

Evidence

A DNA based metabarcoding approach to assess diatom communities in rivers

Project summary SC140024

This project has established a novel, DNA based method to monitor and assess the make-up of diatom communities in rivers. We report the results of the first large scale development and testing of a metabarcoding method (Figure 1); combining DNA barcodes with high throughput next generation sequencing (NGS), for the ecological assessment of diatoms.

We collect data on animal and plant life in the river to help us to understand, assess and manage the health of the environment. Diatoms, with around 2800 UK freshwater species recorded, are a type of microscopic algae used alongside other organisms to assess the ecological status of a river.

Diatoms can be found attached to submerged surfaces such as stones and on plant stems in a river, and are commonly referred to as phytobenthos. They are sensitive to changes in environmental conditions and the diatom community (type of diatom species and their relative abundance) in a river is largely influenced by the surrounding water quality. This means that diatoms are valuable indicators of nutrient enrichment and other pressures. We have used the information from diatom communities to develop indices that allow us to detect the impact of nutrient enrichment, primarily phosphorus, on a river. To determine the make-up of these communities we have to date used a light microscope (LM) approach which is a time-consuming process, requiring highly skilled biologists to identify the diatoms.

This new metabarcoding approach will mean we can analyse a lot more samples much quicker using high throughput technology. For example using metabarcoding, diatom samples will be analysed in batches of around 200. The typical analysis time (Figure 1 Steps 2–5) for 200 samples will be approximately 15 working days. It currently takes 3–4 hours to analyse one diatom sample using LM which adds up to about 100 working days to analyse the same 200 samples.

About the new method

The new method for diatom analysis uses NGS to sequence small pieces of DNA (DNA barcodes) that are unique to different diatom species and which can be used to identify the different diatom species in a community. The new method developed was tested by comparing it to the LM method using approximately 620 diatom samples from UK rivers with different water quality.

Whilst the two approaches measure different things, we have demonstrated good agreement between the two methods (significant correlation $r = 0.75$ and 0.77 in high and low alkalinity rivers respectively). The metabarcoding method uses many of the high priority species that inform our assessments (around 10% of freshwater diatom species described in the UK (Figure 1, Step 5)). We recognise that incremental improvements could be made as more diatom species are added to the barcode database. In addition, we found a lot of information in the metabarcoding data (e.g. other types of algae) that is currently not used as part of the assessment. With further development we could take account of this added information to help improve our understanding of the environmental quality of our rivers.

Further work planned

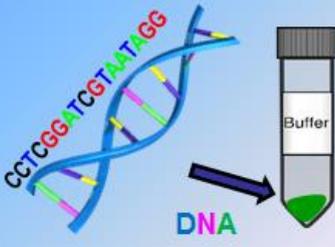
Although we have demonstrated good agreement, there are some differences in the outputs of some samples that require further investigation. Work is underway to explore these. This, alongside further data collection by other UK regulatory agencies in 2017, will refine the method further in 2018.

This project is part of a wider programme of research by the Environment Agency and other UK agencies to explore and develop DNA based methods for ecological assessment. This work will help us to approach the development of DNA based methods for other organism groups and water body types. Examples of other work include assessing the feasibility of using DNA based methods for fish in lakes, macroinvertebrates in rivers and lakes, and for the monitoring and surveillance of non-native invasive species.

Step 1
Diatom samples are scraped from submerged boulders in the river



Step 2
Diatom DNA is extracted along with DNA from other organisms in the sample



Step 3
Target DNA barcodes are separated from the DNA of other organisms that do not have the target barcode using a method called polymerase chain reaction (PCR)



Step 4
Diatom DNA barcodes are sequenced on a high-throughput sequencing next generation sequencing machine



Step 5
DNA barcodes are compared with a barcode reference database (currently includes 10% of recorded UK species) and their identity and relative abundance determined

<i>Cymbella lanceolata</i>			x2
<i>Navicula radiosa</i>			x5
<i>Stauroneis anceps</i>			x12
<i>Pinnularia</i> sp.			x50

This summary relates to information from project SC140024, reported in detail in the following output(s):

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Figure 1: Overview of metabarcoding method developed for diatom assessment in rivers