November 2019

Information note

Developments in the classification of river phytobenthos (diatoms)

Purpose

To inform stakeholders of progress with, and the status of, recent developments in river phytobenthos analysis methods and the classification tool (DARLEQ) for the third River Basin Planning Cycle.

Background

The current UK tool for phytobenthos assessment is DARLEQ2, which was intercalibrated and adopted across the UK for RBP Cycle 2 after consultations held in 2012/13 by UKTAG and the UK Admins. DARLEQ2 uses traditional light microscopy (LM) for sample analysis, based on the standard CEN methods embedded in the WFD. The individual taxon scores used to calculate observed TDI values for derivation of ecological quality ratios (EQRs) for classification are referred to as TDI4 (i.e. Trophic Diatom Index version 4). DARLEQ 2 is described in the method statement published by UKTAG [DARLEQ2 River Phytobenthos method statement], with an accompanying calculator spreadsheet, but is also available as an online tool.

It is acknowledged that DARLEQ2 it does not always classify effectively in high alkalinity river systems, where the current recommendation is that macrophytes alone should be used for reporting the "macrophytes and phytobenthos" Biological Quality Element. In low alkalinity systems the reverse is the case, and DARLEQ2 is primarily used for classification.

Developments during WFD Cycle 2

During the second WFD river basin planning cycle, UKTAG and some of its constituent agencies have explored further developments and improvements to the DARLEQ tool.

a) Use of DNA techniques for sample analysis

As part of a wider programme investigating the potential for use of DNA analysis in environmental monitoring, extensive work has been undertaken by the Environment Agency and SEPA, with support from NRW and DAERA NI, to develop and evaluate a DNA analysis method for benthic diatoms. Results of this work have been published as Environment Agency [A DNA-based diatom metabarcoding approach] and SEPA [Evaluation of benthic diatom classification in UK rivers using LM and NGS methods] reports. A second Environment Agency report is awaiting publication.

b) Improvements to TDI taxon scores

It was recognised that there were some inconsistencies in the TDI4 taxon scores, which have also been addressed through the projects described above. The result of this has been small adjustments to a few scores and the creation of an updated version of the TDI, referred to as TDI5 LM.

c) Development of a DNA based TDI

It was hoped that DNA-derived taxonomic data could be used as a direct substitute of light microscopy in the DARLEQ tool, but comparison of these data from an

extensive set of paired samples showed that there were sufficient differences for this not to be feasible. Further development work was undertaken to derive DNA-based TDI scores. These are based on data obtained by the Next Generation Sequencing technique, and referred to as TDI5 NGS.

d) Evaluation of TDI5 LM and TDI5 NGS

The UKTAG Freshwater Task Team have carried out an analysis of the impact on classification of switching from the current TDI4 to TDI5 LM and TDI5 NGS.
This exercise revealed that the overall impact on phytobenthos classification results is limited (Table 1).

- The change from TDI4 to TDI5 LM results in a slight increase in the percentage of sites at Good or better status across the UK (67 to 68%). This is most pronounced for Northern Ireland (increase from 88 to 92%), although note that NI has the smallest sample size.
- Changing from TDI4 to TDI5 NGS (i.e. DNA analysis) results in a slight increase in percentage of sites at Good or better status overall (67 to 70%).

Table 1. Percentage of classifications at Good or better, and Moderate or worse status using current and proposed new variants of the phytobenthos classification method. Based on site level classification.

	TDI4 (current)		**TDI5 LM** (improved taxon scores, light microscopy analysis)		**TDI5 NGS** (improved taxon scores, DNA analysis)		
	% G or better	%M or worse	% G or better	%M or worse	% G or better	%M or worse	No. samples
UK	67	33	68	32	70	30	686
England	61	39	62	38	65	35	486
Scotland	79	21	82	18	80	20	115
Wales	82	18	85	15	83	17	60
Northern Ireland	88	12	92	8	92	8	25

While the proposed changes have a small overall effect, the impact on classification at the site level is more pronounced, particularly with respect to the use of DNA sample analysis. For the UK as a whole:

- Comparing TDI4 to TDI 5 LM, overall 94% of samples return the same class result, and 100% are the same or only one class different.
- Comparing TDI4 to TDI5 NGS, overall 65% of samples return the same class, and 98% are the same or one class different.
- Similarly, comparing TDI5LM to TDI5 NGS, 67% are the same and 99% the same or one class different.
- There is no bias in the result for those sites that return a different class using the DNA method (i.e. there are approximately the same number of upgrades as downgrades).

In practice this means that there would be some changes in reported site or water body level class that are likely due to the method change rather than a real environmental improvement or deterioration.

Further analysis showed that:

- TDI5 NGS has a similar, but slightly less strong relationship with nutrient pressure (phosphorus) than TDI4 LM
- TDI5 NGS does not provide any improvement in alignment of the phytobenthos class with phosphorus, and worsens the alignment with macrophyte class (although the reasons for this are currently unclear)
- TDI5 NGS does not meet the criterion for a simple intercalibration, and further work would be needed to complete intercalibration of the new method.
- It is clear that our understanding of the nature of DNA data is still evolving, as are DNA analysis techniques and the barcode database relating DNA to species or other taxonomic units.

e) Reference conditions

As part of the development work, we re-visited the model used in DARLEQ2 to derive the reference values used in calculating EQRs. Limitations in the reference model were thought to be part of the reason the tool does not perform well in lowland high alkalinity rivers. An alternative reference model has been produced, but UKTAG felt that the degree of change this would produce in classifications required further evaluation, which was not possible in the time available prior to the new planning round.

f) DARLEQ3

An updated version of the DARLEQ software (DARLEQ3) has been developed which allows calculation of existing (TDI4) and new (TDI5 LM, TDI5 NGS) scores. This version will be used by the UK Agencies to produce new classifications. The software will be freely available to other organisations and individuals.

Decisions on implementation

In consultation with the UK Admins, it has been decided that:

- The change from TDI4 to TDI5 LM represents a method improvement resulting in minor changes to classification. It will be implemented via an update to the tool, and a revised method statement will be produced in due course.

- The adoption of TDI5 NGS is a more substantial change in approach, and while it has a limited impact on classification at the national level, the scale of changes at site/water body level was felt to be too large, and insufficiently understood, to justify adoption for implementation at this stage.

Next steps

UKTAG and its partners will continue to explore options for the use of DNA and environmental DNA in routine monitoring and assessment of the water environment. We will carry out further evaluation of the phytobenthos data to determine whether the technique could be introduced for formal classifications in the future.