believe this is only the beginning of our journey with DNA.

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Front cover image:
Electrophoresis is used to identify, quantify, and purify DNA fragments.

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CIEEM Conferences 2018		
Date	Title	Location
26 April 2018	Irish Section Conference 2018 Making Mitigation Work	Dublin
22 March 2018	Spring Conference 2018 The Nature of Buildings: Designing Effective Mitigation and Enhancement	Birmingham
4 July 2018	Summer Conference 2018 Fit for the future: Developing an Ecologically Resilient Protected Sites Network	Natural History Museum, London
21-22 November 2018	Autumn Conference 2018 Habitat Re-creation and Ecological Restoration	Glasgow

Visit the CIEEM website to book on the Spring Conference and to find out more information.

Relaunching the Academia Special Interest Group

This year sees the relaunch of CIEEM's Academia Special Interest Group (SIG).

The Academia SIG aims to provide support to members working in the academic field and their students through networking, providing a focus for sharing good practice and acting as a valuable consultative and communication function for the Institute in understanding and responding to the needs of academic members.

CIEEM is also committed to supporting students looking to enter the sector and the SIG will provide a link between the Institute and the next generation of CIEEM members.

Find out more at www.cieem.net/academia and sign up to join the SIG via the Members' portal at https://events.cieem.net/Portal/MyDetails.aspx.

CIEEM responded to Natural England consultation on licence charges

CIEEM has responded to Natural England's consultation on charging for licences.

CIEEM understands the reasons for introducing charges for licences and supports Natural England's ambition to improve the licensing system. However, we have pointed out that charging is not the only way to deliver improvements.

In general terms, CIEEM is not against the principle of charging for site-specific mitigation licences. We do however have concerns about charging for individual survey licences.

See the full response at: www.cieem.net/past-consultation-responses

Future *In Practice* Themes

If you would like to contribute an article to a forthcoming edition of *In Practice* please submit it for consideration by the deadline below. We are happy to discuss ideas in advance and welcome suggestions for future themes.

Edition	Theme	Submission deadline
100 - June 2018	Centenary Edition: Big Ideas	n/a
101 - September 2018	Environment and Pollution	28 May 2018
102 - December 2018	Data and Information Management	27 August 2018

Articles for the specific editions below could address, but are not limited to, the following:

- **Environment and Pollution** – all aspects of pollution impacts on the natural environment including water, air quality, eutrophication and enrichment, agriculture, transport, etc.
- **Data and Information Management** – including big data, sharing, storage, management and analysis, etc. in relation to the natural environment

For further information please visit the website (www.cieem.net/in-practice) or contact the Editor (gillkerby@cieem.net).

25-Year Environment Plan

CIEEM has responded to the government's 25-Year Environment Plan, which is for England only. CIEEM applauds the Secretary of State, Michael Gove, for the ambition of the Plan and its long-term vision. There are many aspects of the Plan that CIEEM believes, if properly funded and implemented, could make a real difference to halting the current rate of environmental degradation, helping to build a healthier, more resilient environment for future generations.

CIEEM President, Dr Stephanie Wray, said: *"The Plan rightly sets out the importance of the natural environment to people and the economy, and the huge challenges facing the natural environment. These challenges also unfortunately highlight some of the shortcomings of the Plan, which is an ambitious vision but uses weak, non-committal language and lacks detail on immediate action."*

Read CIEEM's full response to the Plan at: www.cieem.net/news/460/cieem-response-to-25-year-environment-plan

Reducing Plastic

Members will be pleased to see that, in order to reduce our plastic use, we have changed to recycled paper envelopes for mailng *In Practice*. The previously used polybags were made of oxo-degradable low density polythene which breaks down and degrades faster than standard polythene products, however we believe that recycled paper is a more sustainable option.

New publications from Conservation Evidence

In February, Conservation Evidence launched the evidence for two habitat types that are important for CIEEM members; peatlands, and shrublands/heathlands. For each habitat researchers have collected together and summarised the evidence for how well conservation interventions worked to conserve the distinctive vegetation of those habitats. The results are available to read online or can be downloaded for free. This summarised evidence can help ecologists decide which interventions to choose in order to conserve bogs, fens, moorland, and more.

https://www.cieem.net/news/463/moor-evidence-than-ever

Bovine TB control in the devolved administrations

The UK government has published a summary of the approach to the control of bovine TB, including badger culling, in the devolved administrations.

http://researchbriefings.parliament.uk/ResearchBriefing/Summary/CBP-8188

Chief Veterinary Officer's advice on 2017 badger culls

England's Chief Veterinary Officer's advice on the outcome the badger culls in parts of England in 2017 has been published.

https://www.gov.uk/government/publications/bovine-tb-chief-veterinary-officers-advice-on-the-outcome-of-the-2017-badger-culls

UK ecosystem service accounts 1997-2015

This publication presents the UK natural capital ecosystem service accounts, aiming to highlight the relative importance of services provided by the UK's natural assets. It presents 10 service accounts, containing estimates of the quantity and value of services being supplied by UK natural capital. These services include food, water, air filtration and recreation.

https://www.ons.gov.uk/economy/environmentalaccounts/bulletins/uknaturalcapital/ecosystemserviceaccounts1997to2015

Natural Capital Committee's fifth annual report

This report sets out the work done by the committee since March 2017. The NCC reports to the Economic Affairs Committee.

https://www.gov.uk/government/publications/natural-capital-committees-fifth-annual-report

Farming for the next generation

Secretary of State Michael Gove set out his vision on the future of the English farming industry at the Oxford Farming Conference in early 2018. In a wide-ranging speech he stated that that the *"principal public good we will invest in is of course environmental enhancement"* and that the government plans *"to provide new support for those who choose to farm in the most sustainable fashion"* and *"support what economists call the provision of ecosystem services"*.

https://www.cieem.net/news/457/farming-for-the-next-generation

Wildflower planting on farms boosts biodiversity

A study published in *Animal Conservation* has demonstrated the potential for agri-environment scheme land management to substantially enhance the abundance of priority farmland birds and highlights the need for option packages that are resilient to the impacts of variable weather conditions.

http://onlinelibrary.wiley.com/wol1/doi/10.1111/acv.12386/full

New Northern Forest gets Government backing

The Prime Minister has announced plans for a new Northern Forest, along the M62 corridor from Liverpool to Hull, as part of the Government's 25-Year Environment Plan. Spanning more than 120 miles, the proposed Northern Forest will help boost habitats for woodland birds and bats and protect iconic species such as the red squirrel – alongside providing a green space to be enjoyed by millions of people living in the area.

https://www.cieem.net/news/455/new-northern-forest-gets-government-backing

Protecting ancient woodland and veteran trees from development

This guidance, revised twice recently, covers what planning authorities should consider for developments affecting ancient woodland and veteran trees.

https://www.gov.uk/guidance/ancient-woodland-and-veteran-trees-protection-surveys-licences

New EPA Online Mapping Application

The EPA has developed a new online GIS application, which will enable access to all the EPA GIS sites in Ireland from a single application. This has been designed with a strong focus on user experience, creating an application that is intuitive and easy to use. Where possible the EPA has tried to mirror the typical functionality of Google Maps to ensure ease of use.

http://epa.newsweaver.co.uk/general/7co64imwaoh1aoa8sfamq1

New Bat Expert Panel for England

Natural England has appointed a new expert panel to help shape the future of bat conservation in England. See page 47 for more information.

Brexit and local government in England —the challenges ahead

This article, published in *Public Money and Management*, briefly sets out some of the key implications and impacts that Brexit will have on public services, including English local government.

http://www.tandfonline.com/doi/full/10.1080/09540962.2018.1434316

Green Infrastructure Resource Library launched

A searchable database – GIRL – of 800 documents on Green Infrastructure, green spaces, natural capital and related topics has been launched.

http://www.brillianto.biz/girl

Glossary of Terms

Term	*Definition*
Assay	***Investigative procedure to detect the presence of a target species.***
Bioinformatics	***Computational data processing that takes the raw sequence data from high-throughput sequencing and transforms it into usable ecological data.***
Community DNA	***DNA extracted from a mixture of different organisms.*** *Could be eDNA or organismal DNA.*
DNA barcodes	***Short and standardised sections of the genome that can be used for species identifications.*** *The key feature of such DNA regions is that they are the same across individuals from the same species, but differ across individuals from different (even closely related) species. DNA barcodes for animals include CO1, 12S, 16S, 18S, and cytB. For plants, the most commonly used DNA barcodes are matK, rbcL, trnL and ITS.*
DNA degradation	***The process by which strands of DNA are broken up, eventually becoming undetectable.*** *Factors promoting DNA degradation include inappropriate preservation methods, UV radiation exposure, or high temperature. Degradation can be detected using artificial DNA as an internal positive control (IPC). This is added to the preservative solution and extracted in parallel with the sample DNA. If the IPC is not detectable then the DNA in the sample could be degraded.*
Environmental DNA (eDNA)	***DNA deposited in the environment through excretion, shedding, etc.*** *This can be collected in environmental samples (e.g. water, sediment) and used to identify the organisms that it originated from. eDNA is typically sampled in low concentrations and can be degraded, which may limit the analysis options.*
False positives	***Detection of something that isn't there.*** *Can result from contamination or an assay being incorrectly triggered.*
False negatives	***Failure to detect something that is there.*** *Can result from incomplete sampling, PCR inhibition, DNA degradation, or dilute DNA below the limits of detection.*
High-throughput sequencing	***Sequencing technology that produces millions of sequences in parallel.*** *Enables many different organisms to be sequenced at once, so is ideal for use with community DNA. Also known as Next-Generation Sequencing (NGS) or parallel sequencing.*
Inhibition	***Naturally-occurring chemicals/compounds that cause DNA amplification to fail, potentially resulting in false negative results.*** *Common inhibitors include tannins, humic acids and other organic compounds. Inhibition can be detected by adding internal positive control (IPC) DNA to the sample DNA and amplifying it using PCR. If the IPC does not amplify with the expected efficiency then the reaction is inhibited. Inhibition can be overcome by either diluting the DNA (and the inhibitors) or by additional cleaning of the DNA, but dilution carries the risk of reducing the DNA concentration below the limits of detection.*
Limit of detection (LOD)	***The lowest concentration of DNA molecules below which the organism cannot be detected using the assay.***
Metabarcoding	***The process through which whole species assemblages can be identified from community DNA using DNA barcodes.*** *PCR is carried out with non-specific primers, followed by high-throughput sequencing and bioinformatic processing. Can identify hundreds of species in each sample, and 100+ different samples can be processed in parallel to reduce sequencing cost. The output is a species-by-sample table that can be used for community analysis.*
Polymerase chain reaction (PCR)	***A process by which millions of copies of a particular DNA segment are produced. Also known as DNA amplification.*** *A common process in molecular biology and a necessary step for most analyses. PCR relies on primers that target the desired section of DNA for a particular organism or group of organisms. When subjected to a series of heating and cooling steps in the presence of an enzyme called DNA polymerase, the primers bind to the target DNA and create copies of it.*
Primers	***Short sections of synthesised DNA that bind to either end of the DNA segment to be amplified by PCR.*** *Can be designed to be specific to a particular species or to be very general so that a wide range of species' DNA will be amplified.*
Quantitative polymerase chain reaction (qPCR)	***A PCR reaction incorporating a coloured dye that fluoresces during amplification, allowing a machine to track the progress of the reaction in real time. Also known as real-time PCR.*** *When species-specific primers are used, amplification is only expected if the target species is present in the sample. Amplification detected by qPCR is therefore used to infer presence of the target species.*
Reference databases	***Libraries of DNA sequences that have been generated from species of known identity and used to cross-reference and identify unknown sequences.***
Sanger sequencing	***Traditional DNA sequencing that produces a single sequence read per reaction.*** *This is most commonly used to generate reference databases or to check the performance of species-specific primers.*
Sequence reads	*A DNA sequence is made up of four nucleotide bases represented by the letters A, T, C & G. The precise order of these letters is used to compare genetic similarity among individuals or species and to identify species using reference databases. In high-throughput sequencing analyses (e.g. metabarcoding), many identical copies of the same sequence are obtained for each species in the sample. The number of copies obtained per species is known as the number of sequence reads, and this is often – although not always – related to the relative abundance of the species.*

With thanks to Dr Cuong Q. Tang and Dr Kat Bruce at NatureMetrics Ltd for compiling this Glossary.

Developing DNA-based Techniques in Natural England for Surveying and Monitoring Biodiversity

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Keywords: DNA metabarcoding, eDNA, innovation, species survey

Natural England is funding a number of projects examining different applications of DNA technology for the survey and monitoring of terrestrial, freshwater and marine biodiversity. The benefits of using DNA are that we may be able to save money and time, detect species that are difficult to find, and develop new measures of ecosystem health. While there are still issues and challenges associated with this technology, we believe that it will produce significant and exciting improvements to how we monitor and assess biodiversity.

Figure 1. Sampling water from grazing marsh ditches in the Nene Washes SAC. © Keith Porter/Natural England.

Introduction

Biodiversity survey and monitoring has often been limited to recording taxa that are easy to find, or that we have a legal obligation to report on. Identification of some groups relies on a small expert community and it can be a long time between sampling and making records accessible. DNA-based techniques have the potential to significantly change this by reducing costs, reducing sample-to-use times, improving our ability to detect species that are difficult to find or identify, and providing a tool for monitoring functional groups and ecosystem health.

Over the last five years, Natural England has supported research and innovation into the use of environmental DNA (eDNA) to detect the presence of great crested newts in ponds (Biggs *et al*. 2014). Since 2016, we have been funding a number of exploratory projects that are looking at species detection across a range of taxa in different ecosystems (standing and flowing freshwaters, saline lagoons, coastal waters and sediments, terrestrial invertebrate traps, deadwood mould, vegetation and soils). Table 1 lists these projects.

Detecting species that are difficult to find

A number of projects have examined the potential for using eDNA to detect species that are difficult to survey using conventional methods (see Box 1 for work on detecting seahorses *Hippocampus* spp.).

Figure 1 shows a water sample being taken from a ditch on the Nene Washes Special Area of Conservation (SAC) to see if DNA from the spined loach *Cobitis taenia* could be detected. Spined loach is a small bottom-feeding fish listed under Annex 2 of the Habitats Directive, and the Nene Washes SAC was designated because of the high density of this species in one main drainage channel. However, we didn't know if spined loach were present in the smaller ditches because of the difficulties in surveying them. Water samples were taken from five sites. Spined loach DNA was successfully detected from two of the three main drain samples but not in the samples from smaller ditches. This method was cheaper and easier than a conventional survey would have been and also detected a number of other fish species.

Our attempts to detect DNA from the violet click beetle *Limoniscus violaceus* were less successful. This species is also listed under Annex 2 of the Habitats Directive and is known from only three woods in England. It is saproxylic and depends on undisturbed, ancient and decaying beech *Fagus sylvatica* or ash *Fraxinus excelsior* trees where the larvae live and grow in decaying wood mould.

Surveying for the larvae is destructive and so we tested whether DNA from this species could be detected from samples of wood mould. We took samples from trees where the species was thought to occur

Table 1. Projects underway to explore the use of eDNA to detect species across a range of taxa and ecosystems.

Target species/group	Project
Freshwater fish	Testing the use of eDNA to detect spined loach *Cobitis taenia* in grazing marsh ditches in the Nene Washes SSSI and SAC.
	Detecting migratory fish species using eDNA.
Marine fish	Testing eDNA sampling and analysis methods to survey seahorse *Hippocampus* spp.
Terrestrial invertebrates	Metabarcoding of invertebrate samples from malaise, vane and pitfall traps.
	Metabarcoding of soil invertebrates collected from Natural England's Long-Term Monitoring Network.
	Testing the use of eDNA analysis of deadwood/wood mould to detect violet click beetle *Limoniscus violaceus*.
Freshwater invertebrates	Testing the use of eDNA to describe the dragonfly community of ponds.
	Developing eDNA sampling and analysis methods to monitor freshwater pearl mussel *Margaritifera margaritifera* populations in England.
Lagoon invertebrates	Investigating the use of eDNA techniques for monitoring lagoon species assemblages, including protected species.
Marine invertebrates	Testing the potential of DNA metabarcoding to describe the invertebrate community of benthic sediments.
Fungi	Surveying grassland fungi of conservation importance using DNA metabarcoding.
Vascular plants and bryophytes	Detecting vascular plant and bryophyte species from vegetation, turf and soil samples.

using a soil corer. Unfortunately, no DNA of the violet click beetle was detected. This may be because no DNA was present, and the chance of DNA from these rare species being present in a small core is probably low. However, the results could also be false negatives because the target sequences were obscured by much more prevalent bacterial and fungal DNA.

A quicker and cheaper alternative

During surveys and monitoring, sampling effort can be limited by the available capacity to identify specimens collected as well as by costs. This identification bottleneck can mean that data are not made available quickly. DNA metabacoding of invertebrates captured in standard traps on Lampert Mosses SSSI in 2016 successfully identified a range of species and the time taken from sample collection to making data available was only six weeks. As well as being faster, this approach is also cheaper when scaled up and so DNA metabarcoding should make it possible to analyse larger numbers of samples efficiently.

We are also testing DNA analysis of soil samples to detect fungi of conservation importance (e.g. waxcaps *Hygrocybe* spp.). The current approach to establishing which fungi are present on a site involves repeated visits over a number of years in the right season. Fungi may not fruit if the sward is too tall but may still be present in the soil. DNA analysis can detect the presence of species (even if not fruiting) throughout the year.

A third area of investigation is the identification of invertebrates from inter-tidal and subtidal sediment samples. Conventional sampling and species identification is expensive and time consuming. Metabarcoding appears to generate similar species diversity information to morphological analysis across multiple species groups including molluscs, crustaceans and annelids. Ongoing work focuses on the ability of this approach to describe ecological gradients using both the macrofaunal and meiofaunal component of diversity.

Assessing ecosystem health and habitat restoration

Our knowledge of biodiversity outcomes is often constrained by our reliance on data from a small number of popular taxa, rather than on cross-taxa species assemblages or groups which are sensitive to environmental change. Other taxa or groups may tell us more about ecosystem health (e.g. soil biodiversity) and ecosystem services (e.g. pollinators). Defra have recently funded work to generate and collate reference DNA barcode data for key pollinator species (Defra 2017). Figure 2 shows soil being taken from our Long-Term Monitoring Network and we are using DNA metabarcoding to identify soil mesofauna found in these samples (Nisbet *et al*. 2017).

Figure 2. Soil sampling on Natural England's Long-Term Monitoring Network. © Matthew Shepherd/Natural England.

Progress and challenges

Some of our projects have been straightforward and successful while others have presented more challenges. A full report on the first year's projects is in preparation; some of the main conclusions are that:

- eDNA metabarcoding is effective for monitoring fish species in all aquatic habitats although eDNA transport distances may be significant in flowing water.
- Metabarcoding is effective and efficient for characterising terrestrial invertebrate assemblages.
- Plant species can be identified successfully from root material extracted from soil although the extraction process was time consuming.
- More work is needed on the laboratory processes for detecting invertebrates from water samples and substrates (sediment).

Some of the challenges and issues that need to be addressed include testing and developing field methodologies (especially for eDNA approaches); refining laboratory techniques and developing appropriate primers for particular species or groups; being able to estimate abundance or

Box 1. eDNA monitoring of seahorses

There are two species of seahorse in the UK: the short-snouted seahorse *Hippocampus hippocampus* and the spiny seahorse *H. guttulatus*. Both are elusive and live in shallow, weedy areas, particularly eel grass beds. Important populations of both species are present in Poole Harbour and Studland Bay in Dorset, southern England.

Seahorse surveys are difficult because seahorses are small, cryptic, chameleonic animals that live in an expansive, low clarity habitat among dense seagrass/seaweed beds. Diver surveys are the current standard but can only cover very small areas, making comprehensive survey very time consuming and expensive (Garrick-Maidment 2011).

In 2016, we trialled an eDNA metabarcoding approach for detecting seahorses on the basis that this has the potential to be a far cheaper and more efficient tool. We first analysed water samples from tanks housing both species at the Zoological Society of London, and then collected six samples from Poole Harbour and Studland Bay (see Figure 3). eDNA was captured using Sterivex filters (EMD Millipore, Burlington, MA) (three filters per sample), which were filled with ethanol to preserve the DNA during transport to the laboratory.

DNA was separately extracted from each filter and PCR amplified for a short region of the 12S rRNA gene using primers that target fish. Eight of the nine DNA samples (six natural and three tank samples) were successfully amplified for each of the replicates, but one sample from Poole harbour failed to amplify due to Polymerase Chain Reaction (PCR) inhibition despite dilution of the DNA and the addition of various PCR enhancers. Successful PCRs were sequenced on an Illumina MiSeq (Illumina, San Diego, CA) and raw sequence data were processed using a custom bioinformatics pipeline to generate a species-by-sample table.

Both species of seahorse were successfully detected in the aquarium tanks, with each detection supported by around 200,000 sequences. This confirmed that the primers used in this study do effectively amplify seahorse eDNA when it is present. No seahorses were detected in any of the Poole Harbour samples, although these did yield detections of other marine and estuarine fish species including starry flounder *Platichthys stellatus*, sea bass *Dicentrarchus labrax*, allis shad *Alosa alosa*, Atlantic herring *Clupea harengus*, a gurnard species *Chelidonithyes* sp., grayling *Thymallus thymallus*, minnow *Phoxinus phoxinus*, European bullhead *Cottus gobio* and brown trout *Salmo trutta*. All freshwater-associated species were detected in a single sample that was collected from within 1 km of the mouth of the river Frome, and all are typical inhabitants of this river. This provides a useful indication of the typical transport distance of environmental DNA in lowland river systems.

The failure to detect seahorses may have been a consequence of the sampling date, which was later in the year than is advisable for seahorse surveys. It is also possible that greater sampling effort and laboratory replication is required to detect rare species in a marine environment. This is now the focus of ongoing work in the 2017-18 season. Recent work has detected two species of pipefishes (Syngnathinae) from seagrass beds, so we are confident that seahorses can be detected using this approach.

relative abundance; improving DNA databases; and being able to interpret and use this evidence to support site management. Despite these challenges, we expect that DNA applications will produce significant and exciting improvements to how we monitor and assess biodiversity.

Figure 3. Sampling water from Poole Harbour to test for seahorse DNA. © Gavin Black/ Natural England.

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About the Authors

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Kat is co-founder and CEO of NatureMetrics, a company that specialises in DNA-based monitoring of biodiversity. An ecologist by training, her PhD at the University of East Anglia centred on the use of invertebrate metabarcoding to inform environmental management activities and facilitate evidence-based decision-making.

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Pollen Analysis Could Be Revolutionised with DNA Techniques

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Hannah Norman
Imperial College London and
The Natural History Museum

Keywords: DNA metabarcoding, palynology, plant-pollinator networks, pollen analysis

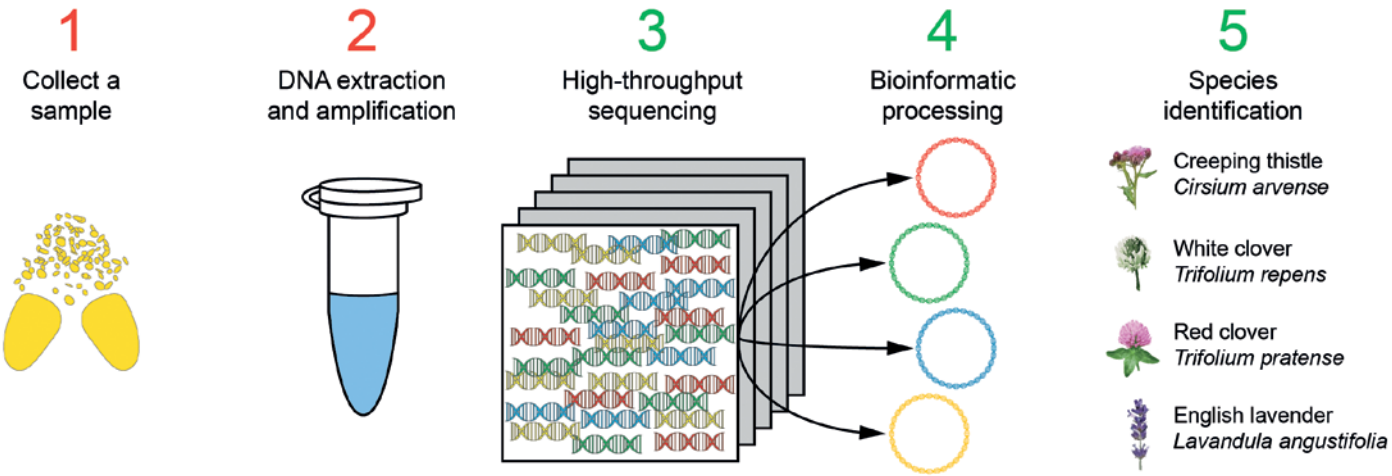

Figure 1. The DNA-based process of obtaining a species identification from pollen requires several steps: DNA must be extracted from the sample and then the DNA barcoding region amplified into millions of copies. These DNA barcodes are then sequenced and the data processed to obtain representative sequences for each species present. These sequences can then be identified against a reference database of known species to give the floral composition of the sample.

Identifying the botanical composition of pollen (palynology) has important implications for environmental management, public health, and even forensics. DNA-based pollen analysis offers significant advantages in terms of cost, scale, and taxonomic resolution over the more conventional morphological methods. DNA-based technologies are now beginning to be applied to palynology and early results are very promising. These approaches could revolutionise the field to facilitate pollination research on an unprecedented scale and provide a deep understanding of plant-pollinator networks.

Introduction

Identification of pollen origin (plant palynology) is a key aspect of pollination ecology and agro-ecological studies. It has been used to link suspects with crime scenes, investigate bee foraging behaviour, provide an early warning system for hayfever sufferers, and even to determine the floral composition of honey. Here we discuss the use of DNA techniques for detecting plant species in pollen from an environmental perspective, outlining current capabilities and limitations.

What is possible and why is it important?

A pollen sample is usually made up of a variety of different botanical sources, which have traditionally been characterised using microscopy. This is labour intensive and heavily reliant on a very specialised group of taxonomic experts (palynology botanists) and an extensive set of reference materials. The identification process is slow, and therefore only small samples tend to be analysed, with low abundance species often undetected. Moreover, the taxonomic resolution to which most plants can be differentiated by morphometrics is relatively broad and subject to observer biases.

Notwithstanding the skill of palynology botanists, current methods of species determination stand to benefit from a DNA-

based approach, which has the potential to be more scalable and cost effective, and to provide more highly resolved botanical data. Indeed, a growing catalogue of literature has shown that pollen DNA metabarcoding provides typically higher taxonomic resolution (e.g. Kraaijeveld *et al*. 2015), higher detection rates at all taxonomic levels (e.g. Smart *et al*. 2017), and higher accuracy (e.g. Vamosi *et al*. 2016) than does morphological palynology.

How does it work?

The DNA contained in a pollen grain can be used to identify the plant species it came from, and high-throughput sequencing (metabarcoding) allows for the analysis of mixed-species pollen samples. Instead of subsampling pollen samples for morphological analysis, DNA can be extracted *en masse* from the whole sample, amplified for plant-specific DNA barcoding regions, and sequenced (Figure 1). Millions of sequences are generated that can then be digitally separated out and quality filtered. Finally, these sequences are compared to an ever-expanding reference database of vouchered sequences to characterise the taxonomic composition of the pollen sample.

Plant-pollinator interactions

Perhaps the most exciting application of DNA-based pollen identification is its use in characterising plant-pollinator foraging networks. These networks are important for establishing how pollinators use floral resources, and in particular whether they are pollinating important plants such as flowering crops. Direct field observations have historically been used to study networks, but these are often limited in scale, with sampling restricted to a very small number of communities, species, or individuals (Vamosi *et al*. 2016) because of the prohibitively high amount of effort required.

Instead of directly observing plant-pollinator interactions, indirect connections can be established by pollen analysis (Figure 2). The use of pollen data, whether identified morphologically (e.g. Bosch *et al*. 2009) or molecularly (e.g. Bell *et al*. 2017, Pornon *et al*. 2016, 2017), reveals more associations than do field observations because significant observer biases are avoided. DNA metabarcoding has advantages in efficiency and resolution over microscopic identification of pollen, and therefore it is feasible to study these networks at the large scales required for macro-ecological research.

DNA-based palynology has already helped to reveal previously unknown plant-pollinator interactions (Pornon *et al*. 2017). It has also provided further insights, including the fact that honeybees only use a small proportion of the plant species that they visit (de Vere *et al*. 2017); that the quality and diversity of honeybee-collected pollen is influenced by season rather than landscape structure (Danner *et al*. 2017); and that Diptera are key pollinators that should not be neglected (Galliot *et al*. 2017).

The majority of research on pollen networks to date has focused on honeybees, principally due to the availability of honey and pollen for analysis. However, our own work at the Natural History Museum focuses on hoverflies (Diptera: Syrphidae). These flies are important flower visitors, and we have been exploring their feeding preferences by metabarcoding pollen sampled from their bodies and intestines. Early results appear to support the hypothesis that many hoverfly species require flat, open flowers and inflorescences such as those provided by daisies and wild carrot. Most

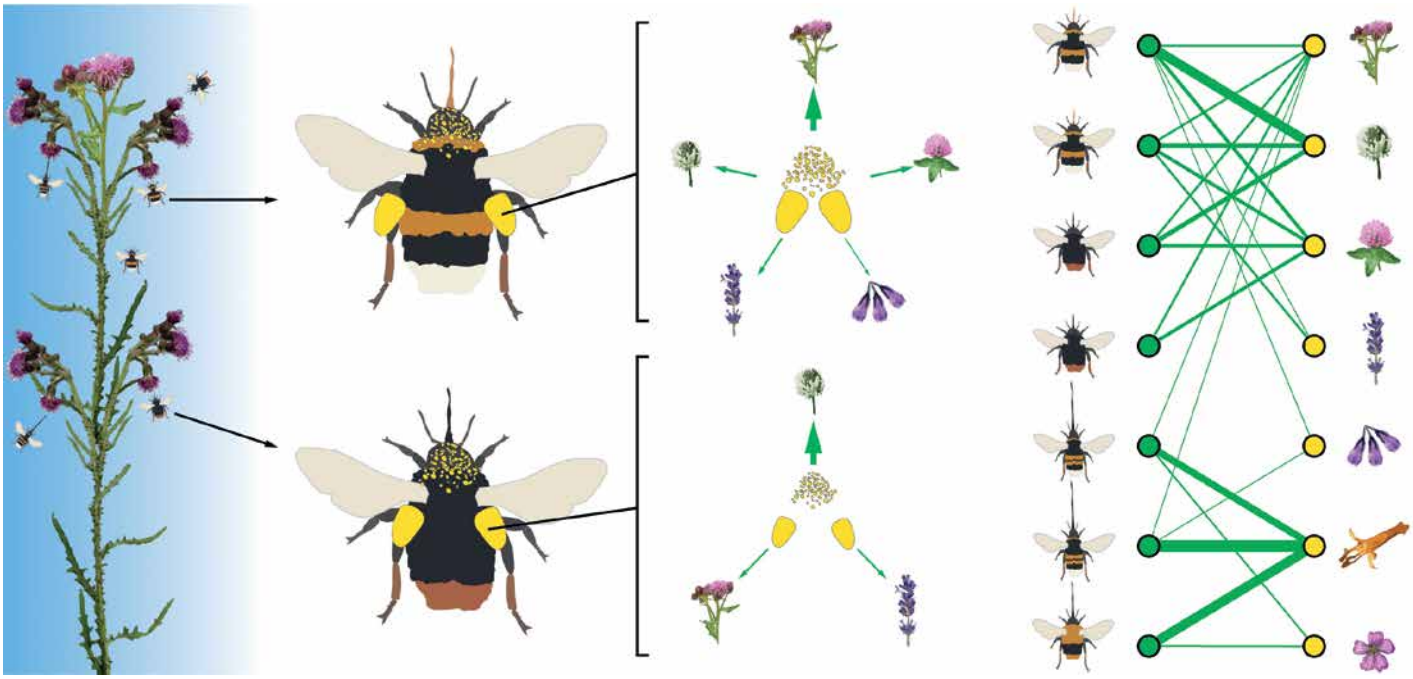

Figure 2. Pollinators collect pollen from the flowers that they visit to provide protein and nutrients for themselves and their young. Different species, in this case two different bumblebee species, both visit the same plant. However, when the species composition of their pollen sacs is analysed we can see that one bee has visited more plant species than the other. By scaling this up the links between all the plants and the individual bees in a network can be seen, with some relying heavily on fewer species and others possessing a large number of links.

hoverfly species have short tongues and so cannot easily access the complicated or tubular flowers that many bumblebees visit. Through our research, we hope to understand how different species of hoverfly use floral resources throughout the year in rural Britain.

Current limitations to DNA-based palynology

Whilst DNA analysis holds many advantages over current morphology-based methods, it is still a developing method that could benefit from further research and development, and remains subject to some limitations. First, although molecular skills are increasingly widespread and more accessible than expertise in morphological palynology, currently there is still a reliance on highly-trained individuals and specialist laboratories to conduct the analysis. Work is already underway to develop technologies that make molecular work simpler to conduct, less prone to human error and more portable, but for the moment these are some way from being operational.

There are also barriers to accurately assessing the quantitative component of pollen species diversity, and a propensity to miss low abundance species. The latter is not unique to DNA methods, but abundance metrics are often considered an important aspect of monitoring, so improving the quantitative capabilities of the approach is a key area for research if the potential of the DNA-based approach is to be maximised.

Conclusion

DNA-based palynology is proving to be a fruitful avenue of research that provides more reliable, sensitive and resolved identifications than conventional morphological methods. Moreover, DNA metabarcoding is not prohibitively slow or costly and so more extensive tests can be performed in a timely, standardised way. While there are limitations to this newly emerging technology, these do not outweigh the high promise afforded by DNA-based palynology. Palynology is an important field with a range of practical applications, including pollinator plant networks, forensic analysis and allergen monitoring, and DNA methods will transform research, monitoring and management in these areas, enabling fast, detailed and reliable decision making.

About the Authors

CT PhD is a senior scientist at NatureMetrics and specialises in DNA-based approaches to characterising biodiversity. He's developed molecular techniques to identify fish communities from water, plants from soil, arthropods from traps, and even bat, bird, and otter diets from their faeces.

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DNA Metabarcoding of Invertebrates to Evaluate Outcomes of Ecological Restoration

Keywords: arthropods, biodiversity, black box thinking, large-scale monitoring, super-indicator, taxonomic impediment

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DNA metabarcoding enables the identification of invertebrate communities at unprecedented scales, effectively overcoming the bottleneck of morphological identification. This unlocks the data potential of highly diverse and responsive groups such as arthropods, which can act as super-indicators of ecological status, facilitating adaptive management during the course of habitat restoration activities in virtually any ecosystem. Ultimately this allows us to learn from failure, improve practice, and obtain better outcomes in biodiversity conservation.

Violet ground beetle *Carabus violaceus*

'Build it, and they will come'

Known as the Field of Dreams hypothesis, an attractive concept in restoration ecology is that once the physical and structural components of a habitat are put in place, the biological elements will inevitably follow. Unfortunately, this does not always hold true (Nilsson *et al*. 2014, Palmer *et al*. 2014), yet it is relied upon to such an extent that only a minority of restoration projects implement robust monitoring programmes to evaluate success (Huddart *et al*. 2016).

Instead, outcomes are frequently assessed on the basis of broad surrogates of biodiversity that are largely structural in nature, with no direct consideration of any aspect of species diversity beyond the classification of vegetation (e.g. Defra 2012).

The lack of monitoring and reporting of restoration outcomes is damaging at two levels. At the project level it reduces the potential for adaptive management, which is likely to be required for a truly successful outcome given the complexity of the natural ecosystems that projects seek to replicate (Cooke and Johnson 2002, Hilderbrand *et al*. 2005). At a higher level, it hampers the development of a conceptual framework for restoration ecology by limiting the ability of the scientific and practitioner community to learn from the successes and failures of past projects (Chapman and Underwood 2000, Lake 2001, Palmer *et al*. 2007, Suding 2011).

Order	Family	Common	Species	Trap 1.1	Trap 1.2	Trap 1.3	Trap 1.4	Trap 1.5	Trap 2.1	Trap 2.2	Trap 2.3	Trap 2.4	Trap 2.5
Coleoptera	Carabidae	Strawberry seed beetle	*Harpalus rufipes*	75,398	56,143	33,546	51,405	17,412	283				
Heterobranchia	Arionidae	European black/red slug	*Arion ater species complex*		186			12,365	54,916	28,284	89,729	47,559	
Heterobranchia	Arionidae	Dusky arion	*Arion subfuscus*		36				58,248	95,460	2,060	5,390	
Polydesmida	Polydesmidae	Millipede species	Polydesmidae species						30,455	9,055	15,395	451	17,522
Coleoptera	Carabidae	Ground beetle species	*Pterostichus melanarius*	1,535	3,523	13,954	1,845	45,491					
Heterobranchia	Agriolimacidae	Grey/Brown field slug	*Deroceras reticulatum/panormitanum*			27	34	22	6,164		3,538	2,699	38,502
Diptera	Tipulidae	Cranefly species	*Tipula paludosa*						1,204			15,175	29,227
Heterobranchia	Arionidae	Hedgehog slug	*Arion intermedius*								1,557	26,505	
Coleoptera	Silphidae	Burying beetle	*Nicrophorus vespillo*	2,406	7,801	2,139	2,110	10,157					
Diptera	Anthomyiidae	Fly species	*Mycophaga testacea*						20,792				
Coleoptera	Carabidae	Ground beetle species	*Pterostichus niger*	69	82	411	21	485					14,853
Coleoptera	Carabidae	Black clock beetle	*Pterostichus madidus*	1,027	3,041						10,617		
Diptera	Muscidae	Muscid fly	*Muscina levida*	27	161	9,297		833					
Diptera	Sarcophagidae	Flesh fly	*Sarcophaga carnaria*	84	90	7,979		674					
Coleoptera	Elateridae	Wireworm click beetle	*Agriotes obscurus*	643	4,646		3,284						
Diptera	Fanniidae	Lesser house fly	*Fannia canicularis*	1,591	3,507		173	2,502					
Araneae	Lycosidae	Wolf spider species	*Alopecosa pulverulenta/cuneata*							7,326			
Isopoda	Philosciidae	Common striped woodlouse	*Philoscia muscorum*	401	1,224								4,978
Heterobranchia	Agriolimacidae	Marsh slug	*Deroceras laeve*							6,507			
Diptera	Muscidae	Muscid fly	*Muscina prolapsa*				138	6,330					
Lepidoptera	Crambidae	Garden grass-veneer	*Chrysoteuchia culmella*						6,247				
Coleoptera	Carabidae	Ground beetle species	*Poecilus cupreus*	1,378	1,970	652	98				2,127		
Eulipotyphla	Soricidae	Pygmy shrew	*Sorex minutus*			5,336							
Coleoptera	Curculionidae	Clover root weevil	*Sitona lepidus*							4,370			
Diptera	Asilidae	Striped slender robberfly	*Leptogaster cylindrica*			4,351							
Polydesmida	Polydesmidae	Millipede species	*Brachydesmus superus*								725	729	2,160
Araneae	Lycosidae	Wolf spider species	*Trochosa ruricola*				1,518			1,988			71
Hymenoptera	Formicidae	Black garden ant	*Lasius niger*					3,149					
Collembola	Isotomidae	Springtail species	*Isotoma viridis*						431		375		2,284

Figure 1. Part of a species-by-site table derived from metabarcoding of pitfall traps. Each of the last seven columns represents a single pitfall trap, and numbers represent the number of sequences identified as each species in each sample.

As the concepts of no net loss, net gain, and biodiversity offsetting gain increasing traction in environmental management, it's vital that we develop the tools to address this issue and ensure that outcomes are properly assessed. Otherwise we risk masking high background rates of biodiversity decline with the appearance of having achieved positive outcomes.

What to monitor?

We need to be able to generate direct biodiversity data across a wide range of taxonomic groups and at large spatial and temporal scales.

That said, we cannot realistically monitor everything, meaning we usually need to choose some representative taxa to serve as indicators of wider biodiversity trends. This is challenging to do well – as is clear from Lake's (2001) summary of desirable indicator attributes: *'they must have no taxonomic difficulties or measuring uncertainties; they need to be sensitive to the restoration measures; they need to respond at different rates over different time spans; and preferably they need to be linked with each other in their ecological functioning'*. In practice, indicator attributes are rarely considered beyond ease of measurement. Most indicator-based assessments measure a narrow group of organisms, with the implicit – and invalid (Lindenmayer *et al*. 2002) – assumption that other groups will follow the same trends.

Thus, the responses of most components of biodiversity usually remain unknown. Importantly, this includes groups such as terrestrial invertebrates and soil biota, which are likely to be of functional importance in the ecosystem and to influence its long-term viability.

Here, we advocate using arthropods as a super-indicator of habitat type and condition. Arthropods are abundant, diverse and can be captured in large numbers from virtually any terrestrial or aquatic ecosystem. They perform many ecological functions, exhibit a wide range of tolerances, and have relatively short generation spans, meaning that they respond quickly to environmental changes. Species turnover along ecological gradients is usually significant, and invertebrate communities are already used to infer ecological condition of aquatic ecosystems. Moreover, the functions and ecology of arthropod species are generally quite well known, which lends additional insight to analysis (e.g. Heaver *et al*. 2017). In short, arthropods meet Lake's (2001) criteria in most respects.

Of course, the problem is identification. There are over 26,000 species of British insects (Buglife 2017), and keying out to species often involves microscopic examination of characters such as distribution of bristles, wing-vane patterns, or genital morphology (e.g. Langton and Pinder 2007). The expertise needed to identify invertebrate specimens trapped in large-scale monitoring programmes is simply not available. Even if it were, conventional taxonomy would be too slow to provide useful data within the timeframe required for decision-making, and the labour involved would be prohibitively expensive.

DNA metabarcoding

DNA metabarcoding is a powerful tool that enables the taxonomic bottleneck to be overcome, so that large and diverse collections of invertebrates can be transformed into useable ecological data within a useful timeframe, yielding large datasets with high statistical power.

The approach uses high-throughput sequencing technology to sequence the barcode genes of many different organisms in parallel, revealing the species composition of complex samples such as those gathered in insect traps (see Box 1 for details of the key steps). Hundreds of samples can be analysed in just a few weeks, and based on the current completeness of reference databases, around 70-80% of terrestrial macroinvertebrates can be identified to species level, with the remainder

assigned to higher taxonomic levels. The key output from metabarcoding is a species-by-sample table that can be used as input for any conventional community analysis (Figure 1).

Our research has shown that the ecological information returned by metabarcoding is at least as good as that derived from gold standard morphological taxonomy, with great advantages in terms of both time and cost (Ji *et al*. 2013), especially when there are large numbers of samples to be processed.

Design & interpretation

Ideally, monitoring in a restoration project (or indeed any management intervention) would follow an extended Before-After-Control-Impact (BACI) design. This means replicated monitoring of arthropod communities before, during and after intervention in:

1. The **restoration** area where modifications are to be applied.
2. A **control** habitat that is equivalent to the starting condition of the restoration area, but with no management intervention applied.
3. A **target** habitat, which should represent a good quality example of the kind of habitat that the restoration hopes to achieve, located as near as possible to the restoration area.

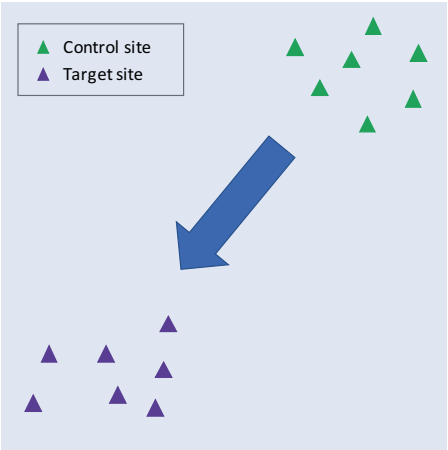

Figure 2. Schematic ordination plot showing the similarity of communities sampled in control and target habitats. The arrow indicates the expected direction of movement of the focal communities, which become more like the target communities over time. See text for further explanation.

Box 1. Key steps in arthropod metabarcoding

1. **Arthropods are collected using conventional trapping approaches**, with some simple modifications to ensure DNA is well preserved (See Box 2). We have worked with samples from pitfall traps, Malaise traps, Winkler traps, vane traps, kick nets, light traps, Surber samplers, sediment cores and others.
2. **Each sample of organisms is ground up** together into a soup-like homogenate. The decisions you take here determine the resolution of the data you obtain and the overall cost of the project. For high-resolution data with maximal statistical power, you should take as many sampling replicates as possible and process each as a separate sample throughout the metabarcoding process. Conversely, to minimise costs you can combine sampling replicates by location or trap type and process them as fewer, larger metabarcoding samples.
3. **Total community DNA is extracted** from the homogenate, and a subsample of this DNA can be archived in frozen storage for future analysis if desired. This could be useful if, for instance, the metabarcoding results suggest the presence of a particularly interesting species with management or conservation implications, in which case you could return to the archived DNA and perform additional species-specific analyses to confirm the detection.
4. **Community DNA is amplified in a process known as Polymerase Chain Reaction (PCR)**. This creates millions of copies of a particular 'barcode' gene across all the target taxa in your sample. Central to this process are primers, which are short fragments of synthetic DNA that bind to either end of the target region of DNA, allowing it to be copied. Primers can be designed to be very general (e.g. to amplify all animal taxa), very specific (e.g. to amplify just a single species even from a mixed sample), or of intermediate specificity (e.g. to amplify just one group of animals). For arthropods, we use very general primers that target the standard animal barcode gene, known as Cytochrome Oxidase 1, or CO1.
5. **Amplified DNA is sequenced on a high-throughput sequencer**, which can sequence the DNA of many different organisms in parallel. The sequencer is relatively expensive to run (around £2000 per run), but tens or hundreds of samples can be processed together on a single run, and thus share the cost. Each run returns around 20 million DNA sequences derived from the organisms whose DNA was amplified in the previous step.
6. **Raw sequence data is processed using a bioinformatics pipeline** to transform it into a recognisable ecological dataset. The pipeline consists of a series of computer programmes that the data passes through sequentially. The key steps are quality filtering, clustering of highly similar DNA sequences, and assignment of taxonomy using a reference library of sequences. The output of this pipeline is a species-by-sample table giving the number of sequences obtained for each species in each sample. This table can be used for familiar community analyses, including ordination and calculation of diversity indices. Data should be interpreted primarily in terms of presence-absence rather than abundance, since the link between the biomass of the species and the number of sequences returned can be affected by the use of very general primers.

Let's assume we've followed the BACI design described above, with several sample replicates collected at each site (restoration, control, target). The samples have been processed following the metabarcoding workflow described in Box 1, and a presence-absence dataset of arthropods has been generated.

We expect that the arthropod communities in the control and target habitats will differ substantially from one another and that this difference will be maintained for the duration of the project. The community in the restoration area should resemble the control community at the outset but become more like the target community over time as a result of restoration activities.

Ordination plots based on methods such as non-metric multidimensional scaling (NMDS) or principal components analysis (PCA) are very useful for visualising this. These plots arrange samples in an abstract

space based on pairwise similarity of their species lists. Thus, samples with many species in common will lie close together in the plot, while those with few species in common will be positioned far apart. If multiple samples are analysed in each of two different habitats, we expect samples from the same habitat to cluster together (as shown in Figure 2), since species composition will be more similar within habitat than between habitats.

We can think of the area where the samples of a particular habitat cluster in the ordination space as the 'biodiversity fingerprint' of the habitat. In reality, this captures the set of species that are characteristic of that environment. What we are trying to achieve through management is to move the biodiversity fingerprint of the restoration area in the direction of the target fingerprint, until they overlap completely and there is no longer any statistical difference between the communities (assessed using permutation tests, multivariate generalised linear models, or similar).

Box 2. Arthropod metabarcoding FAQs

What collecting fluid should be used to preserve DNA?

The most important thing to remember is that formalin should never be used on samples that are intended for DNA analysis. Denatured alcohols and ethylene glycol are poor preservatives in the medium and long term. Ethanol is the ideal preservative but not always practical or possible to use. Other salt- and detergent-based preservatives can usually be supplied by laboratories, and propylene glycol is an effective and accessible alternative.

Does the approach return counts as well as presence-absence data?

The analysis returns the number of sequences identified as each species on a per-sample basis. Although it is tempting to interpret this as a proxy for abundance or biomass, it's important to bear in mind that unavoidable biases introduced during the laboratory process can have a major effect on this relationship. This is primarily due to the fact that some species amplify more readily than others during the PCR step. The most conservative approach is to analyse the data in terms of presence-absence. Due to the number of taxa included in the analysis, presence-absence data still has high statistical power for demonstrating change. If abundance data for particular species is important to the project, then incidence across multiple samples can be used as a proxy for abundance (e.g. a species detected in every sample replicate is likely to be more abundant than one found only in a small proportion of replicates). Alternatively, this may be a scenario in which conventional methods are the best.

What if the reference database is incomplete?

Currently about 80% of terrestrial arthropods in the UK can be identified to species level based on public reference databases. For species that are not in the reference database, identifications are returned at higher taxonomic levels, such as genus or family. These are still presented in the dataset as species-level entities and they will be assigned an identifier so that they can be tracked across different samples. In this way they can be thought of as similar to morphospecies. It is always an option to add to reference databases throughout the monitoring programme by morphologically identifying a subset of samples and sequencing the identified individuals to generate new barcodes where there are gaps. New taxonomic information can be added retrospectively to existing metabarcoding datasets by re-running the last step of the bioinformatics pipeline with the latest reference library, so an incomplete reference database should not be an obstacle to beginning a monitoring programme using metabarcoding.

Does this put taxonomists and entomologists out of a job?

Absolutely not. The best projects will combine the large-scale capabilities of metabarcoding with more detailed morphological analysis of a subset of samples. This allows validation of the metabarcoding output and improvement of local reference databases, as well as acquisition of abundance and trait data.

Can we use eDNA from water to obtain data on invertebrate communities?

This is proving very difficult for a variety of reasons, mostly associated with the taxonomic breadth of the group, which makes it difficult to target macroinvertebrates while avoiding zooplankton and microorganisms. These tend to dominate the data and overwhelm the macroinvertebrate eDNA.

Adaptive management

One of the principal advantages of a comprehensive, on-going monitoring programme is that it facilitates adaptive management. This is the process by which the effects of an intervention are monitored to assess how well they are working, and the management plan is adapted accordingly to ensure the best possible outcome. Figure 3 illustrates a scenario in which one group of organisms (flying insects) has initially responded well to the restoration effort, while another group (ground arthropods) has not.

Recognising during the project cycle that the management plan is not succeeding for ground arthropods allows ecologists to examine the reasons and try to address them. Perhaps the ground habitat is not yet appropriate for the species that characterise the target habitat, or maybe a connectivity issue is preventing these species from colonising the new habitat. Quite possibly some simple adjustments to the management of the site could improve its suitability for this group and deliver a far better overall outcome in terms of the functioning and diversity of the new habitat.

While metabarcoding won't be the ideal approach in all scenarios – for instance where precise abundance data is needed – it allows ecologists to gather and use data on arthropod communities even when they don't have access to expert taxonomists. More generally, it enables direct biodiversity data to be obtained on unprecedented scales in virtually any habitat. This allows us to move towards adoption of a 'black box thinking' approach (Syed 2016), whereby high resolution data is gathered during the course of operations, and used to help understand our failures, adapt our practices, and continually improve our chances of success in managing the natural environment.

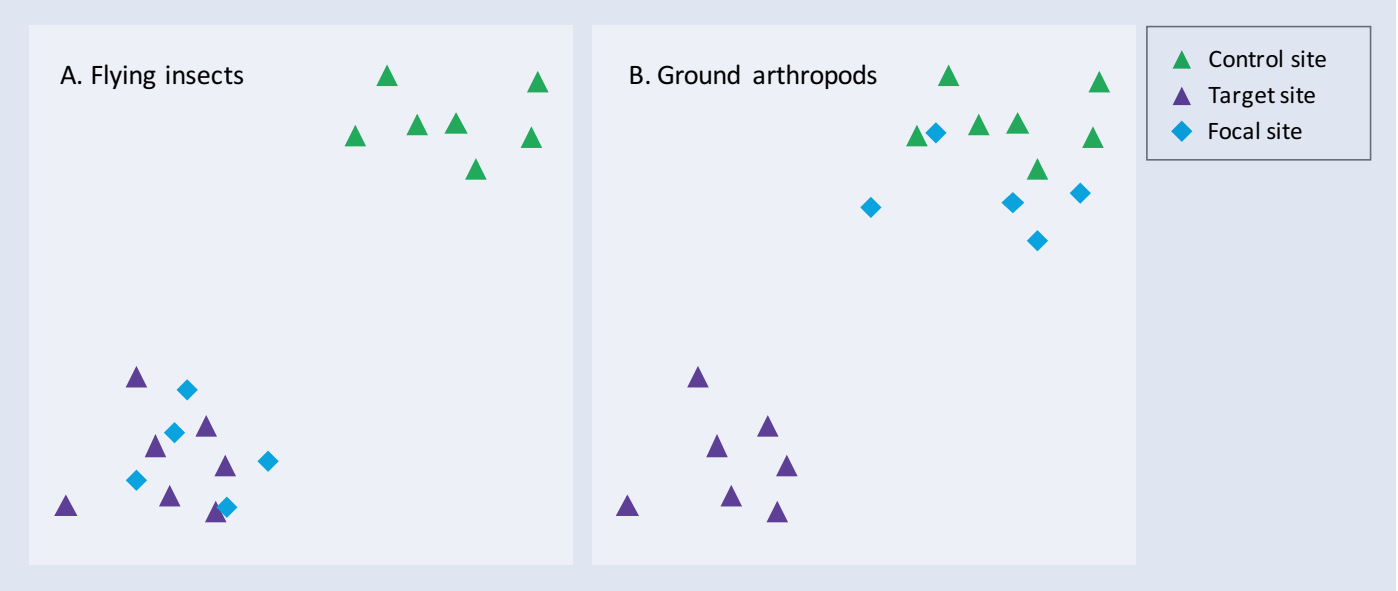

Figure 3. Schematic ordination plots showing communities of (A) flying insects and (B) ground arthropods in Control, Target and Restoration (Focal) sites at a single time point. See text for further explanation.

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About the Author

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Finding Newts – Using Environmental DNA to Detect Great Crested Newts

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Keywords: citizen science, great crested newt, monitoring, ponds

Environmental DNA is a new and potentially revolutionary method for detecting plants and animals in freshwater. In Britain, the use of eDNA for surveying great crested newts has moved quickly from early testing through to practical applications that support the conservation of the species.

The method compares favourably with traditional survey methods and is easy to use. At the same time, current research is further refining the method, including the best times to collect eDNA samples, persistence of eDNA in the water and the use of occupancy modelling techniques to account for false negatives. As a result of effective collaboration between statutory, NGO and research organisations, England now has the world's first national monitoring programme for a protected species using eDNA data collected by citizen scientists.

Introduction

Environmental DNA (eDNA) is changing the way ecologists think about monitoring the aquatic environment, allowing us to identify animals and plants from a simple water sample. First applied by French researchers in the mid-2000s as a technique for detecting the occurrence of non-native bullfrogs *Rana catesbeiana* (Ficetola *et al*. 2008), the technique offers the tantalising possibility of characterising whole communities in ways previously unimaginable.

Detecting great crested newts reliably using the traditional methods of torch counts, bottle trapping and egg searching

Sampling for great crested newt eDNA in a Leicestershire field-drain interceptor pond using the Spygen test kit. The waterbody was created in 2013 to trap polluted sediment and water. No eDNA was detected in this pond which is 1.5 km from the nearest known great crested newt site.

– especially establishing with reasonable confidence that animals are *absent* from a pond – is a time-consuming activity. This has held back the development of national monitoring programmes for the species, despite substantial volunteer monitoring effort by Amphibian and Reptile Conservation Trust (Wilkinson and Arnell 2013). Great crested newts are widespread, found from Devon to the Highlands of Scotland and from mid-Wales to east Kent, but they occur at low density – about 1 in 10 ponds overall. Therefore, monitoring programmes based on stratified random samples of representative sites must include hundreds of locations to reliably detect trends in populations (Biggs *et al*. 2014a). To date, it has proved impossible to resource such surveys using traditional methods – not least because it is difficult to persuade unpaid volunteers to visit lots of sites where there are no newts. eDNA offers a way forward because surveys are relatively quick, large numbers of sites can be sampled and the resulting datasets are big enough to detect trends in the newts' occupancy of ponds or 1 km grid squares.

The development of eDNA for great crested newts from research idea to practical technique has been surprisingly fast. In 2012, researchers in Denmark published the first proof of concept that the DNA of great crested newts and other protected species (e.g. tadpole shrimp *Lepidurus apus*, large white-faced darter dragonfly *Leucorrhinia pectoralis*, weather loach *Misgurnis fossilis*, otter *Lutra lutra*) could be detected in the water (Thomsen *et al*. 2012). Around the same time, Freshwater Habitats Trust (with Defra and Natural England funding) was developing the PondNet project to provide a new, national, citizen-based monitoring programme for ponds and pond-associated protected species, including great crested newts. At Natural England, Pete Brotherton suggested the new eDNA technique should be trialled and by the end of 2012 a pilot project had been set up as part of PondNet, working with the French eDNA pioneers Spygen.

Applying the eDNA technique to great crested newts

The pilot project tested three critical aspects of the eDNA technique for

Instant breeding habitat: this new pond created in Cheshire during the Million Ponds Project was almost immediately colonised by great crested newts. Well grown larvae were found within the first year, even though the pond was completely bare of vegetation. The ability to create new habitats quickly for great crested newts is an important part of new approaches to their conservation.

detecting great crested newts: (i) How does eDNA compare to torching, bottle trapping and egg-searching? (ii) Can we be sure that eDNA doesn't pick up false positives – recording great crested newts where there are none? (iii) Can the method be used by volunteer surveyors (Biggs *et al*. 2015)?

(i) *How does eDNA analysis compare to traditional survey methods?*

Thirty-five ponds known to support great crested newts were surveyed in two areas from mid-April to late June with 2-3 week intervals between each of four survey visits, giving a total of 140 site visits (Biggs *et al*. 2014a). In south Hampshire, England, the work was run by Hampshire Ecological Services Ltd, and near Mold in north-east Wales by a combined professional and volunteer team led by Natural Resources Wales. Both teams compared the traditional 4-visit standard survey method with the eDNA technique for detecting the presence or likely absence of the newts. At each survey visit, torch counts, bottle trapping and egg searches were undertaken and an eDNA water sample was collected from the pond. Great crested newts were detected by torch counting on 75% of survey visits, by bottle trapping on 76% of visits, by egg searching on 44% of visits and from eDNA on 99.3% of visits (Figure 1). Combined, torch counting and bottle trapping detected newts on 95% of survey visits (133 out of 140), close to, but still significantly less than the eDNA

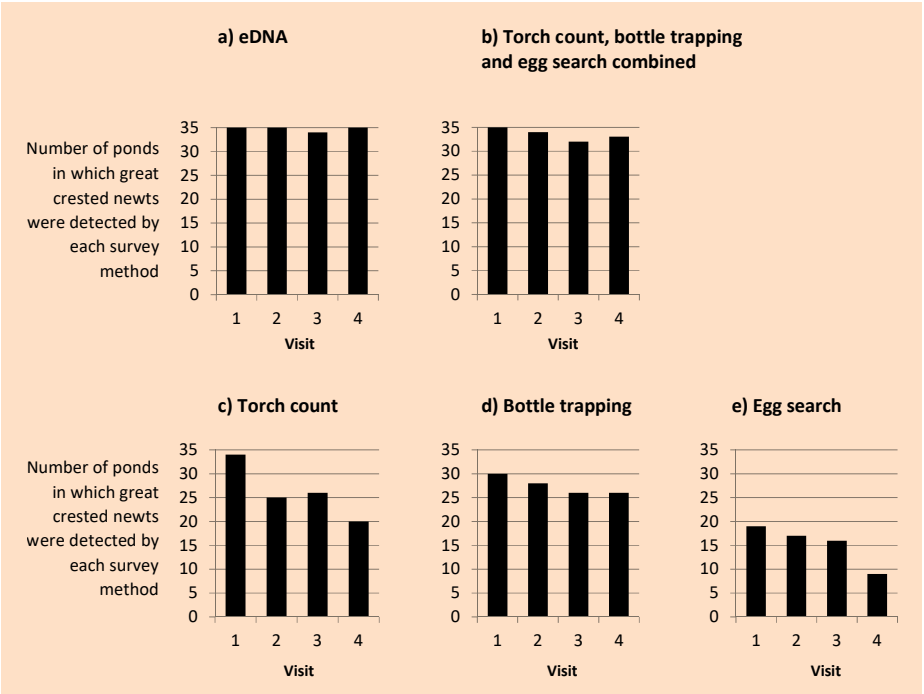

Figure 1. Comparison of the effectiveness of eDNA and traditional great crested newt survey methods. The proportion of ponds (n=35) known to support great crested newts in which the species was detected by: (a) eDNA survey, (b) torch counting, bottle trapping and egg searching combined, (c) torch counting alone, (d) bottle trapping alone, and (e) egg searching alone. The difference between the results shown in graphs (a) and (b) is statistically significant (McNemar's test; $p<0.05$). See text for further details.

method which recorded newts on 139 out of 140 survey visits (McNemar's test, $p < 0.05$). Egg-searching did not contribute extra detections beyond those obtained for torch counting and trapping. These results demonstrated that a single eDNA survey visit was sufficient to detect great crested newts during the breeding season, even though populations were mostly in the small or medium size categories, as defined by English Nature (2001)

(ii) *Does eDNA analysis pick up false positives?*

The project team tested for false positives by collecting water samples from 33 ponds outside the known great crested newt range, three in the Orkney Islands substantially beyond the known range, and 30 in Cornwall, just outside the known range in south-west England. A single water sample was collected using the standard Spygen test kit, all results being negative. A further 30 ponds in southern England with good evidence that newts were absent, but well within the range of the species, were then tested. All these ponds also tested negative for great crested newt eDNA suggesting that false positives were not a substantial problem.

(iii) *Can the method be used by volunteer surveyors?*

We assessed the use of the technique by volunteers who surveyed 239 ponds across England, Wales and Scotland, encompassing a large part of the newt's range (Figure 2). There was recent evidence of great crested newts at all the ponds, based on observations made in the 2013 breeding season. The ponds were concentrated in three contrasting pilot areas within the PondNet project (Hampshire, Cheshire and north-east Yorkshire, n= 40, 31, 27 ponds) with further ponds distributed opportunistically across other parts of England (n= 63) and in Scotland and Wales (n=39, 39). The objective was to expose the eDNA test to a wide range of physical, chemical and climatic conditions, knowing that previous studies had shown inhibition of eDNA detection by environmental factors such as pH and the presence of tannins in the water.

Surveyors were provided with written information on the eDNA survey technique so guidance was comparatively limited. In addition to collecting water samples for eDNA analysis, volunteers recorded the metrics of the Great Crested Newt Habitat Suitability Index, which provides a simple description of the physical characteristics of the ponds and the surrounding landscape, a rough assessment of water quality and observations on the likely occurrence of fish (ARG-UK 2010).

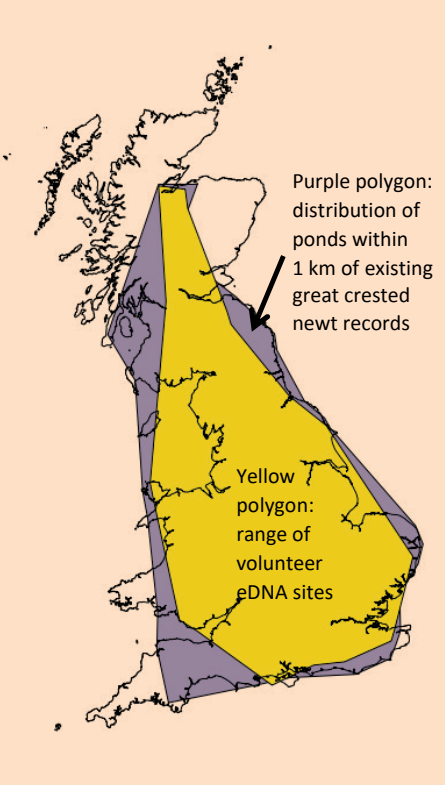

Figure 2. The approximate range of the great crested newt in Great Britain (purple polygon) and the proportion of this range surveyed by volunteers sampling ponds for eDNA (yellow polygon).

In practice, there was little to suggest that environmental factors markedly interfered with eDNA detection because 91.2% of the ponds (218 out of 239) came back as positive. There were no significant correlations between eDNA and pond shade, pond area, pond permanence, abundance of aquatic vegetation, water quality, presence of waterfowl, surrounding terrestrial habitat quality, number of adjacent ponds or pond altitude. At the 21 ponds (8.8%) where eDNA was not detected, there was good reason to think that methodological errors accounted for about half of the apparent false negatives, with the remaining 4-5% being real false negatives where newts were present but the eDNA sample failed to detect them.

False negatives

Three potential causes of false negatives seemed likely: (i) low numbers of newts producing too little eDNA for it to be detected, (ii) wide and shallow pond edges preventing access to the main areas where newts were likely to be active (surveyors having been instructed not to enter the water to avoid cross-contamination and disturbance of sediments), and (iii) surveyors not collecting the 20 water samples from evenly spaced points around the pond (as the method recommended) due to dense scrub or steep banks restricting access to the whole pond margin.

(i) About a quarter of the false negatives were locations where there appeared to be small populations of newts. For example, at the Blackmuir Wood pond in Scotland one local surveyor had checked twice earlier in the year before the sample was collected and had seen no adult great crested newts or eggs and the pond was known to have a diminishing newt population, possibly due to recent fish introductions. Despite this, later in the year, after the negative eDNA test result, a great crested newt larva was photographed, proving presence. Subsequently the surveyor commented: *'The main pond currently has a low water table and maybe where water samples were collected around the pond edge there weren't any great crested newts'*.

(ii) In several survey locations, very shallow water over a wide drawdown zone and/or margins dominated by dense vegetation, reduced the volunteer's ability to collect a water sample from the areas most likely to be used by newts, either because these areas were less favoured by newts, or because the dense vegetation prevented mixing of the water. At Madeley, in Cheshire, we tested this scenario by sampling amongst a dense, marginal, floating mat of grasses over a shallow drawdown zone and compared this to the pond's centre which had deeper water on the edge of the marginal vegetation, reached by attaching the sampler to a long pole. eDNA was not found amongst the floating mat but was detected in the open water.

(iii) There was clear support for sampling right round the pond, as specified in the survey method. For example, at Bowldish in Dorset, where a false negative result was

obtained, the volunteer commented that *'…I was only able to sample a small section in one area as the pond was surrounded by blackthorn bushes growing right down to the edge…'*. Similarly in Scotland, at Dunmore Swamp pond there were access difficulties due to thick vegetation and steep banks, limiting the sampling area to about 10% of the pond perimeter. No eDNA was detected at this pond although newts were known to be present.

Where we are with eDNA and great crested newts at present

Overall, the results of the pilot in 2013 were remarkably successful in demonstrating the value of the eDNA method of detecting great crested newts. In 2014, Natural England rolled out the use of the eDNA test for ecological consultants undertaking great crested newt surveys, with English laboratories offering the Spygen protocol specified in Biggs *et al*. (2014b). Since 2015, with funding from the Heritage Lottery Fund (HLF), Defra and Natural England, Freshwater Habitats Trust has run the first national, volunteer-based great crested newt survey using eDNA as part of the PondNet project. Most recently, Natural England and others have used the method as part of the new District Level Licensing project.

Throughout, the development of the new technique has been a good example of collaboration and partnership. The success is down to the existence of monitoring programmes organised by specialist NGOs with extensive experience of the habitat and the species, expert geneticists, hundreds of volunteers willing to give their time to the project and, of course, government and statutory organisations with the willingness to take a calculated risk with an initially expensive research programme.

Practical limitations

Although the use of eDNA to detect newts has been a game-changer, the method has limitations. At present, it is not possible to routinely describe the abundance of great crested newts with eDNA, although research by the Durrell Institute of Conservation Ecology is addressing this issue (Buxton *et al*. 2017a). Like all methods, the eDNA technique generates false negatives. The reasons will be systematically investigated in due course but, as noted above, the Defra pilot study indicated that false negatives were a combination of methodological errors (due to surveyor inexperience) and 'real' false negatives where the technique was correctly applied but failed to detect newt eDNA. Some false negatives will always occur but the occupancy modelling techniques currently being developed will help to address this limitation (Buxton *et al*. 2017b). Other challenges include the importance of avoiding contamination of eDNA samples by sediments containing great crested newt eDNA (Biggs *et al*. 2104b, Buxton *et al*. 2018) and preventing importation of eDNA to ponds not currently being used by newts. More recently, Buxton *et al*. (2018) have shown variation in great crested newt eDNA detection according to soil type and pond HSI (Habitat Suitability Index) scores, which merits further investigation.

This pond, which is in the suburbs of Oxford and dries out in the summer, at first sight might look unsuitable for great crested newts. In fact, it has three important features needed by the species: clean water, no fish predators and good surrounding scrubby woodland habitat. In the spring of 2017, at the time of the year when the pond holds water, eDNA surveys detected the species, confirming the results of 'traditional' observations of small numbers of newts in previous years.

Our experience of surveying great crested newts using eDNA highlights the importance of following the recommended method. In particular, surveyors must ensure that they sample right around the pond (inexperienced volunteers may be put off by dense scrub that professional surveyors can get through!); use a bamboo cane (one for each site) to reach beyond very shallow water that is hard to sample without disturbing sediments and is probably less attractive for newts; sample all areas where newts are likely to be active; and aim for the peak of the breeding season, within the originally recommended sampling period (Biggs *et al*. 2014b).

The potential of eDNA survey methods

As a result of the work described here, England is running the world's first national monitoring programme for a protected species using eDNA. More generally, the potential of the technique for a huge range of applications is being recognised around the world. The use of eDNA is likely to be particularly valuable in citizen science projects because it can help overcome the 'taxonomy impediment' – the difficulty many inexperienced volunteers face when it comes to learning how to identify cryptic or hard to recognise species. Added to this, the method is very quick to use compared to traditional surveys, and surveyors don't need to obtain a licence to survey protected species. In the case of the great crested newt, the new method has been central to the success of the HLF-funded *People, Ponds and Water* project run by Freshwater Habitats Trust. This includes the first three years of the national great crested newt monitoring programme, with additional support from Natural England and Defra who funded the test kits. More information about the results of this work is available on the Freshwater Habitats Trust website (https://freshwaterhabitats.org.uk/projects/pondnet/pondnet-results-2015-2016-2017/).

Freshwater Habitats Trust is currently planning a new national monitoring programme for freshwaters, especially the smaller waterbodies which are rarely or never monitored in existing statutory programmes, and including a national survey using eDNA survey methods alongside other approaches. We plan to test the use of eDNA to record fish, a range of uncommon invertebrates, amphibians other than great crested newts and water plants. Further work is needed for most of these groups to compare the effectiveness of eDNA detection and routinely used, traditional survey methods. Ultimately, our broader goal is to make use of metabarcoding techniques to describe whole aquatic communities. If you are interested in joining this network as a partner we would be pleased to hear from you.

Acknowledgements

Many people worked together to establish eDNA as a viable survey technique for great crested newts, from a range of backgrounds and organisations. We especially thank the volunteers who collected samples or undertook surveys, including members of the Amphibian and Reptile Groups of the UK, the National Amphibian and Reptile Recording Scheme and the PondNet project. The work was commissioned and funded by Natural England and Defra, who supported the work with enthusiasm and commitment throughout.

About the Author

Jeremy Biggs is a co-founder and Director of the Freshwater Habitats Trust, formerly Pond Conservation. Freshwater Habitats Trust works to protect the freshwater biodiversity of the UK through practical projects, research and policy work and, with many partner organisations, is involved in a wide range of practical species and habitat protection projects. Jeremy has an extensive knowledge of the ecology of freshwater ecosystems, especially ponds, and a particular interest in work to protect small waters. He led the project to test the applicability of eDNA to monitor great crested newts in 2013 and is currently closely involved in the national eDNA monitoring programme for great crested newts and in the development of the South Midlands great crested newt District Licensing pilot project.

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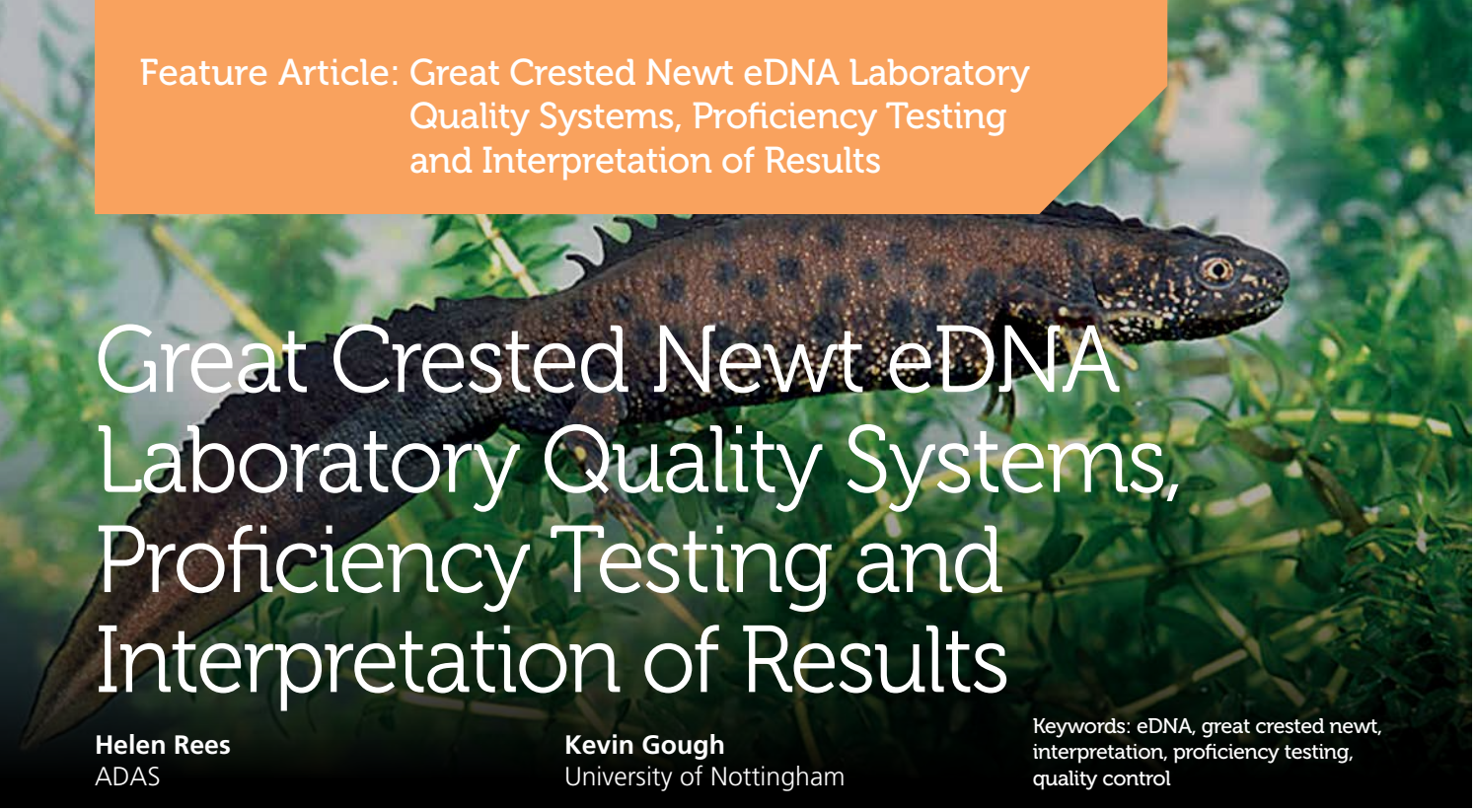

Great Crested Newt eDNA Laboratory Quality Systems, Proficiency Testing and Interpretation of Results

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ADAS

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Keywords: eDNA, great crested newt, interpretation, proficiency testing, quality control

Surveying for great crested newts *Triturus cristatus* using environmental DNA (eDNA) has been carried out in the UK for the past four newt survey seasons. When correctly applied, eDNA testing can be very effective but the methodology is precise and understanding the potential limitations of the technique is important. In order for ecologists to have confidence in the technique, laboratories offering analysis of eDNA must operate to best practice standards and should employ quality control systems at all stages of analysis.

This article is written for ecologists who are already familiar with using eDNA for great crested newt (GCN) presence/absence testing with the aim of enhancing their understanding of how the laboratories offering this service achieve best practice standards. In turn, this will increase ecologists' understanding of the potential limitations of the technique thus helping to achieve more accurate results.

Best practice and quality control systems

In 2014, the results were published from a Defra study: *'Analytical and methodological development for improved surveillance of the Great Crested Newt and other pond vertebrates – WC1067'* (Biggs *et al*. 2014a). As part of this study, an appendix was produced *'Appendix 5. Technical advice note for field and laboratory sampling of great crested newt* (Triturus cristatus) *environmental DNA'*, known as the technical advice note (Biggs *et al*. 2014b), which sets out the required standards, field and laboratory procedures, and provides the basis for consistency and standardisation when performing eDNA analysis for great crested newts. The eDNA survey and analysis methodology in this document was sanctioned by Natural England (NE) in April 2014 for great crested newt presence/absence determination (Natural England 2014).

Laboratories offering eDNA analysis are required to follow this approved methodology and should be able to demonstrate that quality control systems have been put in place. This includes appropriate documentation for sample collection and analysis, and expected outcomes in terms of limits of detection; identification and mitigation of the risks of false positives and false negatives; and, as of 2017, proficiency testing.

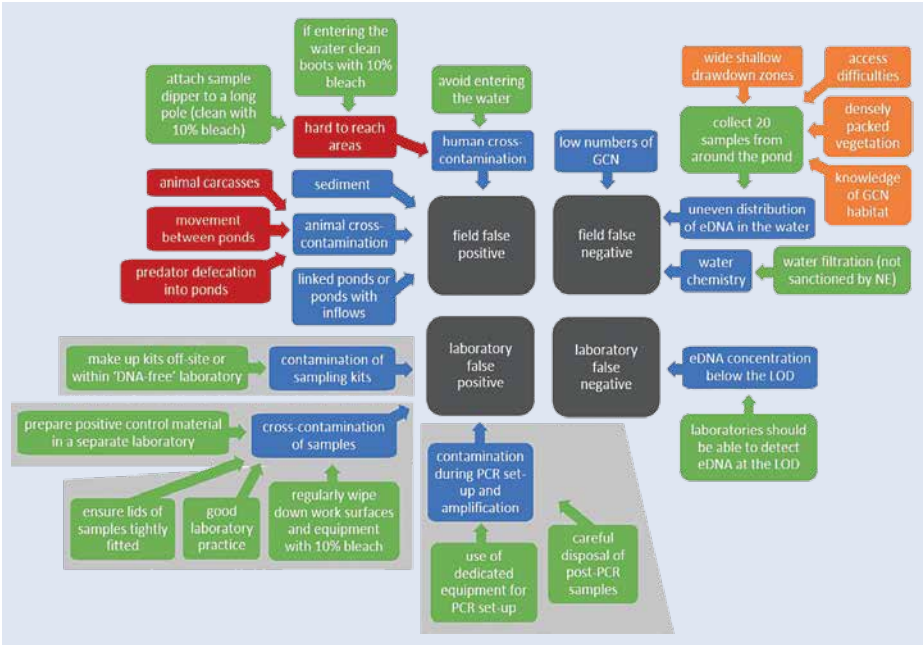

Figure 1. Summary of the different types of error (dark grey boxes), their causes (blue boxes), and mitigation strategies (green boxes). Also shown are factors that generate false errors (red boxes) or that can help or hinder sample collection (orange). The light grey areas represent a physical separation of activities, i.e. separate laboratories and separate staff in each laboratory for kit assembly, eDNA extraction, and PCR amplification. LOD = Limit of detection.

Table 1. Some potential errors and suggested mitigation procedures during great crested newt eDNA analysis.

Potential Error	Mitigation
Incorrect sample collection (a field error)	Licensed ecologists should collect samples according to sanctioned methodology and should identify likely great crested newt location/habitat based on expert knowledge.
Use of faulty equipment	Regular servicing/calibration is needed to ensure reliability/accuracy.
Use of substandard or expired reagents	Use reagent by the expiry date. Record batch numbers in case of manufacturing issues.
Incorrect reagent storage	Store according to the manufacturer's instructions. Temperatures should be monitored to ensure they are within set limits (4°C ± 2 for fridges, and -18°C or lower for freezers).
Non-adherence to standard operating procedures	Staff involved should be suitably trained and identified on sample records.
Inaccurate recording and reporting	Records of sample acceptance should be taken, e.g. include a photograph of the samples in case of any subsequent dispute over sample labelling and to serve as a visual verification of volume and water quality. Data/reports should be double checked for errors.

Laboratory standards and specifications

Establishing and maintaining quality standards is essential for the efficient and effective operation of a diagnostics laboratory. This is important for ensuring the quality and traceability of results and compliance with standard techniques and reagents. All laboratory activities associated with eDNA analysis are subject to errors if quality control is inadequate (see Table 1). The potential for errors can be mitigated by adopting appropriate quality assurance standards via a documented quality management system which follows or shows equivalence to the ISO/IEC 17025 Standard *General requirements for the competence of testing and calibration laboratories* (International Organization for Standardisation [ISO] 2017).

False errors

The technical advice note (Biggs *et al.* 2014b) was designed to limit the risk of false positives and false negatives but there are occasions when an eDNA result will contradict what is known about a pond from other surveys. The eDNA result would not necessarily be incorrect, but false errors are possible and can occur both in the field during sample collection and in the laboratory during sample processing and/or analysis. There are four types of false error which should be considered: field false positives and negatives, and laboratory false positives and negatives (Figure 1). Laboratories should regularly run 'blank' plates (sterile water rather than DNA) to monitor for contamination, either great crested newt eDNA from previous extractions or from polymerase chain reaction (PCR) amplification, a potential cause of false positives. In addition, running field samples known to be positive for great crested newts and also known to be negative/outside their known range will increase confidence in the results and identify potential cross-contamination issues.

Proficiency testing

In 2017, Natural England facilitated the introduction of a proficiency testing scheme for all laboratories providing an eDNA testing service for the detection of great crested newts. Henceforth, Natural England will only accept eDNA results from laboratories that have taken part in the scheme (Natural England 2017) when these data are submitted to support great crested newt licence applications and post-development presence/absence monitoring for mitigation licences.

Proficiency testing is used to provide an independent assessment of competence and is an essential part of quality assurance. The first time a proficiency test is conducted a 'baseline' of laboratory performance can be established. Repetition of the proficiency test, for example on an annual basis, will allow the industry to monitor performance over time. The aim of the great crested newt eDNA proficiency test was to provide evidence of the robustness of the technique and to ensure that laboratories offering this analysis provide a good quality service.

Table 2. Anonymised proficiency testing results for all laboratories taking part in the 2017 scheme facilitated by Natural England. I = Inhibition detected and ND = Inhibition not detected, i.e. whether the sample contains components that inhibit the PCR reactions. Specific conditions tested were: high (A), medium (C), or low (E) concentrations of great crested newt (GCN) eDNA within the sample; blanks (F and G) with no great crested newt eDNA present; and blanks plus inhibitors (B and D) with no great crested newt eDNA present but the sample contained an inhibitory reagent which would prevent the PCR amplification from proceeding properly.

Laboratory Identifier*	A (high GCN eDNA)	B (blank plus inhibitor)	C (medium GCN eDNA)	D (blank plus inhibitor)	E (low GCN eDNA)	F (blank)	G (blank)
001	7/12	0/12 (I)	3/12	0/12 (ND)	0/12	0/12	0/12
002	12/12	0/12 (I)	11/12	0/12 (I)	2/12	0/12	0/12
003	3/12	0/12 (I)	5/12	0/12 (I)	1/12	0/12	0/12
004	10/12	0/12 (I)	11/12	0/12 (I)	1/12	0/12	0/12
005	0/12	0/12 (I)	11/12	0/12 (I)	9/12	7/12	0/12
006	1/12	0/12 (I)	11/12	0/12 (I)	1/12	0/12	0/12
007	12/12	0/12 (I)	4/12	0/12 (I)	0/12	0/12	0/12

* Note: several laboratories have since identified themselves.

Box 1. Understanding the laboratory process

The methodology set out in the technical advice note stipulates both the field and laboratory protocols involved in collecting and processing pond water samples for great crested newt (GCN) presence/absence determination by eDNA analysis. eDNA is the DNA released into the water by an organism via urine, faeces, skin cells, saliva and gametes.

The field protocol uses a simple kit provided by the laboratory of choice (Figure 2) to collect 20 water samples from around the circumference of the pond which are pooled and mixed before transferring 15 mLs into each of 6 tubes of preservative (for full details see Biggs *et al*. 2014b).

The laboratory protocol comprises two main phases:

DNA extraction – DNA is extracted from the water/preservative mixture by high speed centrifugation causing the DNA to collect at the bottom of the tube. The water/preservative mix is then poured off and the DNA 'pellet' resuspended/dissolved in an extraction solution before further purification. The resulting DNA sample can then be stored at -20°C prior to the qPCR phase.

qPCR analysis – Three separate PCR analyses allow the sample to be tested for inhibitory components, potential degradation and/or poor DNA extraction, and presence of GCN eDNA. Together these results determine the presence/absence of GCN within the sample.

The inhibition control is a known fragment of DNA that is added to a sample of the eDNA extract. A PCR specific for the inhibition control DNA is set up. The eDNA sample is considered to contain inhibitors if this DNA amplification was outside of acceptable limits when compared to a similar reaction not containing the eDNA sample.

The degradation control is a piece of DNA which is added to the alcohol preservative before the kit is sent out for use and is a different piece of DNA from that used as the inhibition control. During the DNA extraction, this control DNA is also recovered along with the eDNA. A PCR reaction specific for this degradation control DNA is set up using the DNA recovered from each pond sample. The eDNA within a pond sample would be considered to be degraded (during sampling/storage prior to analysis) if the degradation control DNA amplification differs from an acceptable limit of an un-degraded control. In effect this control does not distinguish 'degradation' from poor eDNA extraction.

The acceptable limit generally refers to a value of ≤ 3 Cq shift in the amplification curve (Figure 3). The Cq or 'cycle threshold' is the cycle number at which the fluorescent signal of an individual PCR reaction is significantly above that of the background (usually calculated by computer software).

Figure 2. eDNA sample collection kit comprises the following sterile equipment: a plastic scoop/dipper, two pairs of gloves, a transfer pipette, and 6 tubes containing preservative within a small box.

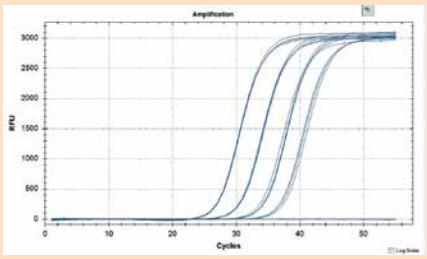

Figure 3. Example of GCN qPCR amplification curves for reactions containing four different concentrations of GCN DNA or no GCN DNA. PCR reactions which are considered to be negative for GCN are flat lines and those considered to be positive for GCN are sigmoidal curves. Higher concentrations of GCN DNA take fewer cycles to generate a signal than lower concentrations. RFU = relative fluorescent units.

The proficiency scheme was conducted by Fapas (FERA's proficiency testing division, www.fapas.com) on behalf of Natural England. During initial discussions between Natural England, Fapas and the eDNA analysis laboratories in March 2017, it was decided that seven samples prepared in simulated pond water would be sent out to the seven independent laboratories offering the analysis in July 2017. These included a range of positive (great crested newt DNA at high, medium and low concentrations), negative (no great crested newt DNA), and inhibited samples (inhibitory reagent added). Following the stipulated methodology, 12 PCR replicates were performed per sample with a result of ≥ 1 out of 12 being considered positive for great crested newt (Box 1).

Four of the seven laboratories taking part correctly identified the samples (Table 2). It is worth noting that due to its stochastic nature, PCR detection can be variable when eDNA is present at very low concentrations (Table 2, column E). This means that different laboratories may return a result where 1, 2, or even 3 of the 12 required replicates are positive, as was the case in this exercise (see also Rees *et al*. 2014), whilst still being considered accurate.

In November 2017, during an appraisal of the proficiency testing process by those laboratories involved, it was decided that future development of the proficiency test via blind testing would include a similar mixture of samples and would use the laboratories' own kits. This was not the case during the 2017 test when kits were supplied by Fapas. It was agreed that the small number of samples sent out in the first assessment was a limitation and that it would be preferable to include more samples in future testing. Additionally, it was suggested that, rather than using simulated pond water, it would be better to use pond water from water bodies either outside of the great crested newt's range or where the water source had been confirmed as negative for newts both by traditional survey methods and by eDNA survey by multiple laboratories.

Interpretation of results

During habitat appraisal and sampling stages of surveys, key factors should be carefully recorded. These include water quality, sources of potential cross-contamination, sampling restrictions

such as access difficulties, and relevant environmental factors such as flow, linked ponds, water levels, turbidity and weather conditions. This information will be relevant to the explanation and interpretation of laboratory results.

The ability of a laboratory to detect great crested newt DNA at the limit of detection (LOD; reported as 3 x 10^{-9} nanograms, Biggs *et al*. 2014) will affect the interpretation of results. If this LOD cannot be attained by detection of known amounts of DNA in control tests then the likelihood of reporting a false negative result increases for samples containing low amounts of DNA. Additionally, the interpretation of results will depend on the inhibition and degradation controls (see Box 1).

An eDNA result may be reported as indeterminate/inconclusive where the degradation control was outside of the acceptable limits for the assay and the great crested newt PCR was negative (0 of 12 positive PCR replicates). For great crested newts, eDNA can remain suspended in water and will persist for approximately two to four weeks (Dejean *et al*. 2011, Thomsen *et al*. 2012, Rees *et al*. 2014), during which time the eDNA will be degraded by biotic and abiotic processes. At the point at which water samples are taken, degradation should be attenuated by a preservative agent, i.e. ethanol. If an eDNA result is indeterminate or inconclusive, the negative result could be due to an absence of great crested newts or the degradation and/or poor extraction of the eDNA during the DNA extraction phase.

Water chemistry (pH, sediment content, salinity, etc.) has been shown to affect PCR amplification (Foote *et al*. 2012) and large amounts of sediment or algae in the water sample can interfere with the eDNA extraction process. Poor extraction results in the degradation control DNA falling outside of acceptable limits. For example, several laboratories have reported receiving samples containing a white precipitate that may not have been apparent during the sample collection. This appears to be the result of a component precipitating out of the water over time, which then interferes with the eDNA extraction process. Chemical analysis of several water bodies where this has occurred indicates high calcium concentrations suggesting that areas that contain large amounts of calcium in their geology may not be suited to this methodology. Surveyors can limit the likelihood of a sample having a high sediment content by not entering the water; this also prevents cross-contamination due to eDNA from another area being transferred into a water body. Sediment can also contain 'historic eDNA', i.e. a trace of great crested newt eDNA from some time ago rather than 'contemporary eDNA' from the present/near present. A study on the bigheaded Asian carp *Hypophthalmichthys* spp. (Turner *et al*. 2014) showed that eDNA recovered from pond sediment was at least eight times more concentrated per gram of sediment than the eDNA recovered from the pond water per millilitre of water. Additionally, eDNA was detected in sediments for up to 132 days after carp removal. This scenario is feasible for great crested newts although a controlled study is needed to confirm this.

Conclusions

The eDNA technique is one of many tools available for great crested newt detection but it is crucial that laboratories operate to agreed standards of best practice. The introduction of the proficiency testing scheme is welcome and will help to drive up standards and competency. In addition, laboratories offering this service must continuously monitor and improve their service so that the ecology community can be confident that this is a robust technique and a useful tool for monitoring presence of great crested newts.

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eDNA Metabarcoding for Non-Invasive Fish Surveys

Keywords: environmental DNA, fish, lakes, metabarcoding, ocean, rivers

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One of the best-developed applications of eDNA is surveying fish communities. Fish are particularly well suited to eDNA monitoring because they are fully aquatic and consistently release DNA into the water, making them detectable year-round. Most UK freshwater fish can be identified to species using the 12S gene, for which well-validated metabarcoding assays exist. This enables all fish DNA in a water sample to be amplified and sequenced in parallel, efficiently yielding whole community data.

Fish in lakes

Extensive testing by the Environment Agency (in collaboration with the University of Hull and CEH) has shown that eDNA metabarcoding is more sensitive than conventional gillnetting for monitoring lake fish communities. In addition, it is far less invasive and substantially cheaper (Environment Agency 2016, Hänfling *et al.* 2016). The Scottish Environment Protection Agency (SEPA) are now employing the eDNA approach on a larger scale to gather data on fish communities in Scottish lakes.

The eDNA in ponds and lakes does not move far from the location in which it was shed (Lacoursière-Roussel *et al*. 2016). Consequently, quite fine-scale data can be obtained on the spatial distribution of aquatic species. At the same time, it makes it vital that samples (or sub-samples) are collected from around the whole perimeter of the waterbody in order to avoid missing species that may have a localised distribution.

Fish in rivers

DNA travels downstream in running water, so an eDNA sample will capture the species in the area directly upstream of the sampling site. Usually the transport distance varies from a few hundred metres to a few kilometres (Civade *et al*. 2016), after which it settles out into the sediment and can no longer be detected in the water column (Shogren *et al*. 2017). The exact distance that DNA travels is influenced by a great many factors including flow rate, depth, substrate, water chemistry and environmental conditions (Jane *et al*. 2014, Shogren *et al*. 2017), so there will always be some level of uncertainty regarding the precise spatial representation of an individual sample. Nonetheless, a sample is likely to be dominated by the DNA of fish that are closer to the sampling point.

Civade *et al*. (2016) found that a single eDNA sampling campaign in an Alpine river captured as much data as 20 cumulative years of conventional monitoring. In particular, the approach is extremely powerful for determining the ability of fish species to migrate upstream and to pass structures that may represent barriers (Jerde *et al*. 2011). In a pilot project currently underway with Natural England, NatureMetrics will monitor key species of migratory fish along rivers using eDNA.

Fish in the ocean

Fish diversity in oceans is high, and eDNA can be moved around by currents and tides leading to some spatial uncertainty. However, studies have shown that communities detected by eDNA are dominated by species typical of the local environment in which the sample was collected (e.g. O'Donnell *et al*. 2017).

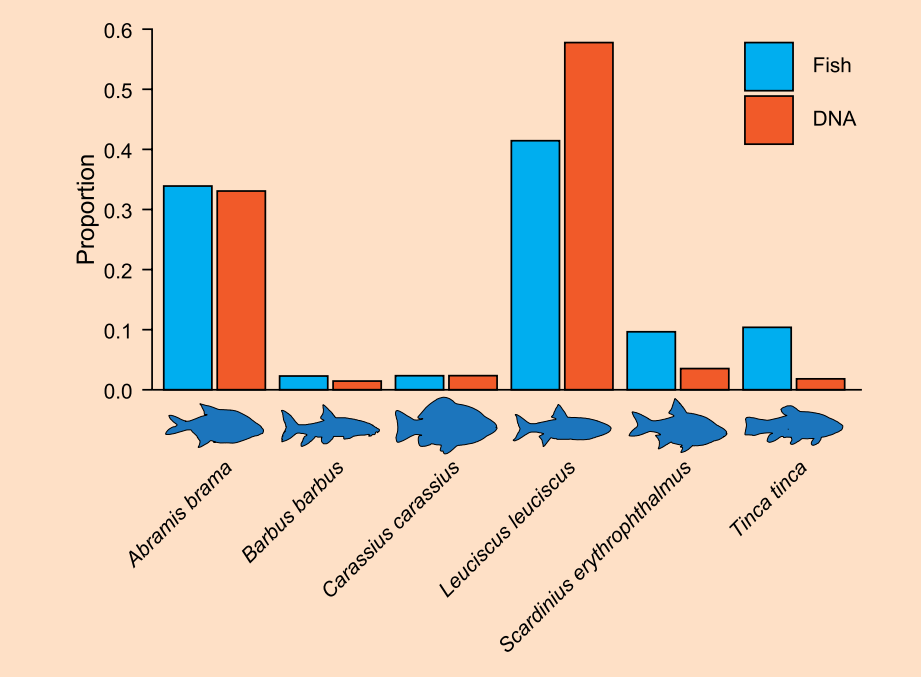

Figure 1. Column chart showing the relative abundance of fish species in a small lake based on counts of fish (blue) and DNA sequences (orange).

Table 1 shows the diversity detected in four seawater samples collected from coastal seagrass beds in Devon in a recent collaboration between the Community Seagrass Initiative and NatureMetrics. A total of 36 different taxa were detected across the four samples, with a maximum of 25 taxa in a single sample. This dataset illustrates several points:

1. Species typical of the seagrass habitat were detected (e.g. pipefish, sand eels) in addition to benthic species (flounder) and coastal pelagic species (e.g. bass, mackerel).
2. In two of the samples (2280, 2281) collected from locations very close to the mouth of a river, we detect freshwater species as well as marine ones. The other samples, which were collected further away from freshwater inflows, did not contain any freshwater species.
3. Not all species can be named. This is largely because the reference database for the 12S gene is incomplete for marine fish species (Miya *et al*. 2016). Additional references need to be generated from tissue samples in order to improve the taxonomic resolution of marine fish metabarcoding.

Can we determine relative abundance of fish from eDNA?

Various factors influence the amount of DNA that fish release into the environment, including size (surface area and biomass), stress levels, life cycle, and behaviour. Indeed, changes in feeding rate can affect DNA shedding rate by an order of magnitude (Klymus *et al*. 2015). Nonetheless, the relative abundance of sequence reads obtained from metabarcoding tends to correlate with the relative dominance of the species, at least for communities in standing water (Hänfling *et al*. 2016, Lacoursière-Roussel *et al*. 2016).

In November 2017, NatureMetrics visited the Environment Agency's fish farm facility at Calverton, Nottinghamshire, to take water samples from a lake on the same day that the fish were removed from it and counted. Figure 1 shows the relative abundance of fish (as counted) against the relative abundance of sequence reads obtained by metabarcoding. It is clear that the sequence data accurately tells us which

Table 1. Species-by-sample table generated by 12S eDNA metabarcoding of water samples collected at four seagrass beds on the south coast of England. Numbers give the percentage of the sequences in the sample assigned to each species.

	Drake's Island	Yealm Cellars Cove	Cawsand Bay	Portland Harbour
	2280	2281	2282	2283
Atlantic horse mackerel *Trachurus trachurus*		3.8		3.8
Atlantic mackerel *Scomber scombrus*	4.5	3.1	5.4	1.4
Bass *Dicentrarchus labrax*	2.8	24.1	2.5	56.6
Black goby *Gobius niger*				1.3
Bream species		2.9		
Brown trout *Salmo trutta*	3.0	5.8		
Carp *Cyprinus carpio*		0.5		
Chabot bullhead *Cottus perifretum*	1.1	1.7		
Common bream *Abramis brama*	1.7			
European eel *Anguilla anguilla*		7.5	1.6	
European pilchard *Sardina pilchardus*	7.1	1.4	5.9	0.5
Flounder *Platichthys flesus*		9.3		
Gilt-head bream *Sparus aurata*		0.1		0.8
Goby species 1	8.6	2.1	2.4	7.4
Goby species 2	8.0			6.4
Goby species 3	3.9	5.7	2.3	
Golden mullet *Liza aurata*		2.0		2.4
Goldsinny wrasse *Ctenolabrus rupestris*		0.9	1.4	
Grass carp *Ctenopharyngodon idella*	4.0			
Long-spined sea scorpion *Taurulus bubalis*		5.8		
Pipefish species 1 *Syngnathus acus/typhle*		1.6		
Pipefish species 2 *Syngnathus acus/typhle*		1.4		
Pollock *Pollachius pollachius*		0.5		
Poor cod *Trisopterus minutus*	0.8			
Roach *Leuciscus rutilus*	2.2			
Rock goby *Gobius paganellus*		0.1		2.7
Sand eel species *Ammodytes tobianus/marinus*		1.4	45.5	
Sand smelt species *Atherina* sp. aff. *presbyter*	45.0	1.1	0.4	1.0
Thin/Thick-lipped mullet *Liza ramado/Chelon labrosus*	3.2	16.7	8.0	2.9
Wrasse species 1 *Labrus bergylta/mixtus*	1.6	0.5	20.1	0.8
Wrasse species 2 *Symphodus bailloni/melops*			4.5	1.2
Wrasse species 3 *Symphodus bailloni/melops*				1.1
Unknown fish species 1				8.2
Unknown fish species 2	2.6			
Unknown fish species 3		0.3		
Unknown fish species 4				1.6

are the dominant fish species and which are present at a lower level. Consistent with published research, this suggests that the laboratory workflow for metabarcoding fish does not introduce large biases.

We would still urge caution in quantitative interpretations of metabarcoding data. In more complex environments – especially running water – the match is likely to be less reliable. Moreover, a single sample can be strongly influenced by localised events such as spawning, so interpretations should always be based on multiple samples. In standing water, the approach taken by the Environment Agency and SEPA is to collect samples from multiple points around the perimeter of a waterbody to analyse site occupancy (Environment Agency 2017). Given that DNA remains quite locally distributed in still water, the more abundant species will occur in a greater proportion of the samples than will the rarer species.

Summary

Fish eDNA metabarcoding is a powerful method that allows fish species composition to be determined without the need for expensive equipment or invasive methods. The relative abundance of sequence reads assigned to each species can be considered a fairly accurate reflection of the relative amounts of DNA captured, and this tends to correlate positively with the relative abundance of fish. When working in running water or oceans, there is inherently a greater degree of uncertainty regarding the relative abundance and spatial distribution of species than is the case in standing water, and this should be taken into account when interpreting results. However, eDNA metabarcoding will allow data on fish species composition to be obtained where it would otherwise have been unfeasible, and for this reason it represents an important new tool in the ecologist's armoury.

Fishing with eDNA - manual filtration of pond water for conducting a fish survey. Photo credit Kat Bruce.

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Kat is co-founder and CEO of NatureMetrics, a company that specialises in DNA-based monitoring of biodiversity. An ecologist by training, her PhD at the University of East Anglia centred on the use of invertebrate metabarcoding to inform environmental management activities and facilitate evidence-based decision-making.

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CT is a senior scientist at NatureMetrics and specialises in DNA-based approaches to characterising biodiversity. He's developed molecular techniques to identify fish communities from water, plants from soil, arthropods from traps, and even bat, bird, and otter diets from their faeces.

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CIEEM Featured Training

Technologies

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Manchester 2-3 May

NEW

This two-day course focuses on using QGIS as a tool for data analysis and producing more complex maps accurately and efficiently. Pitched at intermediate level, the course offers ideal progression from our entry level QGIS training.

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Survey, Ecology and Identification

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Gateshead, 9-10 April

NEW

This unique immersive training event offers an opportunity to understand how bird song works, what it does and how to better identify birds using song as a tool for ornithological survey. The training delivers 14 hours of CPD within a 24-hour period and includes dusk and dawn field sessions. All training is delivered at a residential venue in the beautiful Derwent Valley.

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NEW

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Culross, 17 April

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Stirling, 27-28 March

Our intermediate level EcIA course is suitable for those with some existing knowledge of undertaking EcIA and the legislation and policy drivers behind the process. Understanding is developed using extensive case studies and examples relevant to practitioners in Ireland (21-22 March) or Scotland (27-28 March).

An Introduction to Appropriate Assessment in Ireland

Letterfrack, 12 April

This course has been designed to cover the background to the designations, legislation and steps in Appropriate Assessment (AA). The training provides a basic template for assessments and explores how to carry out a basic assessment. Disucssion sessions, presentations and case studies will consider multiple examples of AA in action and discuss screening, Stage 2 assessments, cumulative impacts and mitigation.

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Practical Application of Genetic Technology in Mammal Research

Denise O'Meara
Waterford Institute of Technology

Keywords: conservation genetics, molecular ecology, non-invasive genetics

DNA techniques were rarely used in mammal research until the late 1990s but they are now more accessible and affordable than ever, and many mammal studies have come to rely on this technology. One of the problems of studying mammals is that they are often elusive, rare and generally difficult to directly observe. DNA technology can help overcome some of these problems especially when people with expertise in DNA methods work with those with expertise on a specific mammal species to help address a research question collaboratively.

Non-invasive genetics

Non-invasive genetics is an example of how DNA technology can complement traditional field survey methods. Species such as pine marten *Martes martes*, otter *Lutra lutra*, squirrels *Sciurus* spp. and bats can be difficult to survey traditionally due to legal protection or their elusive behaviour. Indeed, one of the biggest obstacles for field ecologists is the confirmation of the species in question from field signs such as faecal samples. For example, pine martens are difficult to survey because they are rare or absent in many parts of Great Britain and Ireland, and even where they are found their population density tends to be low. Identification can be further compromised by the risk of misidentification, as fox *Vulpes vulpes* scats are sometimes identified as pine marten, even by the experts! To reduce any level of ambiguity, genetic confirmation of samples is the most accurate form of identification.

Pine marten

Species identification

Samples can be genetically identified in several ways but all rely on the central technique of PCR or Polymerase Chain Reaction. PCR is a process whereby target DNA is amplified with the aid of primers that target the specific section of DNA of interest and an enzyme called DNA polymerase. Using a process involving repeated cycles of heating and cooling, hundreds of thousands of copies of the target DNA are made. The PCR products are then analysed to detect if the target species' DNA is present using a method called gel electrophoresis. This method involves loading the PCR products into an agarose gel which is placed in a tank of liquid buffer. The tank is then connected to a power pack with positive and negative electrodes. This electrical current causes the negatively charged DNA products to migrate towards the positively charged part of the tank. Smaller PCR products move faster than the larger PCR products, and can be visualised and identified with the aid of a DNA binding dye and UV light.

Some research groups will use species-specific primers to target the DNA of the species of interest. In this case, a positive PCR product will mean that only the target species' DNA is present, and identification is then confirmed. In other cases, more generic primers might be used, which will amplify DNA from lots of different species. In this case, the PCR product then needs further analysis to identify the source of the DNA. DNA sequencing facilitates the determination of the nucleotides (Guanine, Gs; Thymine, Ts; Adenine, As; Cytosine, Cs) that comprise the target sequence. The DNA sequence is then compared to a reference library such as that freely available on GenBank (an online DNA sequence library, see https://www.ncbi.nlm.nih.gov/genbank/sequenceids/). As each species has a unique combination of nucleotides, this allows the species to be identified accurately.

However, scat samples and non-invasive DNA samples in general are known to contain small quantities of often highly degraded DNA. One of the biggest obstacles to identifying samples using this approach is that the quantity of DNA present in the sample may be too low to amplify in the process outlined above. In this case, a field survey sample might result in a false negative. This occurs when the target species is not identified as a positive when in fact it should be.

Real-time PCR

Real-time PCR relies on the specific amplification of the target species' DNA. The method relies on the same PCR principle outlined above but it has many advantages. During real-time PCR, the PCR product is monitored and measured as it goes through the PCR cycles and does not require the step of gel electrophoresis or DNA sequencing. As the platform is more sensitive than traditional methods, a much smaller PCR product can be targeted. This

is especially useful for those non-invasive studies with low quantity and highly degraded DNA samples as a smaller PCR product is more likely to amplify than a larger PCR product, and the technology and platform is more sensitive and accurate than conventional PCR methods. At my workplace in Waterford Institute of Technology (WIT), in the south east of Ireland, we now use real-time PCR as a standard approach for species identification for a range of wildlife.

Sex determination

Sex identification relies on the same method as species identification. The difference in this case is that the primers used are designed to target the sex chromosomes. The approach relies on the ability to target a section of the Y chromosome to genetically identify a male sample. After that, some research groups take different approaches such as the simultaneous identification of the X chromosome in males and females or another section of nuclear DNA, present in both males and females that houses DNA of similar quantity.

The DNA in the sex chromosomes is nuclear DNA and this generally degrades faster than the mitochondrial DNA. Mitochondrial DNA is usually targeted for species identification (as there are more copies of this per cell), so in some cases there will be enough DNA present in the DNA extract to verify the species, but not enough to successfully identify the gender of the sample.

Identification of individuals

To identify individuals, microsatellite analysis or DNA fingerprinting is required. A microsatellite is a repetitive section of DNA. The repeat can consist of a section of DNA such as a two base pair repeat, e.g. GT repeated multiple times (between five and 50 repeats). These microsatellites occur at thousands of locations throughout the genome and as they have a very high mutation rate, the length of the repeating section of DNA varies between individual animals. Using a panel of these microsatellites, individual animals can be identified as each animal will have a unique profile of repeat regions or genetic fingerprint, just like your own unique fingerprint. The technique is also used for human forensics and paternity tests, as it is possible to trace relatedness through these microsatellite profiles. It is also possible to assess genetic diversity and in the case of wildlife, assess how genetically diverse or inbred a population might be. Again, these microsatellites are found in the nuclear DNA, so obtaining a reliable DNA fingerprint from a non-invasive DNA sample is not always possible or reliable.

Applications

Pine marten

At Waterford Institute of Technology, Dr Catherine O'Reilly and Dr Peter Turner have developed a method to genetically identify pine marten samples using real-time PCR. This offers a better method than the collection of scats for surveying this elusive species. Long-term collaboration with the Vincent Wildlife Trust led to the development of a 'hair-tube' method that could successfully pluck hair samples from unsuspecting pine martens which visited a section of drainpipe that was tied to a tree and baited with chicken (Figure 1). This worked very well and has since been employed more widely as a survey approach both in Ireland and the UK. With the success of the hair-tube technique came the added bonus of higher quality DNA samples from hairs. Hair DNA is of better quality and quantity than the DNA extracted from scat or faecal samples and, as a result, it is suited for more in-depth DNA analysis such as the genetic identification of the gender of the animal from which a sample originated. This technique was most recently applied to a National Pine Marten Survey in Ireland, where the breeding population of pine marten was estimated to consist of approximately 3000 animals (O'Mahony *et al*. 2017).

Figure 1. A pine marten hair-tube.

Real-time PCR works very well to detect DNA in low quantity or of degraded quality, and can therefore be applied to dietary analysis. We undertook a dietary study of pine marten and used specific real-time PCR tests of pine marten scats to detect mammalian prey including grey squirrels *Sciurus carolinensis* and small mammals. As expected, squirrels occurred at very low frequencies, but items like wood mouse *Apodemus sylvaticus*, bank vole *Myodes glareolus* and pygmy shrew *Sorex minutus* were more common. We were also able to detect the greater white-toothed shrew *Crocidura russula*, a recent introduction and an invasive species in Ireland (O'Meara *et al*. 2014).

Nowadays, many groups are using metabarcoding, a next generation sequencing method which can be used to target all items in the diet of an animal. This, of course, has huge potential in the area of mammal research but real-time PCR is still an accurate and cost-effective approach if the researcher is interested in targeting specific issues such as an invasive species or a small number of target species.

Otter

Hair samples provide the best source of DNA for detailed genetic analysis but, unfortunately, we have not managed to develop a reliable method to obtain hair samples from otters. However, otters are very obliging and leave their droppings (or 'spraints') in obvious spots along river banks, lakes and coastal areas. During the course of the *Mammals in a Sustainable Environment* project (www.miseproject.ie), we collected thousands of spraints from parts of Ireland and Wales. We developed a reliable genetic technique to confirm the species, determine the sex and obtain a unique genetic profile from individual spraints (O'Neill *et al*. 2013). The technique was also used in a citizen science survey of otters in Cork City where we worked with a local volunteer group to collect the samples (White *et al*. 2013). Unfortuntely, spraints are troublesome for this type of analysis and our ability to get good DNA varied considerably. In some surveys, only 10% of the samples yielded good DNA while in others 70% of the samples had good DNA. It seems that diet, weather and a myriad other variables can interfere with the DNA found in an otter spraint!

Red squirrel

I have never found a faecal sample from a red squirrel *Sciurus vulgaris*, so I am fairly confident that hair-tubes are the only way to non-invasively survey for squirrels. Just like the pine marten, they will use a hair-tube but they tend to be a little reluctant and getting a good quality hair sample can be tricky. As part of my PhD research, I developed a hair-tube technique similar to the one used for pine marten to remotely obtain hair samples (Figure 2). Once I had retrieved the sample, I then used real-time PCR to confirm the species (O'Meara *et al*. 2012). If the hair sample contained an adequate quantity of DNA, I was then able to use DNA microsatellite analysis to obtain an individual genetic profile and analyse how many squirrels had visited the tube over time.

It was also possible to assess the genetic diversity of the population. Using a combination of microsatellite data and mitochondrial DNA sequencing (and additional samples from other studies), we established that many populations of red squirrels in Ireland are genetically isolated from one another. They may be at risk of local extinction because many woodlands surveyed are small and geographically isolated. We were also able to examine the genetic heritage of the red squirrel, and could retrace some of the genetic strains or haplotypes back to Great Britain. Most of the red squirrels present in Ireland today were introduced from Great Britain in the 1800s, after the species had previously gone extinct in Ireland some time before. In fact, some of the strains now present in Ireland are most probably extinct in many parts of England. We recommend that the red squirrel in Ireland is managed to conserve their genetic diversity, but they also need to be protected as they may be suitable stock for reintroduction or reinforcement projects in Great Britain in the future (O'Meara *et al*. 2018).

Figure 2. A red squirrel hair-tube.

Bats

We have developed a suite of real-time PCR tests at WIT suitable for identification of bat droppings found in buildings. The method is especially useful for environmental consultants who are interested in identifying the species of bat present at a site that is being assessed for development. The method is fast, reliable and provides clear confirmation of the presence of a particular bat species (see http://swiftecology.co.uk/dna.php).

Work on the lesser horseshoe bat *Rhinolophus hipposideros*, a species whose distribution in Ireland is limited to a few areas in the counties of the west coast, has concentrated on using genetic technology to assess numbers. Lesser horseshoe bats are highly protected and are sensitive to disturbance, particularly in their roost. This presents a problem to the National Parks and Wildlife Service who must report a national population estimate for this species to the European Union under Article 17 of the Habitats Directive. To help provide a non-invasive population assessment, my colleague Andrew Harrington lay down sheets of mesh netting on the floor at a number of roosts near the exit points. When the bats flew out in the evening, the droppings were collected on the sheet. Using a combination of species confirmation, sex determination and microsatellite analysis, Andrew was able to determine the number of bats and the sex ratio of the bats within each of the roosts. Hopefully, this will contribute towards estimating the population size of this species in the future.

Looking towards the future

DNA techniques have an important role in mammal research. They may be applied to a simple survey to confirm the presence or absence of a particular species, or they might be applied to more in-depth analysis to provide an estimate of the number of animals present in a particular population. Following initial surveys, the genetic diversity and genetic heritage of animals and populations can also be explored, providing insight into appropriate management for conservation, mitigation and even enforcement. As the technology moves forward at a rapid rate, it is important that we don't lose sight of the practical applications of the techniques and ensure that the research undertaken benefits the mammals, environment and society in general.

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Use of Environmental DNA Analysis to Detect the Presence of Water Vole

Keywords: conservation, molecular ecology, real-time PCR, species-detection, survey

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The UK water vole population has fallen dramatically in recent years. Accurate and reliable methods of detecting the presence or absence of water vole at specific locations are critical to conservation efforts. Traditional survey methods can, in some cases, be invasive, inaccurate or difficult to carry out. This study aimed to develop a novel method based on identification of environmental DNA (eDNA) to detect the presence of water vole via analysis of water samples. The results demonstrate that the technique offers an accurate method of detection. However, this study was based on a relatively small sample and certain limitations of the technique have been identified, which will be explored with further research. Nevertheless, used and interpreted correctly, the technique can provide reliable evidence of presence or absence.

Water vole *Arvicola amphibius*. Photo credit Peter Trimming.

Introduction

Environmental DNA

Wildlife conservation has entered a new era. The development of molecular genetic tools is providing novel methods to study species, leading to insights and information that would have been unobtainable just a few years ago.

The development of these techniques has been driven by the need for improved biological records and a demand for more effective methods to monitor species' populations. Often, traditional methods of determining presence/absence, based on identification of the physical signs of a species' presence, are expensive, inaccurate or harmful. The analysis of environmental DNA (eDNA) is now well established as an alternative technique that allows researchers to detect the presence of rare, secretive or invasive species, rapidly, non-destructively and accurately.

eDNA refers to the genetic material isolated from environmental samples, such as water and soil. Aquatic species release DNA into the environment that they inhabit in various ways including excretions and by shedding skin cells. This DNA disperses within the body of water as suspended particles, within cells and mitochondria or as free DNA (Turner *et al*. 2014). eDNA analysis involves the collection of an environmental sample (e.g. water, soil) from which the DNA is extracted and analysed to identify the presence of one or more target species (Rees *et al*. 2014).

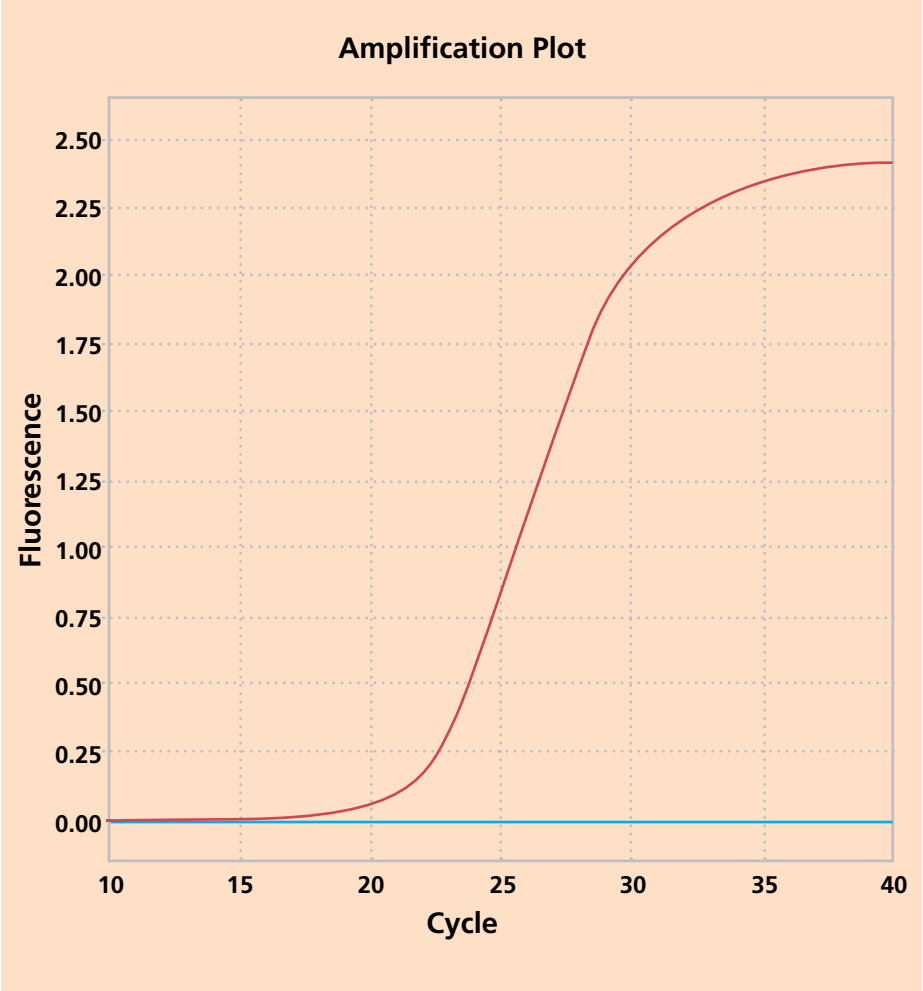

Figure 1. Graphical representation of real-time PCR data. The graph plots the magnitude of fluorescence from the qPCR, measured in relative fluorescence units (ΔR) against the number of qPCR cycles. In samples containing the target DNA sequence, the fluorescence intensity increases due to the release of a fluorescent molecule as the DNA is copied, resulting in an amplification curve (red curve). In negative samples, the DNA target is not present so that there is negligible increase in fluorescence and an amplification curve is not produced (blue line).

The technique relies on targeting the variation in DNA sequences between different species. A sensitive method for the detection and quantification of nucleic acids, known as quantitative real-time Polymerase Chain Reaction (qPCR) is used; qPCR uses a mixture of short DNA molecules and an enzyme to copy specific sections of DNA through the cyclical heating and cooling of the reaction mixture (Heid *et al*. 1996). As the DNA is copied, a fluorescent molecule is released and detected (see Figure 1).

In 2008, eDNA analysis was used to detect a freshwater vertebrate species for the first time when DNA was extracted from water samples and analysed to detect the American bullfrog *Lithobates catesbeianus* (Ficetola *et al*. 2008). The study showed that the detection of eDNA could accurately determine the presence or likely absence of the species. Variations of the technique have since been applied to a range of habitats and species, including amphibian (Thomsen *et al*. 2012) and fish species (Sigsgaard *et al*. 2015), amongst others.

Applying eDNA detection to the water vole

These advances in molecular detection using eDNA have come at an opportune time. Factors including climate change, habitat loss and the spread of invasive species are negatively impacting many species and ecosystems.

The water vole *Arvicola amphibius* (Figure 2) exemplifies this global issue. The population has suffered severe declines over the last century (Jefferies *et al*. 1989) with estimates of a 90% reduction in the UK, attributed to the introduction of the invasive American mink *Neovison vison* and the loss of suitable habitats (Barreto *et al*. 1998). Traditional survey methods rely upon the recording of field signs indicative of the presence of water voles, as described in the *Water Vole Conservation Handbook* (Strachan *et al*. 2011). However, in certain situations, these methods can be inaccurate, labour intensive and expensive to undertake. Consequently, there is a need for additional survey methods. Despite the demonstrated successes of eDNA analysis for the detection of a wide range of species, the technique has, up to now, not been applied to detect the water vole. The way in which their varying habitats, environmental conditions and behaviours would affect the ability to detect the species using this method was largely unknown. In addition, due to several known limitations of the technique, such as the technique not accurately measuring the population size of a target species at a location and the transportation of eDNA in flowing water, it was not known how useful the results would be.

Aim of the study

This study aimed to develop a species-specific qPCR assay for the detection of water vole and to field-test this method, alongside traditional sampling methods, to assess its suitability as a presence-absence survey technique that can ultimately be used to better inform the conservation efforts to preserve the species.

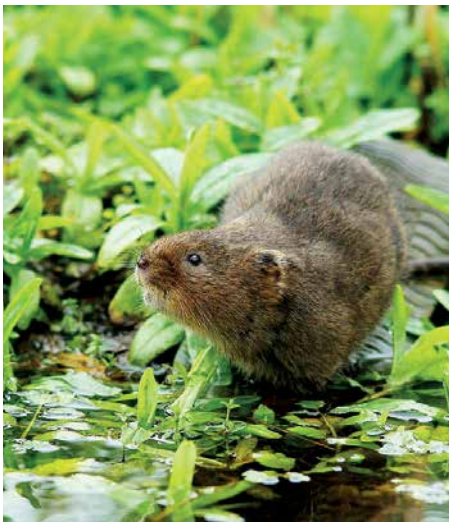

Figure 2. Water vole *Arvicola amphibius*. Photo credit Peter Trimming.

Table 1. List of non-target sympatric species tested during qPCR assay validation.

Species	Latin name	qPCR test result
Bank vole	*Myodes glareolus*	Negative
Field vole	*Microtus agrestis*	Negative
House mouse	*Mus musculus*	Negative
Water shrew	*Neomys fodiens*	Negative
Brown rat	*Rattus norvegicus*	Negative
Wood mouse	*Apodemus sylvaticus*	Negative
European otter	*Lutra lutra*	Negative
Eurasian beaver	*Castor fiber*	Negative
American mink	*Neovison vison*	Negative
Human	*Homo sapiens*	Negative
Domestic dog	*Canis lupus familiaris*	Negative
Domestic cat	*Felis catus*	Negative
Domestic pig	*Sus scrofa domesticus*	Negative
European badger	*Meles meles*	Negative
Pine marten	*Martes martes*	Negative

Methods

qPCR test development

The qPCR test was designed to detect a region of the water vole cytochrome b *(cyt b)* gene and was verified using the PrimerBlast online tool (Ye *et al*. 2012) to confirm that water vole DNA would be specifically targeted. Hair samples were collected by trained staff from eight adult water voles in captive populations at the Derek Gow Consultancy and Wildwood Trust. Hair samples were also collected from a range of non-target species either related to the water vole or likely to be present in the same habitats. DNA was extracted from the hair samples at Crestwood Environmental's eDNA analysis laboratory. These DNA samples were tested by qPCR to ensure that only water vole DNA produced a positive result and that the non-target species' DNA produced a negative result. Table 1 lists the tested non-target species. The assay was then optimised, including altering the reaction mixture and qPCR thermal cycling temperatures to increase the sensitivity of water vole DNA detection whilst maintaining species-specificity by preventing non-target DNA detection.

Field testing

Traditional surveys: Ten sites, situated within the West Midlands, England, were selected for field-testing. Each site was first surveyed for water vole field signs to determine presence or absence. A transect was walked along the survey area to identify and record water vole field signs including droppings, feeding signs such as distinctive vegetation cuttings, burrows and associated vegetation lawns and nests. The field sign search results were withheld from laboratory staff until all laboratory analyses had been completed.

eDNA water sampling: Immediately following completion of the field sign search, the surveyor selected a suitable site for water sample collection, targeting water vole habitat. The surveyor then walked 100 m downstream and using a sterile 30 ml plastic ladle, collected 100 ml of water into a 1 litre plastic laboratory bottle. Nine further 100 ml water samples were collected at 10 m intervals, moving upstream towards the original start point. These were added to the bottle until 1 litre of water had been collected in total. The 1 litre water sample was stored in a Coolbox and transported back to Crestwood Environmental's eDNA analysis laboratory and stored at 4°C for a maximum of 24 hours prior to analysis. All analyses were undertaken by trained and experienced molecular biologists. The surveys and collection of water samples were carried out during August 2017.

Laboratory analysis

Water samples were filtered through a 47 mm, 0.7 µM-pore, glass fibre filter paper to capture the DNA. The DNA was extracted from the filter paper using a modified version of the Qiagen DNeasy Blood and Tissue extraction protocol, as described in Goldberg *et al*. (2011). The DNA samples were then tested for the presence of water vole DNA by qPCR (as described above) using an Aria Mx qPCR system (Agilent Technologies, Santa Clara, CA, USA). Six replicates were run for each DNA sample from each site, with detection of water vole DNA in any of the replicates, shown by the release of a fluorescent molecule (see above), indicating a positive result for that site.

Results

qPCR test development

The qPCR test developed in this study successfully detected water vole DNA from all the DNA samples extracted from known water vole hair samples (n=8). In addition, following optimisation of the assay, there was no detection of DNA from any tested non-target species' DNA samples, extracted from hair samples (Table 1).

Field testing

Ten field sites were assessed (Table 2) and water vole field signs were identified at six of these sites. Water vole eDNA was positively detected at these same six sites. Field signs were not observed at the remaining four sites, indicating that water voles were not present or their field signs were not detected. At three of these four sites, water vole eDNA was not detected, matching the field survey results at those locations. At one site, Newport Brook, field signs were not observed but a positive result was returned for the presence of water vole eDNA. Overall, 90% of eDNA results matched the results from the original field survey with no negative eDNA result from any site with water vole field signs.

Table 2. Presence of water voles at ten sites established through i) traditional field survey and ii) eDNA analysis of water samples according to the protocol described in the text. All samples were taken from flowing (i.e. lotic) water. eDNA results are expressed as the proportion of replicates analysed that gave a positive result, e.g. 4/6 means 4 replicates confirmed presence of water voles out of 6 replicates from each sample; one sample was taken from each site.

Site	Grid reference	Date of survey	Field signs observed	eDNA result	Positive eDNA replicates
Cecilly Brook	SE 525911 015849	03/08/17	Droppings Latrines Vegetation cuttings	Positive	6/6
River Tame	SP 03519 92579	05/08/17	Vegetation cuttings	Positive	6/6
Battlefield Brook	SO 94928 70603	13/08/17	Vegetation cuttings Burrows	Positive	4/6
Newport Brook	SJ 75772 18666	12/08/17	None	Positive	2/6
Staffs and Worcs Canal	SJ 90233 01056	15/08/17	None	Negative	0/6
Spadesbourne Brook	SO 96495 71248	24/08/17	Vegetation cuttings Droppings	Positive	2/6
New Hall Country Park Brook	SP 12957 94922	28/08/17	Droppings Vegetation cuttings Burrows	Positive	1/6
Smestow Brook	SO 89173 99882	29/08/17	None	Negative	0/6
Greenfield Local Nature Reserve	SJ 52942 41645	31/08/17	Vegetation cuttings Droppings	Positive	6/6
Forge Mill Lake	SP 03493 92567	16/08/17	None	Negative	0/6

Discussion

The successful development of an eDNA detection assay for water vole demonstrates the potential of the technique as a reliable survey method for determining the presence or likely absence of water voles at aquatic sites.

The results from the traditional surveys and eDNA analysis were in agreement at nine of the ten sites surveyed by both methods. The exception was Newport Brook where no field signs were found yet a positive eDNA result was obtained. It is possible that the eDNA technique detected the presence of water vole where the traditional methods did not. However, it is also plausible that the water vole eDNA had been transported from a different location by flowing water, resulting in a false positive result. Due to the limitations of this study, it is not possible to differentiate between these causes. Further work is required to confirm the origin of the water vole eDNA detected at this site.

Following these encouraging results, the next step is to identify how to apply the technique to improve the conservation of the water vole. We envisage that sampling for eDNA will be utilised as an additional survey technique for water vole to complement the traditional methods, or as a stand-alone survey method when appropriate. In addition, the technique will be useful when a large geographical area or many separate sites need to be surveyed within a limited time (e.g. along a watercourse). Key advantages include the relative cost, speed and labour efficiency required to collect eDNA water samples when compared to traditional survey techniques.

While the results of this study are very encouraging, several limitations should be noted. For example, the water samples were collected during August, considered to be the time of year when the water vole population is at its peak and is most active (Wildlife Trusts 2017), resulting in higher concentrations of eDNA. Additional field trials are needed to confirm the level of effectiveness and reliability of the technique at different times of the year and to refine the application of the technique.

Water voles are known to inhabit a range of aquatic habitats, including flowing and stationary water systems. It is not yet known how different habitats will affect the transportation and degradation of water vole eDNA and the subsequent effect on detection rates. Therefore, water sampling methods will need to be adapted to different types of habitat, taking into account factors such as the flow-rate and inlet location to ensure that the optimum method is used. Further testing of the technique in a wide variety of aquatic habitats is planned to optimise its use in different situations.

In addition, eDNA analysis cannot give an accurate measure of the water vole population size. Therefore, while it can determine whether water voles are present or most likely to be absent from a location, it cannot provide information on the health of the population. This limits its potential for conservation.

In conclusion, the results presented in this study form part of a growing body of evidence demonstrating that the analysis of eDNA is a very useful tool in

species conservation, although certain limitations of the technique must be taken into consideration. Specifically, we have demonstrated that the detection of water vole eDNA in water samples is an accurate indicator of presence. As the UK water vole population continues to decline, this technique has the potential to contribute important data to conservation strategies aimed at promoting the species' recovery.

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Bringing Beavers Back to Britain – Genetic Considerations in the Restoration of the Eurasian Beaver

Róisín Campbell-Palmer & Helen Senn

Keywords: Eurasian beaver, founder selection, genetic screening, native species, reintroduction, restoration

Hunted to virtual extinction, the Eurasian beaver *Castor fiber* has now been restored to much of its former range across Europe through protection, proactive translocation projects and natural expansion from relict populations. Genetic diversity is crucial to the long-term success of reintroduced populations but has often been overlooked.

Beaver release. Photo credit Steve Gardner, Scottish Beavers (partnership project between the Royal Zoological Society of Scotland and Scottish Wildlife Trust).

Introduction

In recent decades, conservation interventions such as translocations and reintroductions have been increasingly employed to boost species' survival or contribute to ecosystem restoration. Reintroductions can be emotive, generating strong support or objections depending on the species (or perception of the species) involved (Arts *et al*. 2012). Many reintroductions fail, often due to poor planning and implementation. However, better veterinary care, genetic sourcing and behavioural manipulations can all contribute to long-term success (Fisher and Lindenmayer 2000, Germano and Bishop 2009).

Loss of genetic diversity is commonly cited as a potential reason for reintroduction failure. Ideally, genetic information should be taken into consideration both in the planning and monitoring phases (IUCN/SSC 2013); however, many reintroduction projects proceed in the absence of this information or are hampered by a lack of baseline genetic data for the target species (Allendorf *et al*. 2010). Determining if a reintroduction has been successful or not is complex, especially as it may imply that an endpoint has been reached and no further conservation effort is required. Although reintroductions may have mixed outcomes, many species would not currently be present in their native range without such interventions.

Beaver restoration in Britain has been a long, haphazard affair including an official reintroduction of wild animals from Norway into Knapdale in Scotland, and the appearance of significant numbers of unofficially released beavers in various river catchments, notably within Tayside in Scotland (Box 1). In addition, there is a growing interest in enclosure projects which seek to demonstrate the ecological benefits of beavers in a British context.

This article outlines our current knowledge of the genetics of British beavers, focusing on the large population at Tayside. It highlights the steps that can be taken to ensure genetic diversity at the founder stage to ensure that a viable population develops and it also documents the implications of unauthorised releases for population genetics.

Genetic diversity

There are a number of genetic considerations to balance when selecting animals for reintroductions. These may be prioritised differently depending on project objectives and include:

- selection of individuals that are unrelated to reduce inbreeding and maximise genetic diversity,

Box 1. Beaver reintroductions in Britain

The end of May 2009 saw the culmination of many years of work by a diverse range of organisations as four families of Norwegian beavers were released into Knapdale forest, mid-Argyll, as part of the Scottish Beaver Trial (Jones and Campbell-Palmer 2014). This was the first official mammal reintroduction to Britain, concluding in 2015, and offers a model for species restoration programmes in future. As recommended by the IUCN guidelines for conservation translocations, the beaver reintroduction involves a multidisciplinary approach, robust scientific monitoring, community support and engagement, pragmatic animal management and collaboration between a diverse range of organisations and academic disciplines (IUCN/SSC 2013).

The River Otter Beaver Trial in Devon is England's only licenced reintroduction project and is due to conclude in 2020. Other small-scale enclosure trials in Wales and England include the Cornwall Beaver Project, designed to closely monitor specific impacts of beaver on water quality and other riparian issues.

Conservation translocations, including reintroductions, capture people's imagination and can be emotive. The restoration of beavers to Britain has received much attention in the national media, through academic investigation, and in political discussion. Bringing back beavers is not simply about releasing a charismatic mammal for the sake of it; the pivotal role that this species plays in wetland ecology is widely recognised. Centuries of drainage, canalisation of our waterways and replacement of riparian vegetation with monocrops has led to huge losses in biodiversity, flooding and soil erosion. Although beavers have the potential to address some of these issues, the presence of unauthorised populations of beaver living wild in Britain is controversial.

As an island, Britain can be selective about species reintroductions but in comparison to other European countries, there seems to be a general reluctance towards conservation reintroductions. Therefore, when reintroductions are permitted it is imperative that best practice is followed, not only in scientific and animal management but in social communications.

- ensuring that selected individuals are not so genetically different from each other that crosses will have reduced fitness,
- selection of individuals most genetically similar to those historically present at the reintroduction site.

If a small number of related animals are used in a reintroduction project, this will inevitably lead to inbreeding, whereas selecting a large number of unrelated founders can aid reproductive success (Frankham *et al*. 2002). The probability of success under various combinations of genetic diversity/founder number can be modelled in advance using a Population Viability Model to assist with planning. Inbreeding has often been ignored in beaver restoration projects, most probably for reasons of cost and practicality (introducing a small number of animals from the same place is cheaper) and because it is known that large populations have grown from the release of small numbers of animals, with apparently few obvious negative effects (Halley and Rosell 2002, Halley 2011).

Despite rapid population growth in Europe, analysis of archeological records has revealed that modern beaver populations are less genetically diverse than historical populations (Horn *et al*. 2011). There have been no systematic studies on the effect of inbreeding on beavers, but scientific evidence suggests that inbreeding and low genetic diversity are detrimental to population persistence. A good base of genetic diversity will ensure that the population has the greatest potential to evolve and adapt to challenges that it might face in the future, such as disease or climate change.

Genetic constraints

Conservation practitioners are justifiably concerned about the genetic consequences of mixing beavers from different populations. It is possible that interbreeding between animals that have evolved for long periods in isolation will experience negative fitness consequences know as 'outbreeding depression'. In practice, this risk is often overplayed whereas the risk of inbreeding is more serious. For beavers, there is evidence that populations from Eastern and Western Europe were isolated during the last glaciation leading to some genetic differentiation. However, much of the apparent genetic difference seen today between different contemporary populations is attributable to the (human-mediated) reduction to very small 'refugial' populations due to the fur trade (Senn *et al*. 2014). Subsequent (human-mediated) mixing of beavers between eastern and western populations has resulted in successful reintroductions. For example, reintroductions in Bavaria currently number 14,000 – 16,000 beaver of genetically documented mixed descent. Future reintroductions in Britain and elsewhere should aim to mix various source populations from across neighbouring regions to maximize genetic diversity (Senn *et al*. 2014).

Photo credit Steve Gardner, Scottish Beavers (partnership project between the Royal Zoological Society of Scotland and Scottish Wildlife Trust).

Beavers in Scotland

The Eurasian beaver is a native species once widespread in freshwater habitats throughout Britain before being hunted to extinction in the 17th and 18th centuries (Kitchener and Conroy 1997). Radio carbon dating of fossilised remains indicates beaver presence from ~2 million years ago, whilst more recent archaeological evidence, including gnawed timber, dams, lodges, burrows and bones, has been recorded from a number of sites across the country (Coles 2006). The most recent evidence of beavers in the Britain is from the upper Tyne catchment where a beaver-chewed stick was carbon dated to 1269-1396 (Manning *et al*. 2014).

The government-sanctioned, scientific trial reintroduction at Knapdale investigated the feasibility of beaver reintroduction in Scotland using wild Norwegian animals. Separately, a significant population of wild-living beavers are resident throughout the River Tay catchment in Perthshire, east Scotland ('Tayside beavers'). Five years of monitoring the Scottish Beaver Trial, and various studies undertaken in Tayside, are summarised in the Scottish Natural Heritage *'Beavers in Scotland'* report to the Scottish Government (Gaywood 2015). In November 2016, the Scottish Government announced that the Eurasian beaver would be retained in Scotland and it will receive legal protection later this year.

The Tay catchment is over 5000 km^2, with the main land-use in the lowlands being intensive agriculture. The Tayside beaver population was established outside of statutory procedures, and were therefore not subject to the Scottish Code for Conservation Translocations or IUCN reintroduction guidelines, with no baseline data on released individuals. From October 2012 to April 2014, a live trapping programme was undertaken to assess the health and genetics of the Tayside beavers and DNA was extracted from all trapped beavers (blood samples) and from cadavers (muscle sample).

The main purpose of the genetic analysis was to determine beaver species (Eurasian versus North American), assess genetic diversity, estimate molecular relatedness and undertake an origin population assignment. In total, 34 samples were collected from across the Tayside catchment, with all beavers confirmed as Eurasian. The population of origin was found to be Germany (Bavaria) although one individual was assigned to Lithuania/ Poland population origin. Population genetic diversity was roughly equivalent to that found in Bavaria. Taken together with other analyses, this indicated that the release must have been the result of an appreciable number of founders. The majority of individuals (82%, n = 22) were at least as closely related as first cousins.

As part of the Scottish Beaver Trial, a known number of carefully selected beavers were released, following quarantine and robust health screening (Goodman *et al*. 2012). The animals were sourced from Norway where there was no evidence of several diseases and parasites commonly found in central European beavers (Jones and Campbell-Palmer 2014). The IUCN reintroduction guidelines recommend that, as far as possible, the taxonomically closest population should be used in restoration projects. At the time of the Scottish Beaver Trial, few genetic studies had been undertaken on the Eurasian beaver, and numerous reintroduction projects had taken place across Europe with no consideration of the genetic composition of any founders. Expert examination of the skulls from British fossils and extant Eurasian beavers concluded that, morphologically, British skulls were most similar to Scandinavian beavers (Kitchener and Lynch 2000). Unfortunately, as a relict fur trade population, we now know these populations display low genetic diversity. This will be rectified through further reintroductions because the Scottish Beaver Trial has been granted a licence to release a further 28 individuals to augment the original group and improve genetic diversity. Similarly, genetic assessment of beavers within the River Otter catchment in Devon has revealed high levels of relatedness. A licence to release a further five individuals within the lifetime of the trial will address the need to increase the genetic diversity of this population.

With any reintroduction, consideration should be given to whether the conservation objective is to replicate what was formally present, or to restore a population with a broad adaptive potential to current and future environments. Given the physiological, ecological and behavioural similarity between the two extant beaver species, it was particularly important, first and foremost, to confirm species identification genetically. The genetic separation of the two species was only confirmed relatively recently (differing chromosome numbers, Lavro and Orlov 1973).

The current spread of North American beavers *Castor canadensis* in parts of Europe (e.g. Finland) is of serious concern, requiring major management interventions to remove them (Parker *et al*. 2012). In addition, they have been introduced to support fur trade industries in Finland and Russia (Halley and Rosell 2002). More recent escapes from zoos and wildlife parks has seen the North American beaver establish in parts of Belgium, Germany and Luxembourg (Dewas *et al*. 2012). Such introductions, both accidental and planned, impact on native biodiversity, present ecological concerns, and require significant conservation resources for eradication programmes, few of which have been successful. Additionally, negative public reaction to lethal animal control can limit its use, and welfare concerns are

Photo credit Steve Gardner, Scottish Beavers (partnership project between the Royal Zoological Society of Scotland and Scottish Wildlife Trust).

increasingly raised. Early trap and removal attempts for both the Tayside and River Otter beaver populations were met with a public outcry, leading the statutory bodies to terminate the removal programme. It is likely that introduced North American beavers would survive well in the British landscapes so given the animal welfare concerns and likely public reaction to any beaver cull programmes, it is important to ensure that this species does not establish.

Although unlicensed, the Tayside beaver population has been successful in terms of creating a population which is free from disease and genetically viable, composed of individuals that are successfully breeding and apparently well adapted to the environment. There is no evidence to suggest this unauthorised population is not well suited to the Scottish environment or that the welfare of individuals released or born into this habitat is compromised in any way. This may be due to 'good luck' rather than proactive planning but significant resources have been required to answer a range of uncertainties and to establish health and genetic status. The close genetic relatedness may yet have repercussions for the long-term viability of this population. However, the Tayside population could provide a reasonable source of founding individuals for any subsequent reintroductions, although further genetic management to encourage diversity is recommended, including considering the possibility of bringing additional founders to the British Isles in properly licenced translocations.

Conclusions

Reintroduction is an increasing popular wildlife tool, involving the intentional movement and release of species, to re-establish self-sustaining populations within the historical distribution from which it has disappeared. However, despite numerous publications calling for collaboration, there are still fundamental differences in how various disciplines (ecologists, wildlife managers, veterinary surgeons) approach species restorations. Genetic diversity of founders tends to receive less attention than other factors despite its importance in ensuring that any population has the most adaptive potential to evolve and adapt to future changes such as exposure to new parasites or a changing climate.

Active conservation and management measures have ensured that the extinction of the Eurasian beaver was averted. Beaver restoration in Britain has been a long, uncoordinated affair, and the impact of unofficial releases is still being debated. Apart from the Tayside animals, beaver 'populations' in Britain are small and widely distributed such as those in Knapdale and Devon. Small populations are vulnerable to stochastic events and the limited genetic diversity typical of these populations requires management to ensure the best possible chance for future adaptation and long-term survival in Britain. We propose beaver restoration should focus on establishing populations with broader genetic diversity than currently present, within the context of a metapopulation management plan (including conservation translocations) designed to ensure population viability into the future.

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Measuring Connectivity and Gene Flow: An Example in the Hazel Dormouse

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Keywords: connectivity, conservation genetics, dormice, gene flow

Effective management of species relies on functional connectivity between different populations to allow dispersal and genetic mixing. Genetically diverse populations harbour the variability needed to cope with changing environmental conditions such as climate disruption and habitat alteration. This article discusses the use of genetic techniques to assess the level of connectivity between populations of hazel dormice to improve conservation management of the species.

A dormouse in daily torpor on a cold spring morning.

Introduction

A prerequisite of conservation management is an understanding of how animals and plants within an ecosystem interact with one another, both within and between species. As part of this, applied ecologists try to define discrete units or populations and estimate the movement of individuals between them. Conservation genetics can help to establish how plants and animals disperse across the landscape and how this is affected by habitat fragmentation and connectivity.

As part of a project to conserve the hazel dormouse *Muscardinus avellanarius* in the UK, genetic tools were used to assess the level of connectivity between woodland patches; measure the degree to which roads, railways and waterways restrict the movement of individuals; and assess the value of specially created connective habitat such as hedgerows or habitat bridges.

Woodlands are rich wildlife habitats, providing space for thousands of different species. However, the UK has lost most of its ancient woodland due to clearance for agriculture, fragmentation and replanting with timber species. UK woodland cover has fallen to less than 12% of total land cover from an estimated high of around 75% around 6,000 years ago (Watts 2006). This fragmentation of large areas of continuous woodland has altered the distribution and population sizes of woodland species such as the hazel dormouse by reducing connectivity and restricting the ability of individuals to disperse across landscapes.

What is conservation genetics and how can it aid management?

In conservation genetics, we measure how genes move across a landscape, known as 'gene flow'. We can identify populations of conservation concern where genetic variability is limited and relate this to physical barriers such as roads or built-up areas, which are most likely to cause reproductive isolation. This can then help us target conservation activities, such as restoring hedges or creating animal bridges across roads to reconnect vulnerable populations.

Genetic diversity can be measured by investigating highly repetitive regions in our DNA known as microsatellites, where random mutations occur at relatively high rates (O'Meara 2018). Microsatellites are widely used in DNA profiling such as in forensic cases, paternity analysis and cancer diagnosis. In conservation, microsatellites are used to measure the levels of relatedness between groups of individuals and to ascertain if individuals are moving between different areas of suitable habitat or not. Where movement and dispersal is constrained, population subdivision can occur where genes are no longer exchanged. In small, isolated populations, this can result in inbreeding where mating with closely related individuals becomes the norm. This increases the frequency of harmful genes in a population and reduces genetic variability and the potential to adapt to changing environments.

Dormice

Dormice are extremely vulnerable to the effects of habitat fragmentation because they are poor colonisers and tend to avoid moving across open ground. They live naturally at very low densities, so habitat loss or a lack of connectivity between isolated populations may lead to extinction. Since the 1970s, the removal of hedgerows and new road schemes have resulted in the isolation of many dormouse populations. At a molecular level, this has led to reduced genetic mixing and a reduction in genetic diversity.

Dormice are important indicators of ancient woodland health with specific habitat requirements related to traditional woodland management and habitat complexity. Features such as mature shrubs and hazel coppice stands are important, together with year-round availability of food from hazel, honeysuckle, and bramble (Bright *et al*. 2006) (Figure 1). Due to the loss of traditionally managed, ancient woodland, dormice have a limited range and are thought to have a relatively small ecological window for dispersal (Mortelliti *et al*. 2010). UK dormice are clustered into regional genetic groups that are different from one another. This is a consequence of years of physical separation in the landscape leading to very different genetic identities (Combe *et al*. 2016).

Figure 1. Example of dormouse habitat: a dense hazel coppice (left) and an open area (right). Clearing woodland in order to allow new growth is a good management practice for dormice.

Investigation of the effects of landscape features on the dispersal and isolation of dormice can help to identify vulnerable populations, which may already be reduced to relict populations suffering from genetic inbreeding. More generally, genetic information can be used alongside regular population monitoring to inform dormice protection and conservation.

In order to investigate the genetic make-up of dormice populations, a small amount of DNA can be obtained non-invasively from hair samples collected during routine monitoring using hair-tubes (O'Meara 2018). The root cells of hair contain large amounts of DNA which can be extracted for analysis of microsatellite markers (Figure 2).

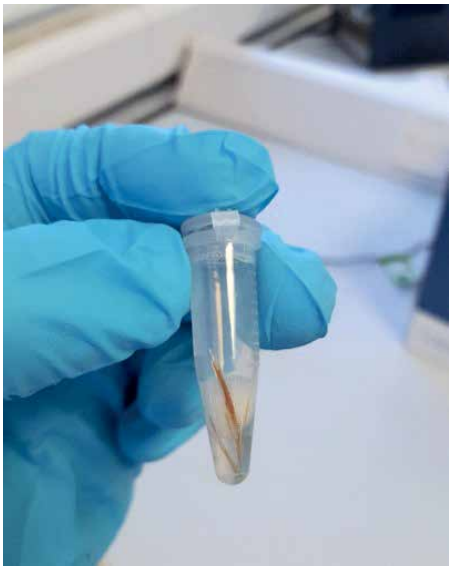

Figure 2. A hair sample collected from a dormouse; we break down the keratin and tissue to extract the DNA from the cells.

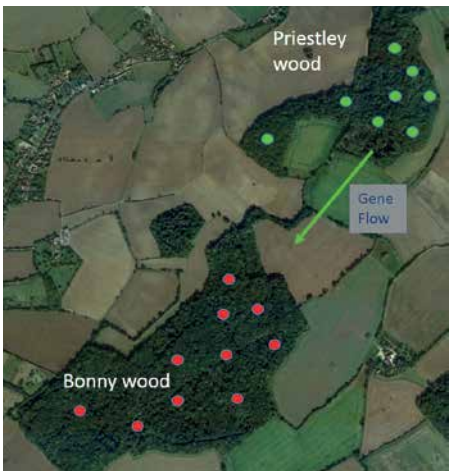

Figure 3. Priestley and Bonny Woods each support a distinct dormouse population, shown by red and blue dots.

Effects of landscape features

Dormice are the focus of a nationwide, long-term monitoring programme of more than 700 sites, co-ordinated by the People's Trust for Endangered Species. As part of this broader project, we are working with the Suffolk Wildlife Trust to investigate the role of landscape features and how they impact dormice movements in Suffolk. The Trust monitor many different populations that vary in size and degree of possible isolation due to roads, railways and other physical barriers. Our work focused on two populations, 100 m apart, at Priestley and Bonny woods (north-west of Ipswich) (Figure 3). The dormice at Bonny Wood are part of a wild population whilst dormice were reintroduced to Priestley Wood in 2000. Since the reintroduction, hedgerow

Figure 4. Connective hedgerow in Suffolk that provides a dispersal route between two woodlands.

restoration has improved habitat connectivity between the two populations (Figure 4).

Hair samples from 60 dormice were collected by Suffolk Wildlife Trust. Analysis of the DNA from individuals in each wood confirmed that Bonny and Priestley are separate populations in genetic terms but there is a small amount of gene flow between them, sufficient to sustain high levels of genetic diversity. The results also indicated that movement is predominately from the reintroduced population in Priestley Wood in the north to the established population in Bonny Wood (Figure 3).

It seems likely that an improvement in landscape connectivity between the populations has contributed to the dispersal and movement of dormice between the two sites. Further monitoring of genetic diversity is needed to establish if genetic mixing increases over time.

A hazel dormouse during routine monitoring of dormouse boxes.

Did the dormouse cross the road?

Roads are known to be significant barriers to animal movement but in some circumstances dormice will cross roads (Chanin and Gubert 2012). However, the degree to which roads impact on movement among populations is not known. DNA microsatellite analysis allows comparison of the genetic signatures of populations to measure the level of gene flow between separate populations including those isolated by physical barriers. In addition, through comparison with other healthy populations, it allows us to assess whether the level of gene flow is sufficient to avoid inbreeding and improve the long-term population viability.

Our research results to date have established that there is gene flow between dormice populations separated by both minor and major roads, and also by housing. The level of gene flow is lower than that recorded between dormouse populations separated by natural landscape features such as hedgerows (see above), but sufficient to maintain moderate levels of genetic diversity. Future work will measure genetic diversity more precisely to quantify the level and direction of gene flow between neighbouring dormouse populations.

Conclusion – monitoring, genetics and management

Conservationist management of isolated or reintroduced populations should ensure that there is sufficient habitat connectivity to allow gene flow between different populations. Genetic analysis of DNA samples from neighbouring wildlife populations allows us to quantify the success of connecting corridors or the negative effect of barriers within the landscape. Evidence that specific management actions – such as hedgerow restoration – can increase dispersal and improve genetic connectivity is invaluable to practitioners responsible for drawing up wildlife mitigation or management plans. The integration of genetic information with population monitoring data will help to conserve species such as the dormouse that are suffering from the effects of anthropogenic habitat change.

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DNA Working Group for the UK

The UK DNA Working Group provides an open forum for government agencies, academics and other stakeholders to discuss priorities for DNA-based method development, share learning and progress, explore technical challenges, develop collaborative opportunities and leverage research funding. The group holds an annual conference and the most recent one in December 2017 brought together over 120 delegates from around the world at the University of Salford.

The Working Group began with a focus on the use of eDNA in aquatic environments but now covers all applications of this technology to monitor and understand biodiversity and ecosystems. A number of Technical Groups have been established to look at fish, pond biodiversity, invasive non-native species, marine and terrestrial applications. Groups to look at standards and reference databases are also being discussed.

The priorities of the Working Group are informed by a smaller group of end users comprising staff who are leading the development of DNA-based methods within the UK environmental and conservation agencies. In May 2017, the end users group produced a summary of areas of interest for method development and key questions that they wanted to explore with the research community.

If you want to know more about the UK DNA Working Group please contact Vicki Rhodes (Vicki.Rhodes@environment-agency.gov.uk) or Andy Nisbet (Andy.Nisbet@naturalengland.org.uk).

Natural England Appoints New Panel on Bat Conservation

Natural England has appointed a new expert panel to help shape the future of bat conservation in England.

The legal protection of bats commenced following the Wildlife and Countryside Act 1981 and was further strengthened by the Habitats Directive and subsequent Conservation of Species and Habitats Regulations. Since this legislation has been in place, national monitoring data suggests that populations of most bat species have been stable or increasing – although this is recognised as being set against large-scale historic declines. This improvement for certain bat species may be due in part to successful implementation of the above legislation. Over this time, the bat conservation movement has developed enormously and survey technology has moved on, advancing our understanding of bat ecology.

As Natural England considers innovative approaches to licensing across a range of species, it is looking at how the implementation of protected species legislation could be improved in its delivery for conservation and ensuring that regulation is applied proportionately. The Bat Expert Panel will provide a forum for generating ideas and testing Natural England's thinking with the aim of securing better outcomes for bats and stakeholders.

The panel is chaired by Natural England's Chief Scientist, Dr Tim Hill, and includes experts with a strong track record of research or achievement in bat conservation from across academic, commercial, NGO and statutory sectors. It will shape Natural England's bat reform programme and help to ensure the reform projects are informed by the best available evidence, and based on sound judgement of what is achievable. In this way it will play an important role in developing consensus and partnerships for bat conservation.

The members of the Bat Expert Panel are:

Name	Position and Organisation
Professor Kate Jones	Professor of Ecology and Biodiversity, University College London
Professor Fiona Mathews MCIEEM	Professor of Environmental Biology, University of Sussex
Jean Matthews	Former Mammal Ecologist, Natural Recourses Wales. Retired
Dr Stuart Newson	Senior Research Ecologist, Population Ecology & Modelling, British Trust for Ornithology
Professor Paul Racey	Emeritus Professor, University of Aberdeen
Paola Reason CEcol CEnv MCIEEM	Technical Director, Arcadis
Dr Peter Shepherd MCIEEM	Partner at BSG Ecology
Dr Stephanie Wray CEcol CEnv FCIEEM	President of CIEEM, Partner at Tyler Grange
Dr Carol Williams	Director of Conservation, Bat Conservation Trust
Dr Matt Zeale MCIEEM	Research Associate and Lecturer, University of Bristol

CIEEM's Policy Activities

Jason Reeves MCIEEM
Policy and Communications Manager, CIEEM

Brexit Activities

Since last November CIEEM has been busy promoting our Brexit key messages[1] to MPs and Peers in Westminster. At the time of writing, we have met with 20 parliamentarians from across the party-political spectrum, and have more dates in the diary. See the full list of who we have met on the website[1].

CIEEM's key messages on Brexit are that we believe government should:

- Establish a new, independent scrutiny body to provide appropriate enforcement of environmental legislation after we leave the EU.
- Transform land and marine management policies by using 'biodiversity net gain' as the driver to halt biodiversity loss and rebuild our stocks of natural capital.
- Introduce a new Environment Act, envisioned jointly by all countries of the UK, to provide the legislative framework for a new, bold, shared ambition for the environment.

We have completed two briefing papers (on Net Gain and a new scrutiny body) to complement the key messages leaflet. We are working on further details for a new Environment Act, but this is a much more complex issue and a longer-term goal that the Institute's Strategic Policy Panel[2] is working on. The two completed briefings will be available on the website[1] and add the necessary detail to our key messages as we discuss them with parliamentarians and others. We intend to add further briefing papers subsequently.

Since drafting our key messages, the policy environment has evolved and Environment Secretary Michael Gove has agreed to consult on a new scrutiny body for the environment and has seemingly indicated that he agrees that a new Environment Act is required. This is great news and the environmental sector must keep pushing to ensure that this ambition is delivered.

CIEEM is grateful to the following organisations, which have invested in our Brexit engagement activities:

The basis of many of our discussions with parliamentarians has been how we can use the opportunities presented by Brexit to help to deliver the best possible outcomes for the environment.

The UK government has published its 25-Year Environment Plan. CIEEM applauds the ambition of the Plan and its long-term vision for England. The Plan rightly sets out the importance of the natural environment to people and the economy, and the huge challenges facing the natural environment. These challenges also unfortunately highlight some of the shortcomings of the Plan, which is an ambitious vision but uses weak, non-committal language and lacks detail on immediate action. The Plan sets out a "blueprint" but no legally-binding targets, and where there are targets they are too far into the future. Action needs to start now, during this Parliament, and we want to see what this Government is proposing to deliver by 2022. Read CIEEM's full response to the 25-Year Plan on the website[3].

Beyond parliamentarians, CIEEM is also engaging with civil servants. The first stage of this process was two meetings with Defra directors and teams in December 2017 to discuss a new regulatory body, a single marine plan, reform of agricultural subsidies, biodiversity, and international leadership. We are following up these meetings with more on specific topic areas so that we can delve deeper into the details.

Other Policy Engagement

CIEEM had its latest 6-monthly high-level meeting with Natural England in November 2017. The meeting focused on Natural England communications, wildlife licensing reform, working together to deliver more for the environment through the planning system, and future roles for ecologists. We continue to work with Natural England on their Earned Recognition schemes and have responded to the consultation on charging for licences[4].

Diana Clark, CIEEM's Wales Project Officer, has been making useful contacts in Welsh Government and Natural Resources Wales, and is enthusiastically pushing ahead with policy engagement in Wales.

Elizabeth O'Reilly has only recently joined CIEEM as the new Ireland Project Officer, but is also keen to engage with policy issues, particularly with the support of the Irish Policy Group and Jenny Neff (Ireland Vice President).

Our engagement in Scotland has been through myself, Kathy Dale (Scotland Vice President) and other senior members. During 2018 we are hoping to be able to recruit a Scotland Project Officer to help with further policy engagement in the country.

The work in the devolved administrations and in Ireland is something that we are keen to push ahead with and this links with the new Country Policy Working Groups[5] – which will have their first meetings in the spring. Thank you to all the members who put yourselves forward to contribute to CIEEM's policy engagement at the national levels.

Alongside the policy work that CIEEM does unilaterally, we also continue to work in collaboration with partners[6] including the Association of Local Government Ecologists (ALGE), the Environmental Policy Forum (EPF), Greener UK, and Wildlife and Countryside Link.

Looking Ahead

In 2018, we will be focusing on further engagement with the devolved administrations and Ireland, progress with the EU Withdrawal Bill, responding to Defra's consultation on a new environmental scrutiny body, and responding to the Fisheries and Agriculture Bills consultations.

Notes

1. www.cieem.net/eu-referendum
2. www.cieem.net/strategic-policy-panel
3. www.cieem.net/news/460/cieem-response-to-25-year-environment-plan
4. www.cieem.net/past-consultation-responses
5. www.cieem.net/country-policy-groups
6. www.cieem.net/partnerships

Further Information:
For more information on CIEEM's policy activities or to get involved please contact: JasonReeves@cieem.net

Employment and Salary Survey – a Snapshot

Sally Hayns CEcol MCIEEM
Chief Executive Officer, CIEEM

Over 1,300 members participated in our recent survey – a fantastic response – and you have given us a wealth of data and information, which we are currently busy analysing. In the fullness of time we will publish a full report but here are some headlines.

Let us start with some positives. Figure 1 shows the extent to which respondents describe themselves as an ecologist or environmental manager or neither of these.

Regardless of role type, almost 85% of respondents (across all grades) are happy in their chosen profession (6% are unhappy) and almost the same proportion would recommend our profession to others. Many respondents cited the variety of work, the feeling of doing something good for the environment and for society, working with committed and passionate colleagues and the opportunity for continually learning more about the subject and acquiring new skills as the main reasons why it is a good career to be part of. However, many respondents, even those who enjoy their job and would recommend the profession to others, noted the long hours/unsociable hours and concomitant impact on family life, the relatively low pay and a lack of respect from others.

Worryingly, almost 10% of respondents had been unemployed for all or part of the past 12 months despite 46% of employer respondents stating that they had recruited additional staff over the course of the year. Of those respondents currently in full-time employment, 93.5% work 40 hours or less but 75% regularly work in excess of these hours, typically between 3 and 10 hours a week but sometimes more. Sixty-two percent of part-time employee respondents regularly work in excess of their contracted hours, again typically between 3 and 10 hours a week.

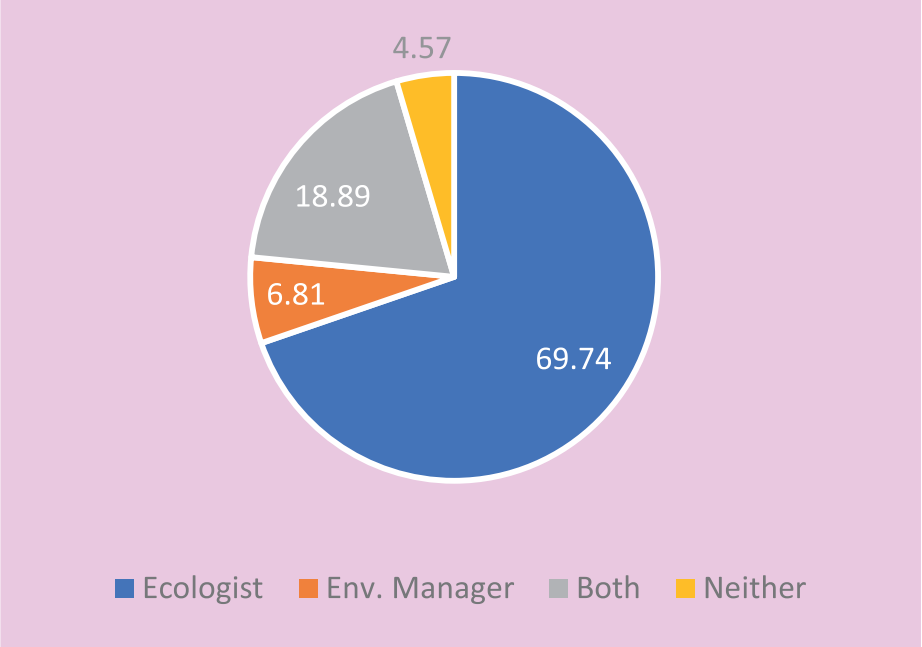

Figure 1. Percentage of respondents regarding roles

Most employed respondents receive some recompense for these hours, although only in 27% of full-time employee cases is this recompense in full (35% for part-time employees). Shockingly, in over 30% of cases respondents do not receive any form of recompense. The most common form of recompense by far is 'time off in lieu' (TOIL), although a much smaller proportion of respondents do receive overtime payments.

Over 60% of contract workers regularly work more than their contracted hours and for the majority of respondents (74%) these are recompensed in full or part.

Reassuringly, over 50% of employed respondents had seen their salaries increase over the past three years although the size of the increase has, in most cases, been modest (see Figure 2).

For self-employed respondents, 53% work less than 40 hours a week whilst 47% regularly work more than this. Generally self-employed respondents have seen their income increase (41%) or stay the same (39%) over the past 3 years. The three main benefits of being self-employed were felt to be the flexibility of working hours, independence/choice over what work to do, and the absence of interference/regulation from managers/colleagues. The main disadvantage was, not surprisingly, income uncertainty.

Almost 70% of respondents had not changed their employer or employment status over the past 3 years. Those that had had primarily stayed within their employment sector (e.g. private, public, academia, NGO, industry) with less than 25% moving between sectors. Over 50% of movers had moved to a position of higher seniority, 37% had better hours and 70% had a higher income.

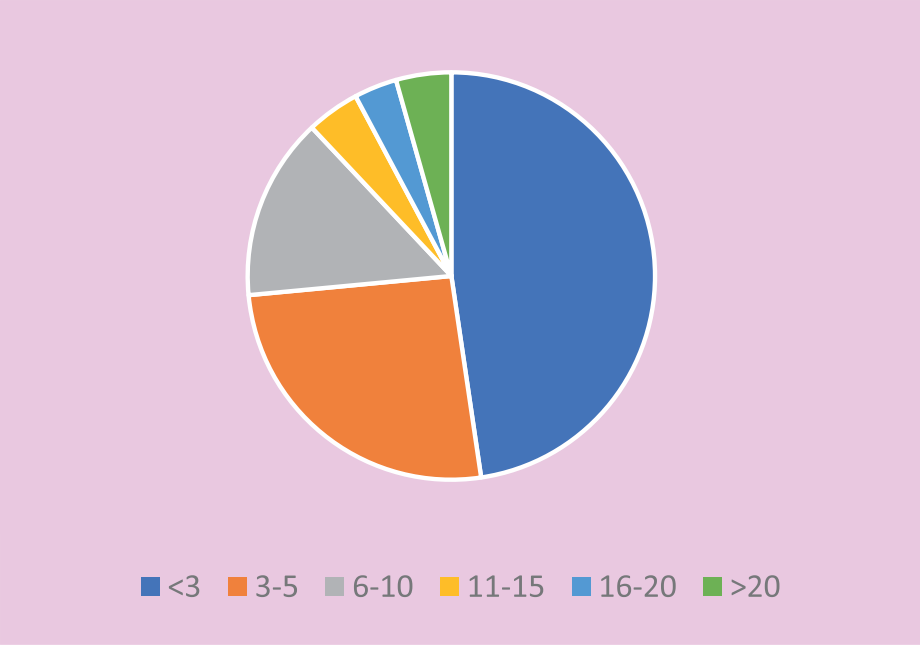

Figure 2. Percentage Salary Increase

For those who had moved from employed to self-employed status there was clear evidence that, whilst overall earnings were higher (67%), stress levels were also slightly higher (42.5% compared to 32% reporting lower stress levels) but job satisfaction was more commonly higher (64.5%).

Figure 3 illustrates the benefits, in addition to salary, that permanently employed respondents receive or would like to receive. Pension and annual leave in excess of any statutory minimum were the most common additional benefits, together with flexible working. Additional benefits that are available in some instances or would be welcomed included taking some of the listed benefits as cash equivalents, access to pool vehicles, training grants, childcare vouchers and home-working.

Overall the survey paints a picture of a committed workforce, still passionate about the work you do and frustrated by the relatively low salaries or income. However, there is also evidence to suggest that it is the un-family-friendly hours, the long working hours and the lack of consideration/support from some employers (but by no means all) which drives people from the profession. We undoubtedly need to look at ways of making the profession more attractive financially, but improving working conditions would not be a bad way to start. Certainly food for thought for the next Advisory Forum meeting.

There is still a lot of work to do on the data, including segmentation of responses by grade, geography and chartered status. We will be crunching the numbers over the next few weeks and months and will provide members with a downloadable summary later this year.

Thank you again to all those who took part.

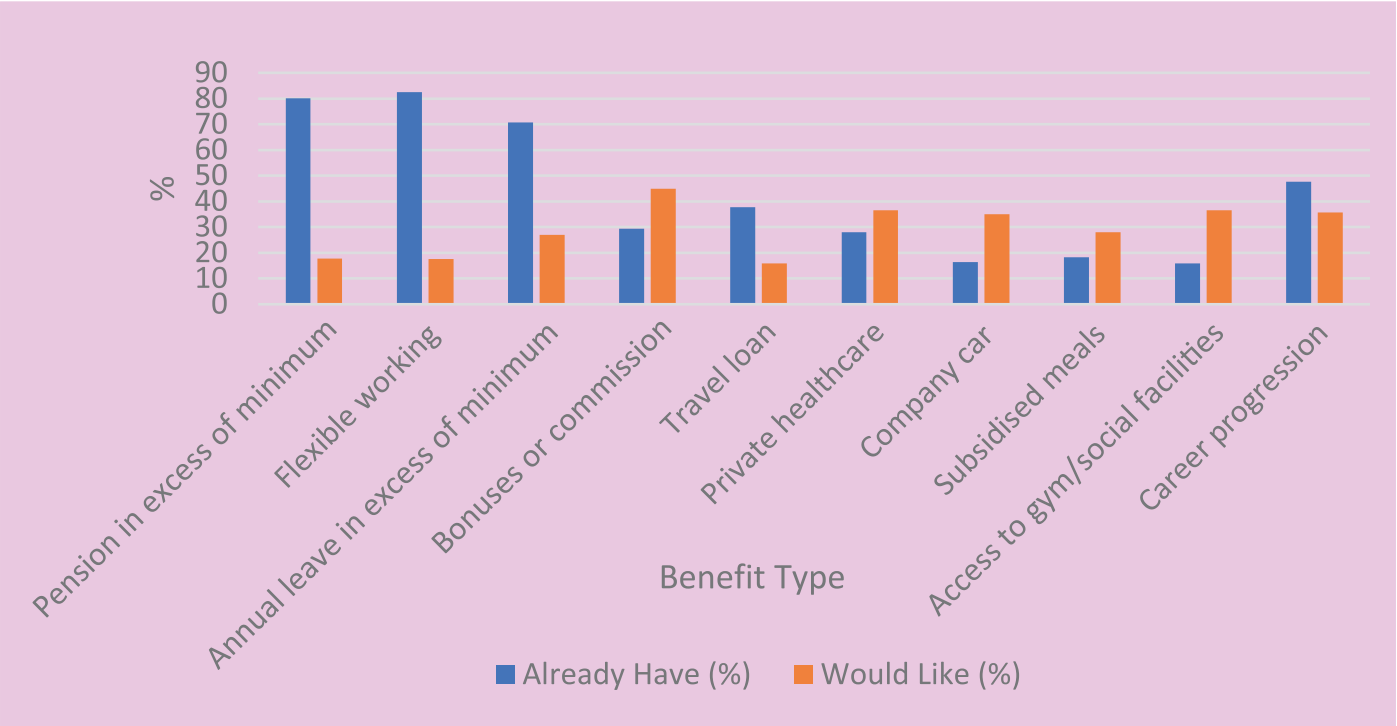

Figure 3. Benefits In Addition To Salary

New Special Interest Group:

Ecological Restoration and Habitat Creation

Nick Coppin MCIEEM, John Box CEcol CEnv FCIEEM(rtd), Penny Anderson CEcol FCIEEM(rtd) and David Parker CEcol CEnv FCIEEM

Habitat restoration on the A338 Bournemouth Spur Road has benefited reptiles including sand lizard © Chris Gleed-Owen, CGO Ecology

Why this new Special Interest Group?

Conservation of nature is of vital importance, but the 'defensive' approach (involving protected areas and species) that underpins much of our nature conservation policy and regulation is not, on its own, sufficient to halt, let alone reverse, the erosion of our natural assets. The Lawton Report (Lawton *et al*. 2010) argues that we need a step-change in our approach to wildlife conservation, involving habitat restoration and re-creation, underpinned by the re-establishment of ecological processes and ecosystem services to create a more resilient and coherent natural environment (more, bigger, better and joined).

In 2017 the CIEEM Governing Board approved the new Special Interest Group as a focal point for those whose work and interests will be important to making this happen, and to enable members to support the policy work of CIEEM in this area.

One of the three key CIEEM policy statements on Brexit 'asks' that the biodiversity net gain approach should be used to underpin the transformation of land (including planning and agriculture) and marine management policies. To achieve this net gain will require much more emphasis on ecological restoration, reclamation, transformation and habitat creation in both urban and rural environments. This has to go far beyond mitigation and offsetting when existing assets are threatened.

Recently, the Government has published *A Green Future: Our 25 Year Plan to Improve the Environment* (HMG 2018), which sets out proposals to better protect threatened species and provide richer wildlife habitats, and calls for an approach to agriculture, forestry, land use and fishing that puts the environment first (some of which is UK-wide and some will be implemented differently by each devolved country). This gives further motivation to our profession, to influence the way that this vision is turned into policy, regulatory and financial instruments, and then to provide the skills and expertise to help it happen in a sustainable way.

What is its scope and remit?

As well as biodiversity outcomes, the objectives of restoration and habitat creation will include a range of ecosystem services with applications for mitigation and compensation, re-establishing ecological networks, reconnecting people and nature, flood management, urban and post-industrial regeneration, living landscapes/seascapes, green/blue infrastructure, natural flood management and 'eco-engineering'.

The scope of this can be very broad:

a) Restoration and management of degraded or damaged ecosystems to natural or semi-natural reference ecosystems.

b) Rehabilitation or transformation of agricultural, forestry and other habitats subject to land use change or intensification.

c) Habitat creation of anthropogenic or 'novel' ecosystems, particularly through the furthering of natural processes, where the original ecosystem no longer exists.

d) Habitat and species translocation and reintroduction as part of a functioning ecosystem.

Achieving these outcomes involves an understanding of appropriate ecosystem objectives and functions, as well as the design and practical implementation processes involved.

What will the Special Interest Group be doing?

The first opportunity to 'launch' the Group was at CIEEM's 2017 Autumn Conference in Manchester. This was also an opportunity to get feedback from members, through a workshop, on the immediate priorities for the Group to address and take forward. The conclusions of the workshop, attended by about 150 delegates, are summarised in Box 1.

The Group Committee will prioritise and progress these through a range of activities, in conjunction with the Secretariat and Standing Committees, such as:

- events and field visits, in conjunction with Geographical Sections and jointly with other relevant organisations;
- developing a readily accessible knowledge base of good practice, guidance and case studies;
- developing CPD resources through seminars and webinars; and
- contributing to policy development and assisting the Strategic Policy Panel in relevant areas.

The Group will also be involved in organising the 2018 Autumn Conference on 'Habitat Re-creation and Restoration'.

Box 1. Autumn Conference Workshop Conclusions, Manchester, 22 November 2017

Four discussion groups considered the priorities for the Special Interest Group in terms of knowledge (research), evidence, policy and practice – what are the main challenges that members want to see the SIG concentrate on? There are common themes running though the feedback from each of the groups, so the conclusions are combined and summarised here.

1. Restoration Success

a) Improved understanding of the timelines and trajectories for success of restoration outcomes. Expectations are often not met. Standardised protocols for monitoring and data gathering would help.

b) Consistency in setting restoration and habitat objectives, not just plant community but ecosystem functions and services. Extend this to follow-up monitoring and enforcement, against planning conditions and ecological management plans (i.e. holding planners, developers and implementers to account).

c) Success also depends on close involvement with local communities; social functions are just as important as ecological ones.

d) Record outcomes and success of schemes, delivery mechanisms and management; make this more available to others as evidence. Make better use of existing outlets for case studies such as Conservation Evidence, anecdotal evidence as well as formal research; develop other outlets and databases (e.g. the County Biological Records Centres)?

e) Noted that the Chartered Institute for Archaeologists require that all archaeological investigations are written up and reported – should all restoration, creation, offsetting, mitigation and reclamation projects have a similar obligation?

2. Working with other disciplines

a) Fundamental for large scale projects and smaller scale work such as GI – ecological input is essential, in many cases projects should be ecology led. Ecologists need to understand engineering and hydrological processes, and how other professionals approach the built environment.

b) Ecologists working with Landscape Architects is commonplace, yet there is so often a disconnect between the LA and ecologist approaches, which needs to be explored and understood.

c) More ecological input is required into non-native landscaping, particularly in GI and making space for nature in urban settings.

d) We must move away from 'one size fits all' standard specifications for habitats such as ponds, grasslands and tree planting, involve more ecological (natural) processes and succession.

3. *Gaps in knowledge of practitioners*

a) Ecology practitioners need a broader understanding of soils and hydrology. Perception that they are focussed on plant communities or protected species and do not think of the ecosystem as a whole. Need to look at the CIEEM competencies and course accreditation to see why this might be. Mentor early-stage practitioners in these skills, and provide CPD?

b) Need a better understanding of how to work with (enable) natural succession and manage natural processes, the functioning of ecosystems, ecosystem services and how these relate to land cover and plant communities.

c) Share and develop best practice for translating opportunity mapping into practical solutions on the ground, minimum habitat size and dispersal distances, and now to engage with and involved local people.

d) The marine environment – restoration and habitat creation are just as important here but are much less well understood. Link with Marine and Coastal SIG.

e) High priority is providing a knowledge base for good practice, guidance and case studies, and providing a forum for exchange of ideas and for practitioners to request and share experience.

f) Communication between practitioners and researchers, both ways, is essential; how should we support the existing links between CIEEM, BES and the research community? Maintain a list of potential research needs and ideas for BSc/MSc/PhD projects.

4. *Policy drivers and instruments*

a) CIEEM could consider a Best Practice 'Ecological Restoration' Award?

b) The BREEAM process provides an opportunity to influence the nature of greenspace around development, so we should explore how this can be achieved.

c) Offsetting, as part of a development's planning obligation, also provides an opportunity for larger scale restoration and habitat creation.

d) The post-Brexit environmental policy regime is being addressed by CIEEM and should provide opportunities for more ecological approaches to urban, upland and lowland land management. We need to position ourselves and invest now in skills and professional infrastructure, PR and marketing, to be able to respond to the increasing demand for ecological services, well as seeking to influence how the policy statements are put into practice.

Get Involved

How the Group progresses will be down to the involvement of members. If you are interested in being directly involved we welcome additional Committee members. Details are on the CIEEM website under Member Networks.

If you wish to be kept informed and updated on the activities of the Group through the mailing list, you can elect to join by registering your preference in the Members' Area on the CIEEM website (select 'Update Personal Preferences').

Group activities will also be promoted through the usual CIEEM channels (website, *In Practice* and social media).

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CIEEM

Awards 2018

Be part of something amazing

Join us for a day of celebration.

Each year, CIEEM presents a series of awards with the overall aim of celebrating the achievements of both the profession and of individual practitioners. The 2018 celebrations will take place on Thursday 21 June at Merchant Taylors' Hall, London, where we will reveal the winners of our Award categories with a presentation by our Guest Speaker.

Tickets will be available via: www.cieem.net/cieem-awards-2018

Student Hub:
Gaining Valuable Work Experience

Amidst all the exams, the coursework and celebrations, it's easy to forget that soon you will be catapulted into a highly competitive job market and looking for that dream role. As Ollie Ramm, Director of RammSanderson notes: *"Employers receive hundreds of applications from graduates every year. There are fewer jobs available than there are candidates, meaning competition between candidates is rife. You need to stand out from the crowd, show an interest in the company or make contact to be selected over your peers."*

Employers are looking for candidates who can demonstrate at least some basic skills in technical and transferrable activities. CIEEM's Competency Framework is a great starting point to help you see what kind of skills employers are looking for. If you don't feel like you're there yet, work experience can be a great way of defining your skill set and showcasing your potential. You could gain relevant skills in paid work, volunteer or intern roles within or beyond the environmental sector; read on for our suggestions on how to get the most from your experience.

- ✓ List the skills you want to develop.
- ✓ Expect employers to offer you training, management and support to develop the skills and knowledge you want to gain.
- ✓ Plan interview questions to find out if an opening really will help you build the skills you need and how you'll be supported.
- ✓ However exciting an organisation may seem, avoid accepting a role that doesn't offer you scope to build the skills and knowledge you need.
- ✓ Keep a portfolio documenting the skills and experience you are building – many online websites can help you prepare a basic, shareable portfolio which allows you to set up a basic professional portfolio for free and include images, files, weblinks and videos that you can share with potential employers via one URL.
- ✓ Make sure you get your portfolio endorsed by relevant staff during work experience.
- ✓ Find out as much background as you can about projects you're involved in and further work that's planned. Read follow up reports and talk to other project staff to develop your understanding of the sector.

Additional benefits of work experience include:

- Gaining an insight into workplace etiquette and conducting business relationships.
- Acquiring hands-on skills and knowledge that you might not have had the chance to develop at university.
- Finding out what you're actually interested in! There are a range of roles and opportunities in the profession; work experience enables you to figure out the career direction you want to follow.
- Building connections who you can approach for recommendations and guidance when you graduate and start applying for vacancies.

Box 1. Where can you find out about work experience opportunities?

- Environment Jobs – **www.environmentjob.co.uk**
- Countryside Jobs Service (CJS) – **www.countryside-jobs.com**
- Conservation Jobs – **www.conservationjobs.co.uk**
- Direct approaches to employers in the CIEEM Professional Directory
- CIEEM Jobs board – **www.cieem.net/jobs**
- Linked In – Search for or request work experience opportunities

Robyn Thompson is studying on a CIEEM-accredited degree BSc Biological Sciences (Ecology and Environmental Management). Robyn undertook work experience within the Estates department at Nottingham Trent University:

"During my work experience as the 'Environmental Engagement Assistant' in the Environment Team at Nottingham Trent University, I was actively involved in maintaining and updating the institution's ISO 14001 and EcoCampus Environmental Management Systems. I also supported staff and student sustainability engagement initiatives; creating and managing my own projects, for example; new social media channels and a medium-sized allotment volunteering scheme. This fantastic experience greatly developed my knowledge in numerous areas of sustainability which has both supported my final year studies and helped me recognise the type of environmental management career I intend to pursue post-graduation!"

Sponsorship & Exhibition Opportunities

Your organisation could be engaging directly with ecologists and environmental managers at one of our conferences:

Irish Conference: *Making Mitigation Work* - 26th April, Dublin

Summer Conference: *Fit for the future: Developing an ecologically resilient protected sites network* - 12th July, London

Autumn Conference: *Habitat Recreation and Ecological Restoration* - 20th-21st November, Glasgow

If you would like information and costs about exhibiting at one of our conferences or becoming a sponsor, please contact emmadowney@cieem.net

Why should you be a Chartered Ecologist?

Stuart Parks
Membership Manager, CIEEM

CIEEM's register of Chartered Ecologists is still very much in its infancy, but is growing with each new set of applications into a body of professionals increasingly recognised as being at the top of their game. As we continue to meet with parliamentarians and work with sector partners to develop informed, sensible proposals to meet the challenges posed by the UK's decision to exit the European Union, it is vital that this work is underpinned by harnessing the skills and experience of our members at the highest level. The Register of Chartered Ecologists, as an obvious benchmark of the professionalism and expertise of our members, plays a key role in reinforcing CIEEM's reputation as a body that is worth both working alongside and listening to. At the moment the Register makes up just a small percentage of our overall membership. We know that many of you are ready to become a Chartered Ecologist and have yet to take the next step, so to help you decide if it could be for you, we asked the current Chair of CIEEM's Registration Authority, Penny Anderson, why experienced ecologists should consider becoming Chartered (see box on right).

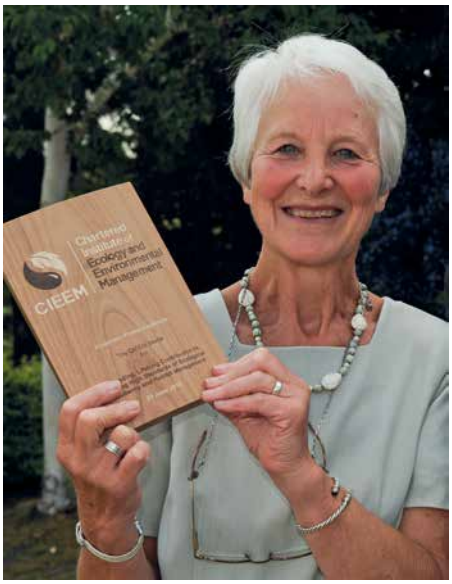
If this strikes a chord with you and you have in the region of 10 years' relevant experience (don't forget relevant voluntary work counts too) why not take a look at the application process and see whether the time has come for you to become a Chartered Ecologist? Our Registration Officer, Michael Hornby, is available to support you through the process, answer any questions you may have and to help you achieve Chartered status.

More information:
www.cieem.net/chartered-ecologist

Ways to get started:

- read the comprehensive guidance materials and watch the short introductory video on the CIEEM website;
- look out for a CEcol workshop or webinar on how to apply;
- familiarise yourself with the competency framework if you've not yet done so - using it successfully is the key to a good application;
- consider whether a mentor would help - we can try to organise one for you if you need it;
- have a look at the next deadlines for applications and give yourself plenty of time to complete an application - you need to do yourself justice!
- if it is a long time since you have had an interview, try to organise some practice with your line manager, peers, sponsors or mentor.

We look forward to receiving your application and hope to be welcoming you to the Register soon.



Penny Anderson

"Two fundamental reasons spring to mind immediately: for the good of the profession and for your own good. We as ecologists and environmental managers work regularly with other professions, most of which have had their own chartered process for a very long time. It may not be the same as ours, but there is normally a Chartership ladder to climb. Being able to award Chartership for our members puts us firmly into the professional arena – we are seen to have reached the required benchmark; to be able to compete in terms of respect and professional competence with other professions; we can be listened to with greater belief, confidence and assurance – this takes us completely out of the realm of tree-huggers or whatever else comes to mind as the proverbial image. We are working as equals on the same stage. I assure everyone reading this who is under 40, that you might be taking these things for granted now (although there is still some way to go) but those of you in an older age bracket will remember how we struggled to be heard, struggled to have our say, were subservient to other professions who took it upon themselves to speak for us (not very effectively) and ecologists and environmental managers were not seen as important or game changing, as they now can be."

"Being Chartered is also a major personal achievement. It shows that you have good levels of competence across a broad range of subject areas and it demonstrates that you work to a high level of professional conduct. These help you gain authority, ensuring others listen to your views or give credence to your suggestions, opinions and judgement, for example in a public inquiry arena or when working with multi-disciplinary teams. Sometimes the award of our Charter enables you to climb up the professional ladder, bringing with it a better salary, more responsibility and the opportunity to help and support others."

Meet the Member Networks Team

Around half of CIEEM's 325+ volunteers form the committees that run our increasing number of Member Networks – both Geographic Sections and Special Interest Groups (SIGs). The Member Networks Team works to coordinate, support and promote these groups and our wider membership on a range of activities, including local events, student engagement and policy work.

Member Networks Coordinator **Vicky Bowskill** joined CIEEM in Winchester in June 2013, just after we became Chartered, to take up a new post tasked with supporting our established Geographic Sections and the first SIG. At the outset this involved getting out and about across the UK and Ireland to meet each of the committees to find out who they are and what sort of support they needed. Since then our Member Network activities have grown, with new SIGs being set up and Sections becoming increasingly active. Keeping pace with the support needs for this enthusiastic community of volunteers is no mean feat! Vicky is also part of CIEEM's Volunteer Working Group and has recently become a member of the Association of Volunteer Managers. The Volunteer Working Group aims to raise the profile of all our volunteers, and to provide consistency in how our volunteers are supported and managed.

After studying practical conservation at further education, Vicky went on to a career spanning both training administration in the public and private sector, and 10 years in countryside access for various local authorities in England, most recently Hampshire Country Council in Winchester. This involved working with many planning committees and Local Access Forums. In 2012 she took the plunge and began a BSc (Hons) Environmental Sciences with the Open University, which she will (finally!) complete in 2018.

Outside work and study Vicky enjoys anything creative and can be found handling socialised wolves a couple of weekends a month near Reading!

Diana Clark has been a CIEEM member since 2005, has been an elected member of both the East Midlands and Welsh Section Committees. She joined the Secretariat as member of staff in August 2016, working two days a week as Project Officer (Wales) based in Swansea. This is a wide-ranging role that sees Diana working closely with colleagues in Winchester, the Welsh Section Committee, the CIEEM Vice President (Wales) and our wider membership in the country. The aim of the role is to raise CIEEM's profile in Wales and to increase our engagement across the sector, including training provision, student engagement and policy work, with the founding of a new Welsh Policy Group early in 2018.

Diana studied for her BSc (Hons) Environmental Biology at Swansea University and MSc Plant Diversity (Vegetation Survey and Assessment) at Reading University and has experience working in ecology in both the UK and New Zealand. Alongside her two days a week with CIEEM, Diana runs her own freelance ecological consultancy (Koru Ecology Associates), and outside work she enjoys messing about in kayaks, cycling and playing traditional fiddle in a ceilidh band.

Elizabeth O'Reilly joined the team in November 2017, working two days a week as Project Officer (Ireland), based in County Louth. Her role is very similar to Diana's, forming an important on-the-ground link between the CIEEM team in Winchester and our volunteers, members and potential members in both Northern Ireland and the Republic of Ireland.

Prior to joining CIEEM, Liz spent time travelling the world, which included working on dairy farms along the way, something she still does closer to home alongside her CIEEM work. Before that Liz gained experience in marine and freshwater policy with the Socio-Economic Marine Research Unit (SEMRU), the Sustainable Water Network (SWAN) and the Marine Institute. She has experience as a volunteer committee member for the Environmental Science Association of Ireland (ESAI) and gained a Bachelor of Arts in Science, Zoology at Trinity College Dublin and an MSc in Ecological Management and Biological Conservation at Queens University Belfast. Outside work Liz enjoys crafting and working in her garden, growing vegetables and tending to chickens.

Vicky, Diana and Elizabeth are always keen to hear from members who are interested in getting involved in CIEEM activities, whether that is running an event, joining a committee, talking to students or feeding into policy engagement work. They especially enjoy meeting members at our conferences, Annual Members' Meetings and other CIEEM events. We hope to grow the team to include a Project Officer post in Scotland during 2018.

You can reach the current team at:
vickybowskill@cieem.net
dianaclark@cieem.net
elizabetho'reilly@cieem.net
Find out more about Member Networks at:
www.cieem.net/member-networks

British Ecological Society

Richard English
Communications Manager

A very big, belated Happy New Year to our colleagues in CIEEM! We are excited to be working with you on a number of initiatives this year; we love collaborating, so drop me a line if you would like to chat through an idea.

Our Annual Meeting, in Ghent before Christmas, was a prime example of successful collaboration. Ecology Across Borders was organised with three international organisations: Gesellschaft für Ökologie (GfÖ) and NecoV, in association with the European Ecological Federation (EEF). One thousand five hundred international delegates braved airport closures, road blockages and snow drifts to join us for 13 parallel sessions, two poster sessions, 13 career-progressing workshops and a fun, inclusive social programme that was a perfect segue into Christmas. Our plenaries speakers' (Iain Couzins, Sue Hartley, Carlos Herrera and Louise Vet) talks and our oral presentations will be online, so do check out our YouTube channel.

We are committed to ensuring our high quality, peer-reviewed content is accessible to a broad audience – and all five of our journals publish blogs giving background and context to the work we publish, as well as highlighting key pieces of research using non-specialist language. All articles published in *Functional Ecology* also have a dedicated plain language summary (fesummaries.wordpress.com), published alongside the article, ensuring that the key messages of the article are accessible to all. We also have a series freely available guides (www.britishecologicalsociety.org/publications/guides-to) to better science, which provide practical information on various aspects of publishing. Our most recent guide to reproducible code was published in December 2017 and is the latest in the series that also covers data management, getting published and peer review. Our publications team regularly runs training workshops on all aspects of academic publishing so, if your organisation is interested in publishing your research in academic journals and need any advice, please do get in touch.

Don't miss the fantastic Special Feature 'Functional traits in agroecosystems' in Issue 1 of *Journal of Applied Ecology* (http://bit.ly/JAPPL55_1). You can also visit the journal blog for a variety of interesting posts, videos and infographics on the papers (https://jappliedecologyblog.wordpress.com). Also in this issue, a study by Soanes *et al*. highlights the importance of using genetic techniques not just to evaluate the success of road-crossing structures for wildlife, but also to guide their placement within the landscape.

Keeping with the theme of this *In Practice*, a recent open access Review article in *Methods in Ecology and Evolution* (http://bit.ly/MEEeDNA) presents guidelines created by environmental DNA researchers from around the world for implementing eDNA methods to detect aquatic macro-organisms.

Our global reach is seen in our journal impact, but also in our membership. We have over 6,000 members located in 120 countries, so joining us will allow you to become part of our worldwide community of ecologists – as well as access to discounts on all our events and member-only research grants.

Membership starts at £21.00 at our student or concession rate, and is **FREE** for undergraduate, master's or first-year PhD students for their first 12 months. If you have access to a UK bank account or are based in the Single Euro Payment Area (SEPA), you can pay by Direct Debit – taking the effort out of renewals. Email Helen, our Membership Manager, for more information: helen@britishecologicalsociety.org

Our policy team continues to facilitate the engagement of ecologists with policy-making, and recent key topics include sustainable land management and marine conservation. Briefings on each issue, informed through member consultations and workshops, have been in development. With the expected Agriculture Bill and the ambitions set out in the 25-Year Environment Plan for both marine and agriculture, these briefings will be used to highlight important ecological issues and needs with key decision- and policy-makers. Through the Environmental Policy Forum, BES and CIEEM will continue to collaborate on key environmental policy topics.

Our policy team continues to develop further material in the Policy Guide series. Policy Guides provide an accessible resource about the legislative and policy formulation processes across the UK. Future Policy Guides may look at international policy processes, as well as producing targeted information on topical policies. The Guides aim to improve communication between members and policy-makers, and therefore help increase the impact of ecological research and support evidence-informed policy-making. However, if any of CIEEM's membership are interested in getting involved in writing a Guide or being interviewed to provide their tips on policy engagement, Camilla (camilla@britishecologicalsociety.org) would love to hear from you.

Contact
richard@britishecologicalsociety.org
www.britishecologicalsociety.org
@BritishEcolSoc

Member Network News

CIEEM has regional Geographic Sections across England and national Sections in Wales, Ireland and Scotland. Special Interest Groups (SIGs) provide a focus for activity in particular topic areas of ecology and environmental management.

Each is run by a volunteer committee, providing opportunities to network, share knowledge and learn more about the science and practice of our profession.

There are currently about 160 Member Network volunteers. For further information about what they get up to and how you can get involved, please visit www.cieem.net/member-networks.

WEST MIDLANDS

Urban Ecology: Bats, Otters and Waterways

23 November 2017, Birmingham

The event was very well attended with almost 30 ecologists present to hear a series of talks on the ecology of waterways in the Midlands region. Three speakers presented inspirational talks covering: research on Midlands otters (Samantha Mason); the urban ecology of our waterways (Paul Wilkinson); and development of a 'Batlas' for urban bats of the Birmingham and Black Country area (Charlene Jones).

Samantha Mason presents her Masters research on Midlands otters

SOUTH WEST ENGLAND

South West England Section Conference

Life in Earth: Soils, the Forgotten Science in Ecology

held in association with South West Soils Discussion Group

6 December 2017, Okehampton

This conference, organised jointly with the British Society of Soil Society (BSSS) South West Soils Discussion Group, was held at the prestigious North Wyke Rothamsted Research site. Seventy delegates gathered to hear about the importance of soils and their structure to ecologists when planning and implementing schemes on the ground and when addressing problems that sometimes arise. We were also treated to a tour of the labs and farm site at this world-leading, non-profit research centre that focuses on strategic agricultural and soil sciences.

You can find presentations from the day at www.cieem.net/previous-conferences.

SCOTLAND

Scottish Conference 2018

Wildlife tourism in Scotland: A wildlife destination or a destination for wildlife?

24 January 2018, Aberdeen

Scotland was recently voted the most beautiful country in a Rough Guides readers' poll and this conference looked at a range of terrestrial and marine case studies focussing on some of Scotland's most iconic species to see how they are faring under this growing attention.

You can find presentations from the day at www.cieem.net/previous-conferences.

Look out for upcoming events in your area and keep up to date with what's been going on at www.cieem.net/member-networks.

For information on vacancies in your Member Network committees visit www.cieem.net/cieem-committee-vacancies.

JOIN OUR NETWORK

- **Enhance your CV**
- **Gain professional recognition**
- **Network with others in your field**
- **Get involved with projects**

New Members

The decision on admission (except for Chartership and Student members) is usually taken by the Membership Admissions Committee under delegated authority from the Governing Board but may be taken by the Governing Board itself.

CIEEM is pleased to welcome the following individuals as new and Chartered members:

ADMISSIONS

Chartered Ecologist (CEcol)
Neil Beamsley, Stewart Clarke, Jan Collins, Emma Hatchett, Morgan Hughes, John Jones, Jamie Woollam

Chartered Environmentalist (CEnv)
Katherine Baggaley, Sharon Bayne, Emily Castel, Christopher Gerrard, Rachel Hardy, Ellen Harpham, Andrew Nyul, Anna Parry, Morgan Taylor, Leyton Williams, Lorraine Woolley, Nicholas Wright

Full Members (MCIEEM)
Samuel Arthur, Sara Blackburn, Dr Anna Gilchrist, Joanne Gilvear, Philippa Hamshaw, Christopher Rodger, Dr Toru Tsuzaki, Nancy Wilkinson

Upgrades to Full Membership (MCIEEM)
Aislinn Harris, Gabrielle Horup, Kylie Jones, Hannah Tracey

Associate Members (ACIEEM)
Tas Adcock, Callum Gilhooley, Sam Mardell, James Spencer, Andrew Stanger, Rosalind Watkins

Upgrades to Associate Membership (ACIEEM)
Joanna Barratt, Thomas Clemence, Rhiannon Ferguson, Joshua Mitchell, Cerian Thomas, Jordan Todd

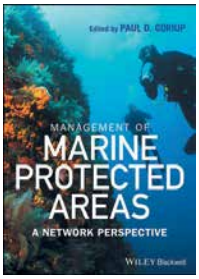
Graduate Members (Grad CIEEM)
Helen Calver, Laura Goble, Rosie Harper, Amy Harris, Ashleen Higgins, William Hurry, Adam Jamieson, Ross Johnson, James Pickerin, Ellen Quinton, Amy Smith, Christopher Stone, Darren Storey, Katie Watson

Upgrades to Graduate Membership (Grad CIEEM)
Ben Lappage, Russell Mansfield, Robert Monje, Ben Payne, Amelia Reddish, Melissa Sullivan, Trystan Thomas

Qualifying Members
Marc Anderton, Lucy Grable, Kirandeep Johal, Jill Marples, Sharon O'Neill, Dr Jeremy Palmer, Emma Randall

Student Members
Kate Adams, Samantha Ambler, Iona Anderson, Lewis Badham, Mohammad Badiuzzaman, Kerry Baker, Maya Baker, Lara Bates-Prior, Richard Batey, Fiona Bell, Caitlin Bell, James Bellis, Archie Bird, Grace Bishop, Ellie Booth, Nils Bouillard, Toni Bradley, Fay Brotherhood, Alexandra Bull, Elliott Burns, Florence Butler, Christian Cairns, Thomas Casey, Joseph Chidzey, Manuel Chopitea Kober, Andrew Coe, Euan Connell, Sophia Couchman, Kyle Cullen, Michaeljohn Cullen, Fintan Damer, Hayley Dean, Lynsey Devine, Lisa Douglas, Paulina Drozdowska, David Eastwood, Natalie Elms, Tracy Evans, David Feitschinger, Kate Flood, Loren Freslaud, Bianca Gal, James Gamble, Martin Garea-Balado, Dan Gibson, Jack Glover, Carl Goldsack, Helen Greaves, Rachel Harper, Natalie Harvey, Laura Harvey, Mara Hernandez, Ben Hinder, Catherine Hinds, Robbie Holden Cooper, Natalie Hoyland, Rossanne Imaita, Claire Inglis, Gareth James, Stephanie James-Melling, Rebecca Jennings, Julia Jung, Ilona Kater, Lois Kelly, Colleen Layton, Kirsty Lee, Jakob Leigh, Kelly Lench, Elinor Lewis, Leith Livingstone, Sam Lloyd, Elliott Lloyd, Rebecca Lloyd, Matthew Lory, Diana Luke, Nicholas Lumley, Remi Maeda, Emma Magee, Sabah Malik, Rebekah Mayhew, Cian McGlinchey, Kieran McGranaghan, Robert Middleton, Nicola Milburn, Catriona Miller, Jake Modica, Kate Morgan, Nina Mulligan, Sylvia Myers, Sarah Nicholson, Dylan Owen, Kelly Park, Charlie Patel, Theo Patten, Mohammad Patwary, Bethany Pearson, Richard Penrose, Horaine Pickersgill, Mohammad Rahman, Shona Redman, Jack Riggall, Elena Ruiz, Dylan Sach, Elysia Salmon, Emma Scotney, Prateek Sharma, William Silkstone, Neil Smith, Fraukje Sportel, Melissa Stephenson, Alytta Teuber, Bethany Thompson, Emily Thomson, Owen Thorogood, Will Townsend, Emma Ulyatt, Sophie Vickress, Lindsay Walker, Dominic Wallace, Lyndsay Wayman-Rook, Rachel West, Jack Wheeler, Rebecca White, Gabriella White, Alexander Willey, Benedict Williamson, Margerita Wilson, Kayleigh Winch, Jennifer Wrayton, Louise Wyatt

Recent Publications



Management of Marine Protected Areas: A Network Perspective

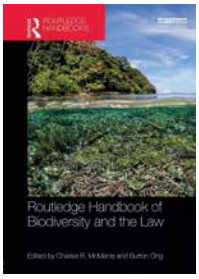
Editor: Paul D. Goriup CEcol CEnv FCIEEM

ISBN: 978-1-119-07577-6

Available from: www.wiley.com

Price: £89.95

This publication draws on the results of a major EU-sponsored research project related to the establishment of networks of MPAs in the Mediterranean and Black Seas that transpired from February 2011 to January 2016. Featuring contributions by leading university- and national research institute-based scientists, chapters utilise the latest research data and developments in marine conservation policy to explore issues related to ways in which networks of MPAs may amplify the effectiveness and conservation benefits of individual areas within them.



Routledge Handbook of Biodiversity and the Law

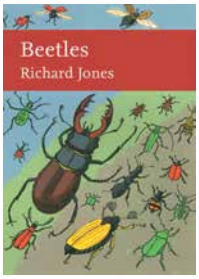
Editors: Charles R. McManis and Burton Ong

ISBN: 9781138693302

Available from: www.routledge.com

Price: £175.00

This publication provides a reference textbook and comprehensive compilation of multi-faceted perspectives on the legal issues arising from the conservation and exploitation of non-human biological resources. Contributors include leading academics, policy-makers and practitioners reviewing a range of socio-legal issues concerning the relationships between humankind and the natural world. The Handbook includes chapters on fundamental and cutting-edge issues, including discussion of major legal instruments such as the Convention on Biological Diversity and the Nagoya Protocol.



Beetles (New Naturalist Series Volume 136)

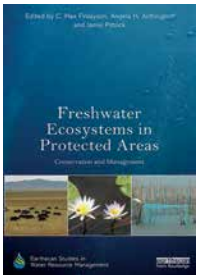
Author: Richard Jones

ISBN: 9780008149505

Available from: www.nhbs.com

Price: £27.99

Beetles are arguably the most diverse organisms in the world, with nearly half a million beetle species described and catalogued in our museums, more than any other type of living thing. This astonishing species diversity is matched by a similar diversity in shape, form, size, life history, ecology, physiology and behaviour. Beetles occur everywhere, and do everything. And yet they form a clearly discrete insect group. Richard Jones' New Naturalist volume on beetles provides a comprehensive natural history of this fascinating and beautiful group of insects.



Freshwater Ecosystems in Protected Areas: Conservation and Management

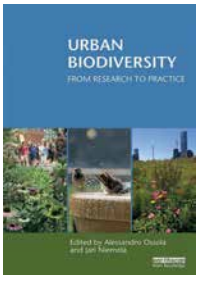
Editors: C. Max Finlayson, Angela H. Arthington and Jamie Pittock

ISBN: 9780415787147

Available from: www.routledge.com

Price: £39.99

This book shows that, rather than being a marginal part of terrestrial protected area management, freshwater conservation is central to sustaining biodiversity. It focuses on better practices for conserving inland aquatic ecosystems in protected areas, including rivers, wetlands, peatlands, other freshwater and brackish ecosystems, and estuaries.



Urban Biodiversity: From Research to Practice

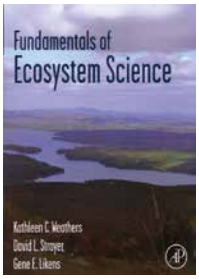
Editors: Alessandro Ossola and Jari Niemelä

ISBN: 9781138224391

Available from: www.routledge.com

Price: £36.99

This book is designed to fill this cultural and communicative gap by discussing a selection of topics related to urban biodiversity, as well as its benefits for people and the urban environment. It provides an interdisciplinary overview of scientifically grounded knowledge vital for current and future practitioners in charge of urban biodiversity management, its conservation and integration into urban planning. Topics covered include pests and invasive species, rewilding habitats, the contribution of a diverse urban agriculture to food production, implications for human well-being, and how to engage the public with urban conservation strategies.



Fundamentals of Ecosystem Science (2nd Edition)

Editors: David L. Strayer and Gene E. Likens

ISBN: 9780128127629

Available from: www.nhbs.com

Price: £68.95

This is a solid introduction to modern ecosystem science covering land, freshwater and marine environments. This edition covers major concepts of ecosystem science, biogeochemistry, and energetics. Case studies are also included, offering insights into how adopting an ecosystem approach has helped to solve important intellectual and practical problems.

Ecology (International 4th Edition)

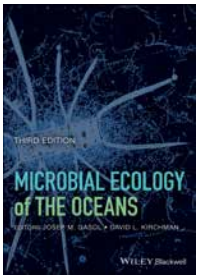
Authors: William D. Bowman, Sally D. Hacker and Michael L. Cain

ISBN: 9781605357973

Available from: www.nhbs.com

Price: £88.99

The new fourth edition of Ecology maintains its focus on providing an easy-to-read and well-organized text for instructors and students to explore the basics of ecology. This edition also continues with an increasing emphasis on enhancing student quantitative and problem-solving skills.



Microbial Ecology of the Oceans (3rd Edition)

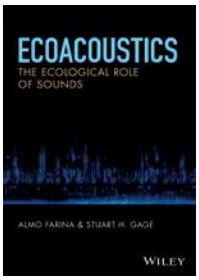
Editors: David L. Kirchman and Josep M. Gasol

ISBN: 9781119107187

Available from: www.nhbs.com

Price: £104.00

This third edition features new topics as well as different approaches to subjects dealt with in previous editions. Chapters discuss ecology, diversity and function of microbes and of microbial genes in the ocean, as well as the structure of the microbial ecosystem, discussing in particular the sources of carbon for microbial growth.



Ecoacoustics: The Ecological Role of Sounds

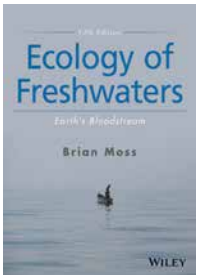
Editors: Almo Farina and Stuart H. Gage

ISBN: 9781119230694

Available from: www.nhbs.com

Price: £99.99

This comprehensive book collates and reviews the science behind ecoacoustics; illustrating the principles, methods and applications of this exciting new field. This invaluable resource provides an unrivalled set of ideas, tools and references based on the current state of the field.



Ecology of Freshwaters: Earth's Bloodstream (5th Edition)

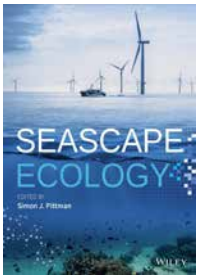
Author: Brian R. Moss

ISBN: 9781119239406

Available from: www.nhbs.com

Price: £59.95 (due May 2018)

This established textbook continues to provide a comprehensive and stimulating introduction to rivers, lakes and wetlands and is written as a basis for an entire course on freshwater ecology.



Seascape Ecology

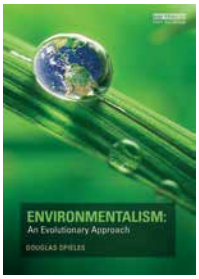
Editors: Simon James Pittman

ISBN: 9781119084433

Available from: www.nhbs.com

Price: £79.95

This publication provides a comprehensive look at the state-of-the-science in the application of landscape ecology to the seas and provides guidance for future research priorities. It is comprised of contributions from researchers at the forefront of seascape ecology working around the world, presenting the principles, concepts, methodology, and techniques informing seascape ecology and reports on the latest developments in the application of the approach to marine ecology and management. A growing number of marine scientists, geographers, and marine managers are asking questions about the marine environment that are best addressed with a landscape ecology perspective. Seascape Ecology represents the first serious effort to fill the gap in the literature on the subject.



Environmentalism: An Evolutionary Approach

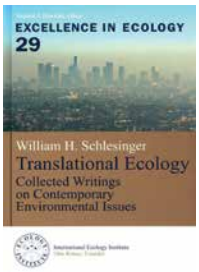
Author: Douglas Spieles

ISBN: 9781138502420

Available from: www.routledge.com

Price: £36.99

The premise of this book is that our environmental dilemmas are products of biological and sociocultural evolution, and that through an understanding of evolution we can reframe debates of thought and action. The purpose is to explain the wide variety of environmental worldviews, their origins, commonalities, points of contention, and their implications for the modern environmental movement.



Translational Ecology: Collected Writings on Contemporary Environmental Issues

Editor: William H. Schlesinger

ISBN: 9783946729297

Available from: www.nhbs.com

Price: £29.99

The author believes that scientists have a duty to translate scientific research for non-specialists. This engaging book draws together many of his general-interest writings, on topics ranging from climate change and species extinction to human population dynamics. In accessible language peppered with personal anecdotes he explores the origin of contemporary environmental problems and in many cases offers solutions.

Copying ancient woodlands: a positive perspective

Smith, P.L.

Biodiversity and Conservation 2017, https://doi.org/10.1007/s10531-017-1494-6

This paper makes a case for the deliberate creation of woodlands with ancient characteristics. Some facets of ancient woods are irreplaceable and their destruction is discouraged. Nevertheless, as human population and impact increase, this resource will degrade. Woodland with many characteristics of ancientness can be deliberately created. The intricacy that defines ancient woodland could be assembled in timescales of less than a century.

Ascension Island's anthropogenic cloud forest provides a precedent. For utilitarian purposes, human-mediated plant dispersal to the island, led to the transformation of species-poor, fern-dominated hillsides to species–rich cloud forest in about 100 years. How much more could be achieved with appropriate ecological intention?

Read at: http://rdcu.be/C9RD

Reintroducing the Eurasian beaver *Castor fiber* to Scotland

Gaywood, M.J.

Mammal Review 2018, 48: 48–61. doi:10.1111/mam.12113

This review looks at the issues surrounding beaver reintroduction to Scotland between 1995 and 2015. This was one of the most detailed assessments carried out for any species reintroduction proposal. It was confirmed that beavers have a positive influence on biodiversity and ecosystem services, and have the potential for socio-economic benefits. However, there are exceptions that will need to be considered. A strategic approach to developing management throughout Scotland will need to be progressed in partnership with key stakeholders.

Open access: http://onlinelibrary.wiley.com/doi/10.1111/mam.12113/full

Comparing methods suitable for monitoring marine mammals in low visibility conditions during seismic surveys

Ursula K. Verfuss *et al*.

Marine Pollution Bulletin 2018, 126: 1-18

Regulations typically prescribe marine mammal monitoring before and/or during offshore industrial activities that emit loud sounds. The authors review which monitoring methods are suitable and compare their relative strengths and weaknesses. Passive acoustic monitoring has been implemented as either a complementary or alternative method to visual monitoring in low visibility conditions. Other methods such as RADAR, active sonar and thermal infrared have also been tested, but are rarely recommended by regulatory bodies. The efficiency of the monitoring method(s) will depend on the animal behaviour and environmental conditions, however, using a combination of complementary systems generally improves the overall detection performance. The authors recommend that the performance of monitoring systems, over a range of conditions, is explored in a modelling framework for a variety of species.

Open access: https://www.sciencedirect.com/science/article/pii/S0025326X17308809

Special Issue: Translational ecology

Frontiers in Ecology and the Environment 2017, Volume 15: 537–612

This special issue of *Frontiers in Ecology and the Environment* looks at translational ecology. Never has there been a more appropriate time for ecology and its complementary disciplines to take leadership roles in addressing the urgent environmental threats faced by our planet. It is neither morally acceptable nor in society's best interest to continue business-as-usual trajectories of climate-altering emissions, species loss, and degradation of ecosystem services. It is equally questionable whether society can afford the luxury of business-as-usual science that disproportionately values the discovery of new knowledge and understanding, but lacks sufficient commitment or clear pathways to translate knowledge into practice. Translational ecology provides an appropriate model for this linkage of scientific discovery with practical application.

Open access: http://esajournals.onlinelibrary.wiley.com/hub/issue/10.1002/fee.2017.15.issue-10/

Building partnerships with communities for biodiversity conservation: lessons from Asian mountains

Mishra, C. *et al*.

Journal of Applied Ecology 2017, 54: 1583–1591. doi:10.1111/1365-2664.12918

The need for engagement with local communities is embedded in the 2020 Aichi biodiversity targets and is widely thought to be critical to the success of conservation efforts. However, ecologists and practitioners often have little formal training in how to engage with local communities and to recognise the pitfalls and opportunities provided by developing genuine partnerships. This Practitioner's Perspective seeks to focus on the elements that practitioners and researchers need to consider when engaging with communities to effect conservation. The authors have outlined eight principles for community-based conservation that build on ideas developed in fields as diverse as applied ecology, conservation and natural resource management, community health, social psychology, rural development, negotiation theory, and ethics. They have been developed, challenged and tested through 20 years of community experience and the authors' own research on the snow leopard *Panthera uncia* and its mountain ecosystems in South and Central Asia. The authors propose that with contextual adaptations, their relevance for applied ecologists and practitioners may be universal.

Open access: http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12918/full

The importance of trees for woody pasture bird diversity and effects of the European Union's tree density policy

Jakobsson, S. and Lindborg, R.

Journal of Applied Ecology 2017, 54: 1638–1647. doi:10.1111/1365-2664.12871

Recent Common Agricultural Policy reforms aim to green the subsidy system. For example, the tree density limit for pastures to qualify for EU subsidies has been increased from 50 to 100 trees per hectare. However, recent studies show that the high biodiversity values of these habitats may be threatened by these limits. Little is known about the direct effects of tree density limitations on bird communities in woody pastures. The authors investigated how bird diversity and species composition are affected by tree density in woody pastures along a gradient of trees per hectare. The results show that tree density is not the limiting factor, but rather a driver of bird diversity and species composition in woody pastures and that tree density limits may fail to capture the whole range of biological values.

More information: http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12871/full

Forest restoration as a double-edged sword: the conflict between biodiversity conservation and pest control

Kärvemo, S. *et al*.

Journal of Applied Ecology 2017, 54: 1658–1668. doi:10.1111/1365-2664.12905

Forest restoration is increasingly implemented to counteract the negative effects of commercial forestry on biodiversity. However, restoration measures aimed at mimicking natural disturbance regimes could simultaneously increase the risk of unwanted negative effects, such as damage by forest pest species. This study compares the effect of two restoration methods (prescribed burning and gap-cutting), on both biodiversity conservation and pest control, to provide a basis for solutions to this potential conflict. The number of trees that died post-restoration was highest on burned sites, whereas no difference was found between gap-cut and reference stands. The authors demonstrate the potential for a conflict between forest restoration for biodiversity conservation and the potential risk for tree mortality caused by forest pests. The results suggest that this conflict can be moderated by the choice of restoration method. The restoration method gap-cutting had a similar positive impact on bark beetle species richness as compared to the burning method, but did not increase tree mortality as burning did. Thus, in areas where there is an apparent risk for pest outbreaks, the data suggest that gap-cutting should be the chosen method to avoid an unwanted increase in tree mortality at the stand level.

More information: http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12905/full

A national-scale model of linear features improves predictions of farmland biodiversity

Sullivan, M.J.P. *et al*.

Journal of Applied Ecology 2017, 54: 1776–1784. doi:10.1111/1365-2664.12912

The authors assessed whether a novel spatial dataset mapping linear and woody-linear features across the UK improves the performance of abundance models of 18 bird and 24 butterfly species across 3,723 and 1,547 UK monitoring sites, respectively. Although improvements in explanatory power were small, the inclusion of linear features data significantly improved model predictive performance for many species. For some species, the importance of linear features depended on landscape context, with greater importance in agricultural areas. This study demonstrates that a national-scale model of the extent and distribution of linear features improves predictions of farmland biodiversity.

Open access: http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12912/full

Urban development, land sharing and land sparing: the importance of considering restoration

Collas, L. *et al*.

Journal of Applied Ecology 2017, 54: 1865–1873.

There is limited knowledge of how best to reconcile urban development with biodiversity conservation, and whether populations of wild species would be greater under low-density housing (with larger gardens), or high-density housing (allowing more area to be left as undeveloped green spaces). The land sharing/sparing framework can be applied to address this question. The authors sampled the abundance of trees in Cambridge (UK) along a gradient of human density. They designed different scenarios of urban growth to accommodate the human population predicted in 2031. For each scenario, they projected the future city-wide tree population size and quantified its carbon sequestration potential. Although both tree populations and carbon storage appear to benefit from land-sparing development, the risk that this might widen the existing disconnect between people and nature must also be addressed. In regions which have already been cleared of intact habitat, a combination of land-sparing urban development with the restoration of green space could accommodate urban population growth whilst dramatically improving the existing status of local tree populations. Where cities are expanding into intact habitat, the merits of urban development by land sparing may be even more pronounced.

http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12908/full

Seals and shipping: quantifying population risk and individual exposure to vessel noise

Jones, E.L. *et al*.

Journal of Applied Ecology 2017, 54: 1930–1940. doi:10.1111/1365-2664.12911

Usage maps characterising densities of grey and harbour seals and ships around the British Isles were used to produce risk maps of seal co-occurrence with shipping traffic. Across the British Isles, rates of co-occurrence were highest within 50km of the coast, close to seal haul-outs. Areas identified with high risk of exposure included 11 Special Areas of Conservation (SAC; from a possible 25). Risk to harbour seal populations was highest, affecting half of all SACs associated with the species. The authors present a framework to allow shipping noise, an important marine anthropogenic stressor, to be explicitly incorporated into spatial planning. Potentially sensitive areas are identified through quantifying risk to marine species of exposure to shipping traffic, and individual noise exposure is predicted with associated uncertainty in an area with varying rates of co-occurrence.

Open access: http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12911/full

Making rewilding fit for policy

Pettorelli, N. *et al*.

Journal of Applied Ecology 2018, 00:1–12. https://doi.org/10.1111/1365-2664.13082

Rewilding is increasingly considered as an environmental management option, with potential for enhancing both biodiversity and ecosystem services. Despite burgeoning interest in the concept, there are uncertainties and difficulties associated with the practical implementation of rewilding projects, while the evidence available for facilitating sound decision-making for rewilding initiatives remains elusive. The authors identify five key research areas to inform the implementation of future rewilding initiatives:

1. increased understanding of the links between actions and impacts;
2. improved risk assessment processes (e.g. through better definition and quantification of ecological risks);
3. improved predictions of spatio-temporal variation in potential economic costs and associated benefits;
4. better identification and characterisation of the likely social impacts of a given rewilding project; and
5. facilitated emergence of a comprehensive and practical framework for the monitoring and evaluation of rewilding projects.

Global environmental change is driving some ecosystems beyond their limits so that restoration to historical benchmarks or modern likely equivalents may no longer be an option. This means that the current environmental policy context could present barriers to the broad implementation of rewilding projects. To progress the global rewilding agenda, a better appreciation of current policy opportunities and constraints is required. This, together with a clear definition of rewilding and a scientifically robust rationale for its local implementation, is a prerequisite to engage governments in revising legislation where required to facilitate the operationalisation of rewilding.

Open access: http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.13082/full

Embracing uncertainty in applied ecology

Milner-Gulland, E.J. and Shea, K.

Journal of Applied Ecology 2017, 54: 2063–2068. doi:10.1111/1365-2664.12887

Applied ecologists often face uncertainty that hinders effective decision-making. Common traps that may catch the unwary are: ignoring uncertainty, acknowledging uncertainty but ploughing on, focussing on trivial uncertainties, believing your models, and unclear objectives. The authors integrate research insights and examples from a wide range of applied ecological fields to illustrate advances that are generally under-used, but could facilitate ecologists' ability to plan and execute research to support management. Recommended approaches to avoid uncertainty traps are: embracing models, using decision theory, using models more effectively, thinking experimentally, and being realistic about uncertainty.

Open access: http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12887/full

Integrating invasive species policies across ornamental horticulture supply chains to prevent plant invasions

Hulme P.E. *et al*.

Journal of Applied Ecology 2018, 55:92–98. https://doi.org/10.1111/1365-2664.12953

Ornamental horticulture is the primary pathway for invasive alien plant introductions. The authors critically appraise published evidence on the effectiveness of four policy instruments that tackle invasions along the horticulture supply chain: pre-border import restrictions; post-border bans; industry codes of conduct; and consumer education. The authors suggest that closing the plant invasion pathway associated with ornamental horticulture requires government-industry agreements to fund effective pre- and post-border weed risk assessments that can be subsequently supported by widely adopted, as well as verifiable, industry codes of conduct. This will ensure producers and consumers make informed choices in the face of better targeted public education addressing plant invasions.

Open access: http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12953/full

The impact of even-aged and uneven-aged forest management on regional biodiversity of multiple taxa in European beech forests

Schall, P. *et al*.

Journal of Applied Ecology 2018, 55:267–278. https://doi.org/10.1111/1365-2664.12950

For managed temperate forests, conservationists and policy-makers favour fine-grained uneven-aged (UEA) management over more traditional coarse-grained even-aged (EA) management, based on the assumption that within-stand habitat heterogeneity enhances biodiversity. There is, however, little empirical evidence to support this assumption. The authors investigated how differently grained forest management systems affect the biodiversity of multiple above- and below-ground taxa across spatial scales. The results show that a mosaic of different age-classes is more important for regional biodiversity than high within-stand heterogeneity. The authors suggest reconsidering the current trend of replacing even-aged management in temperate forests. Instead, the variability of stages and stand structures should be increased to promote landscape-scale biodiversity.

Open access: http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12950/full

Marine mammals and sonar: Dose–response studies, the risk-disturbance hypothesis and the role of exposure context

Harris, C.M. *et al*.

Journal of Applied Ecology 2018, 55:396–404. https://doi.org/10.1111/1365-2664.12955

Marine mammals may be negatively affected by anthropogenic noise. Behavioural response studies (BRS) aim to establish a relationship between noise exposure conditions (dose) from a potential stressor and associated behavioural responses of animals. This study reviews the current state of understanding of naval sonar impact on marine mammals and highlights knowledge gaps and future research priorities. Despite data gaps, the authors believe that a dose-response approach within a risk-disturbance framework will enhance our ability to predict responsiveness for unstudied species and populations. The authors advocate for (1) regulatory frameworks to utilise peer-reviewed research findings when making predictions of impact, (2) regulatory frameworks to account for the inherent uncertainty in predictions of impact, and (3) investment in monitoring programmes that are both directed by recent research and offer opportunities for validation of predictions at the individual and population level.

Open access: http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12955/full

Eco-engineering urban infrastructure for marine and coastal biodiversity: Which interventions have the greatest ecological benefit?

Strain, E.M.A. *et al*.

Journal of Applied Ecology 2018, 55:426–441. https://doi.org/10.1111/1365-2664.12961

Along urbanised coastlines, urban infrastructure is increasingly becoming the dominant habitat. These structures are often poor surrogates for natural habitats, and a diversity of eco-engineering approaches have been trialled to enhance their biodiversity, with varying success. The authors undertook a quantitative meta-analysis and qualitative review of 109 studies to compare the efficacy of common eco-engineering approaches (e.g. increasing texture, crevices, pits, holes, elevations and habitat-forming taxa) in enhancing the biodiversity of key functional groups of organisms, across a variety of habitat settings and spatial scales. The efficacy of eco-engineering interventions varies among habitat settings and functional groups. This indicates the importance of developing site-specific approaches that match the target taxa and dominant stressors. Furthermore, because different types of intervention are effective at enhancing different groups of organisms, ideally a range of approaches should be applied simultaneously to maximise niche diversity.

Open access: http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12961/full

Artificial light at night alters grassland vegetation species composition and phenology

Bennie, J. *et al*.

Journal of Applied Ecology 2018, 55:442–450. https://doi.org/10.1111/1365-2664.12927

The worldwide growth of human settlements and transport networks has been accompanied by increasing illumination of the environment at night. Consequently, a growing proportion of the world's ecosystems are exposed to artificial light at night, profoundly altering natural cycles of light and darkness. While in recent years there have been advances in our understanding of the effects of artificial light at night on the behaviour and physiology of animals in the wild, much less is known about the impacts on wild plants and natural or semi-natural vegetation composition. In a long-term experimental field study, the authors exposed a semi-natural grassland to artificial light at intensities and wavelengths typical of those experienced by roadside vegetation under street lighting. They found that lighting affected the trajectory of vegetation change, leading to significant differences in biomass and plant cover in the dominant species. The results demonstrate that artificial light, at levels equivalent to those in street-lit environments, can affect species composition in semi-natural vegetation. This highlights the importance of considering artificial light as a driver of vegetation change in urban, suburban and semi-natural ecosystems, and where possible, of minimising or excluding artificial light from habitats of conservation importance.

Open access: http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12927/full

Forthcoming Events 2018

For information on these events please see *www.cieem.net*.

Conferences		
Date	Title	Location
26 April 2018	Irish Section Conference 2018 – Making Mitigation Work	Dublin
20 March 2018	CIEEM Spring Conference 2018 – Nature of Buildings: Designing Effective Mitigation and Enhancement	Birmingham
12 July 2018	CIEEM Summer Conference 2018 – Fit for the future: Developing an Ecologically Resilient Protected Sites Network	London
20-21 November 2018	CIEEM Autumn Conference 2018 – Habitat Re-creation and Ecological Restoration	Glasgow

Training Courses		
March 2018		
13-14	Water Vole Live Trapping, Handling, Practical Care and Re-establishment	Lifton
13-14	Intermediate QGIS for Ecologists and Environmental Practitioners	Athlone
15	Introduction to Protected Species Law and Policy	Bristol
20	Peregrine Falcon – Ecology, Survey and Mitigation	Tamworth
21-22	Developing Skills in Ecological Impact Assessment (EcIA)	Dublin
21	Habitats Regulations Assessment (HRA) of Projects	Birmingham
22	Habitats Regulations Assessment (HRA) of Plans	Birmingham
22	Barn Owl: Ecology, Surveying and Mitigation	Birmingham
27	Otter Ecology and Surveys	Cirencester
28	Badger Mitigation	Dorchester
27	Badger Ecology and Survey	Dorchester
27-28	Developing Skills in Ecological Impact Assessment (EcIA)	Stirling
28	Trees and Bat Roosts	Dorking
29	BS42020 Biodiversity: Code of Practice for Planning and Development	Newcastle
April 2018		
6	eDNA and Traditional Techniques for Effective GCN Surveys	Horndean
9-10	Getting to Grips with Bird Song for Identification and Survey	Gateshead
11	Effective Communication for Women	Swindon
11	Calculating and Using Biodiversity Units	Central London
12	An Introduction to Appropriate Assessment in Ireland	Letterfrack
11-12	Introduction to Great Crested Newt Ecology and Field Survey Techniques	Dorking
17	Breeding Bird Surveys and checks	West Bromwich
17	Great Crested Newt Ecology and Surveys	Airdrie
18	Great Crested Newt Assessment and Mitigation	Airdrie
May 2018		
2-3	Intermediate QGIS for Ecologists and Environmental Practitioners	Manchester
4	Introduction to Green Infrastructure in an Urban Environment	Central London
9	Introduction to Bats and Bat Surveys	Forest of Bowland
10	Early Season Grass and Sedge Identification	Salisbury
22	Water Vole Ecology and Surveys	Cirencester
23	Water Vole Mitigation	Cirencester
24-25	Surveying for Bats in Woodland	Gloucester
25	Field Skills and Resources for Identifying Annex 1 Habitats in Ireland	Dublin
28-29	Introduction to Phase 1 Habitat Survey	Livingston
June 2018		
9	Bat Handling and Identification	Herne Bay
12	Otter Ecology and Surveys	Cannock
13	Otter Mitigation	Cannock
13	Grasses and Sedges – Neutral and Calcareous Grasslands	Salisbury
14	Grasses, Sedges and Rushes – Heaths and Acid Grassland	New Forest
25	Using Indicator Species for Habitat Assessment (Phase I and NVC) – Grasslands	Salisbury

Our Ecology team work throughout the year carrying out surveys to identify, record, and monitor species and their habitats. As we enter spring we are looking for enthusiastic and capable new people to join our growing team of experienced ecologists.

Seasonal Roles: Spring and Summer are always busy seasons so we are in search of seasonal field surveyors and experienced ecologists to support our national team, with particular focus on Edinburgh, Newcastle, Belfast, Manchester, Leeds, Leicester, Southampton, Guildford, London, Cardiff and the South West.

Permanent Vacancies: In addition to seasonal vacancies, we also have permanent vacancies from Assistant to Associate Director level across the national team.

To see full information on all the vacancies and how to apply see **www.wygcareers.com**. You can also make a speculative application by sending your cv to **ecology@wyg.com**

Our ecologists work on projects across multiple sectors in the UK. Examples include:

- minimising impacts on biodiversity whilst maximising the developable area in Brookfield Garden Village, Hertfordshire
- great crested newt translocation and creation of enhanced newt habitats at Jodrell Bank, Macclesfield
- providing advice and support regarding appropriate sensitive mitigation for roosting bats at Bitts Park, Carlisle
- developing wintering bird mitigation strategies along the Solent coastline
- providing ecological support for a new highway which will deliver economic and social benefits for Cardiff Airport and St Athan Enterprise Zone in Wales
- supporting the DIO to relocate 4,000 Service personnel and families to Salisbury Plain including biodiversity offsetting and assessing impacts on internationally designated sites, for which we received a Sanctuary Award for protecting the environment.

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