

# Evidence

## Developing DNA techniques to identify freshwater invertebrates for environmental monitoring

Project summary SC110014

A PhD student at Bangor University, co-funded by the Environment Agency, has taken us closer to using DNA analysis for routine monitoring of freshwater macroinvertebrates (animals a few millimetres long such as insect larvae). She has successfully used new techniques to analyse environmental DNA (eDNA) released by organisms into water, for example in skin or faeces, to identify invertebrate species that are used as indicators of water quality. With further developments this approach should offer a quicker, cheaper and more effective way to carry out this important part of our environmental monitoring work. The project was part of a wider programme of research by UK agencies to develop DNA-based methods for environmental monitoring.

Until now, identification of macroinvertebrates has required time-consuming microscope work by highly skilled ecologists. The automated methods we are investigating involve sequencing DNA to identify the fragments of DNA, or 'barcodes', that characterise different species. This has recently become much faster using 'high throughput sequencing' in a process known as 'metabarcoding'. Using this method to identify species requires a DNA reference library and as part of the study this was established for 94 macroinvertebrate species.

The project tested the metabarcoding method on the aquatic larvae of a group of non-biting midge species (chironomids) in Llyn Padarn in Wales. It found that the eDNA in water samples and the DNA in shed pupal skins showed the seasonal changes in species expected from traditional methods. This means that by just taking a water sample from the lake it is possible to identify what midge species are present and how they change over time. In the future this could make monitoring macroinvertebrates in lakes easier and cheaper.

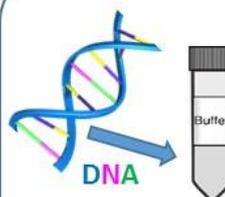
As well as understanding the species that are present or absent in a sample, having a measure of their relative abundance (the proportion of a sample made up by each species) is also a useful indicator of water quality.



Organisms shed cell debris into their surrounding environment creating a "soup" of floating eDNA.



Floating eDNA from all different organisms in the lake is collected and filtered..



DNA from all the organisms in the sample is extracted and then the macroinvertebrate DNA is separated, amplified (make copies of it so there is enough to analyse) and sent for DNA sequencing.



Thousands of DNA sequences are analysed and compared against a reference database to determine the identity of individual species.

Figure 1 Simplified overview of eDNA metabarcoding. Steps to determine what species of macroinvertebrates are present in a sample.

The project also tested the performance of two DNA methods in determining the relative abundance of artificially made up macroinvertebrate communities in the laboratory. The results suggest it is possible to determine both species presence/absence and their relative abundance.

Overall, the research showed the potential to use eDNA analysis for routine monitoring of macroinvertebrates in lakes. The results from this work will help the Environment Agency to decide how it can best develop more cost-effective environmental monitoring programmes.

This summary relates to information from project SC110014, reported in detail in the following output:

**PhD Thesis:** Defining a high throughput sequencing identification framework for freshwater ecosystem biomonitoring

A thesis submitted for the degree of Doctor of Philosophy in the School of Biological Sciences, Bangor University by Iliana Aglaia Bista:

<http://e.bangor.ac.uk/9811/>

The results have also been reported in Nature Communications:

<http://www.nature.com/articles/ncomms14087>

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**Research Contractor:** Bangor University, School of Biological Sciences, Molecular Ecology and Fisheries Genetics Laboratory

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