Assessing river nutrients using diatom DNA: further development of an operational method

Project summary SC160014

This project has improved a DNA based method developed earlier (SC140024/F) to monitor and assess the make-up of diatom communities (a type of microscopic algae) that we use to assess nutrient enrichment in rivers.

About the new method

The new method for diatom analysis characterises small pieces of DNA that are unique to different diatom species and which can be used to identify the diatom community in a water sample. The method uses modern DNA sequencing technology called next generation sequencing (NGS) and the unique pieces of DNA are called DNA barcodes. When you use NGS to identify many DNA barcodes in a sample the approach is known as metabarcoding. Metabarcoding enables rapid identification of multiple species and is less reliant on taxonomic expertise. This new DNA method is different to the existing method that uses light microscopy (LM).

The new DNA method works in the same way as the LM method and uses the composition of diatom species to calculate a metric called the Trophic Diatom Index (TDI), which is designed to measure the trophic status (extent of nutrient enrichment, primarily phosphorus) of the river. Fundamental to this metric are diatom sensitivity values that indicate how sensitive (1 = very sensitive) or tolerant (5 = very tolerant) the diatoms are to nutrients and these are used to calculate the TDI.

The DNA method is designed to produce comparable results to the current LM method. Both methods measure different components of the diatom cell. LM uses the cell wall or parts of it (for example, the valve) and the DNA method uses a gene involved in energy production.

This report builds on earlier work and describes:

- Improvements in the sampling approach
- Further refinement of the metabarcoding method and
- The feasibility of extending the approach to lakes

Although there are areas within the new DNA method that could be improved further, the project has developed a pragmatic approach that balances the need to produce a cost-effective method for routine monitoring with one that provides good resolution compared with the traditional LM approach.

Method improvements: sampling approach

The DNA method offers greater sensitivity for species detection, but with this comes the potential for cross-contamination if existing sampling methods are used. Testing the potential for cross contamination on 2 contrasting rivers showed the scale of contamination to be low. However, sensible precautions are recommended to reduce the effect from both the stream water and sampling equipment. Toothbrushes should be used once and then cleaned in a dilute bleach solution or single use toothbrushes should be used. While stream water is unlikely to be a major source of error, brushing cobbles into distilled water rather than river water is encouraged.

Method improvements: recalibration

Earlier work using a dataset of 620 samples demonstrated good agreement between the LM and DNA method. However, there were still some differences in the output from individual samples and it was apparent that some species sensitivity values (scale of 1-5) used to calculate the LM based TDI might be too stringent and so were adjusted prior to recalibrating the DNA TDI metric. Using the adjusted sensitivity values and a larger dataset of 1,367 samples, different statistical approaches were adopted to obtain the strongest-possible relationship between the TDI outputs from both methods. There was a small overall improvement in the relationship between the 2 methods and their response to inorganic nutrients was similar which was reassuring.

There were individual differences in some sample outputs between the 2 methods. The way in which the relative abundance of taxa are established and gaps in the DNA barcode reference database are likely to be significant reasons contributing to these differences. As more taxa are incorporated into the reference database, there should be further improvements in the method over time.
Response to environmental pressures
To understand how the LM and DNA methods work at a local scale in response to a pressure, the 2 approaches were applied to a case study in a Devon river which receives effluent from a small sewage treatment works, as well as from storm sewers.

Both methods gave ambiguous results, reflecting a catchment experiencing a variety of ecological stresses. Although there was not complete agreement between the 2 methods, both indicated that there were issues upstream and downstream of the sewage treatment works, which was the primary focus of concern.

In general, the diatom communities were similar between the 2 methods but differences were apparent in the relative abundance of certain taxa. Some of the differences observed followed already understood patterns, while others were much harder to explain. It will therefore be important to adjust our understanding of how different taxa are represented in the DNA method and how they combine to give an indication of the condition of a water body.

Developing the method for use in lakes
The DNA method developed for rivers was tested on lakes using 162 samples from 42 lakes in England. While the DNA method yielded slightly higher TDIs, the results were encouraging, with TDIs for both methods showing good agreement Therefore, it would be relatively straightforward to produce a lake DNA method following the same principles used to derive the method for rivers.

Further work
It is recognised that incremental improvements could be made as more diatom taxa are added to the barcode reference database. Around 40% of DNA sequences generated by the method cannot be assigned to a species in the barcode reference database and are not used as part of the assessment. With further exploration of this unassigned data and incorporation into the method, there is the potential to develop new insights about how diatom communities reflect environmental change.