# ANNEX 10 – LAKES – Macrophytes & Phytobenthos – DARLEQ

## Contents



## A1 Description of method

### Sample collection and analysis

Samples of the biofilm which covers the upper surfaces of five submerged cobbles or small boulders from the littoral zones of lakes are collected between spring and autumn, by brushing or scraping with a toothbrush. The biofilm is the thin coating on such submerged surfaces, composed of algae and other microorganisms in a polysaccharide matrix with organic and inorganic particles. Sampling sites are away from inflowing streams and obvious human impacts. If there are no cobbles or small boulders present at the sampling site, and where bottom sediments are dominated by fine sediments with only a few large stones, five submerged stems of emergent macrophytes, such as *Phragmites australis*, *Sparganium erectum*, *Glyceria maxima* or *Typha* species are sampled instead. The sampling method conforms to EN 13946: 2003 Water quality – Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers. The resulting suspension is collected in plastic bottles, fixed with Lugol's iodine and stored prior to analysis.

The samples are subsequently digested in order to remove the organic matter. There are a number of digestion methods available. Permanent slides are then prepared from each sample, which are analysed using high power microscopes to identify the presence and number of valves of each of the diatom taxa present. A valve is one half of the silica shell which surrounds the cell contents of an individual diatom, which generally separates during the digestion process. The analytical method conforms to EN 14407: 2004 Water quality – Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters. The slides are retained for future reference.

### Calculation of the Ecological Quality Ratio

The Trophic Diatom Index was originally an expert-derived riverine index, in which all diatom taxa were assigned a score from 1 (nutrient sensitive) to 5 (nutrient tolerant) but was subsequently recalibrated by an iterative weighted averaging procedure and applied to lakes. The resulting index is termed the Lake Trophic Diatom Index (LTDI) and is similarly highly correlated with the first axis of a Canonical Correspondence Analysis constrained by soluble P and nitrate-N, suggesting that the LTDI also reflects the underlying inorganic nutrient pressure gradient (Kelly et al., 2008).

The LTDI for each sample is calculated using equations 1 and 2:

$$\text{Observed value of river trophic diatom index} = (W \times 25) - 25 \qquad \text{Equation 1}$$

where:

"W" is given by the equation:

$$W = \frac{\sum_{j=1}^{n} a_j \times s_j}{\sum_{j=1}^{n} a_j} \qquad \text{Equation 2}$$

where:
"$a_j$" is the number of valves of taxon j, and
"$s_j$" is the nutrient sensitivity score corresponding to the taxon represented by j.

The expected value of the LTDI value was derived for each of 3 lake types (low alkalinity, <10 $CaCO_3$ mg/l, medium alkalinity 10-50 $CaCO_3$ mg/l, and high alkalinity >50 $CaCO_3$ mg/l) as the median of the LTDI values of reference lakes for that type. The range of LTDI values for reference lakes belonging to each lake types were 22, 35 and 42 for LA, MA and HA types, respectively. Marl lakes are included with HA types, but peat and brackish systems are not covered under the tool.

The ecological quality ratio for the parameter should be calculated using the following equation:

EQR = (100 – observed value of lake TDI) ÷ (100 – reference value for lake TDI) Equation 3

Calculated EQR is set to "1.0" in instances where calculated EQR is > 1.00.

## Status class boundaries

The boundary between 'High' and 'Good' status was defined as the 25$^{th}$ percentile of EQRs of all reference sites. The boundary between 'Good' and 'Moderate' status was set at the point at which nutrient-sensitive and nutrient-tolerant benthic diatom taxa were present in equal relative abundance (Figure 1), which details the distribution of sensitive and tolerant benthic diatom taxon groups along the EQR gradient. The cross-over point on these plots allowed the 'Good' /

'Moderate' boundary for each alkalinity type to be derived. The 'Moderate'/'Poor' and 'Poor'/'Bad' boundaries were then defined by equal division of the remaining EQR gradient. Full details are provided in Environment Agency report SC070034/TR3 (https://brand.environment-agency.gov.uk/mb/DCJHIl).

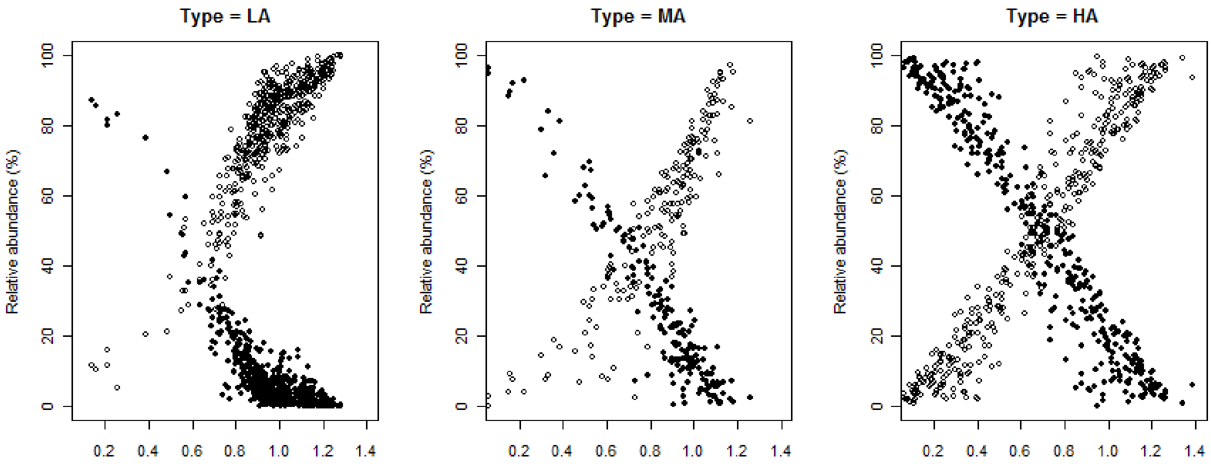


**Figure 1. Abundances of sensitive (open circles) and tolerant (closed circles) taxa along the EQR gradient for the three alkalinity classes. (LA = low alkalinity, MA = medium alkalinity, HA = high alkalinity; EQR is on the x-axis).**

The resulting boundary values for three alkalinity types are shown below (Table 1). The Poor/Bad boundary is not applied in UK classification. Sites are classified only as High, Good, Moderate and Poor for diatom status.

**Table 1. EQR class boundaries for 3 alkalinity lake types.**

| | H/G | G/M | M/P | P/B |
|---|---|---|---|---|
| **Low alkalinity** | 0.92 | 0.70 | 0.46 | 0.23 |
| **Medium alkalinity** | 0.93 | 0.66 | 0.46 | 0.23 |
| **High alkalinity** | 0.92 | 0.70 | 0.46 | 0.23 |

## Worked example

The following taxon list was obtained from an analysis of a sample collected from a lake in northern England in July 2006.

When using the tool for purposes of classification the ecological status or potential, the annual mean of the EQR should be used. It is also of note to remember that marl lakes are included together with high alkalinity types, and peat and brackish systems are not covered under the use of this tool.

| Taxon identified as counted from permanent slide | Number of valves (*a*) | Nutrient sensitivity score (*s*) | *a* * *s* |
|---|---|---|---|
| Achnanthidium minutissimum type | 187 | 2 | 374 |
| Brachysira styriaca | 2 | 1 | 2 |
| Brachysira vitrea | 48 | 1 | 48 |
| Caloneis silicula | 1 | 3 | 3 |
| Cyclotella sp. | 3 | 0 | 0 |
| Cymbella affinis | 7 | 2 | 14 |
| Cymbella cistula | 1 | 3 | 3 |
| Delicata delicatula | 1 | 1 | 1 |
| Denticula tenuis | 13 | 2 | 26 |
| Encyonema gracile | 1 | 2 | 2 |
| Encyonemopsis microcephala | 7 | 2 | 14 |
| Eucocconeis flexella | 3 | 3 | 9 |
| Eunotia pectinalis | 5 | 1 | 5 |
| Eunotia sp. | 3 | 1 | 3 |
| Fragilaria perminuta | 8 | 3 | 24 |
| Gomphonema acuminatum | 1 | 3 | 3 |
| Gomphonema gracile | 4 | 3 | 12 |
| Gomphonema parvulum | 1 | 5 | 5 |

| | | | |
|---|---|---|---|
| Gomphonema sp | 1 | 3 | 3 |
| Nitzschia frustulum | 1 | 4 | 4 |
| Nitzschia gracilis | 1 | 3 | 3 |
| Nitzschia sp. | 2 | 4 | 8 |
| Rossithidium lineare | 1 | 3 | 3 |
| Sellaphora pupula | 1 | 4 | 4 |
| Synedra tenera | 3 | 3 | 9 |
| Tabellaria flocculosa | 2 | 1 | 2 |
| Tryblionella acuminata | 4 | 4 | 16 |
| | ∑a = 312 | | ∑as = 600 |

The observed value of lake trophic diatom index for this sample is calculated as follows:

sum *as* for all taxa in sample = 600

sum *a* for all taxa in sample = 312

Calculate W = sum *as* / sum *a* = 1.92

Calculate the observed value of lake trophic diatom index for the sample using the equation:

$(W \times 25) - 25 = (1.92 \times 25) - 25 = 23.1$

The mean total alkalinity for the lake in 2006 was 3.1 mg/l $CaCO_3$. This means that the lake is in the 'low alkalinity' type. The reference value for the LTDI in the lake is therefore 22.

Applying this, the ecological quality ratio for the sample is:

(100 – 23.1) / (100 – 22) = 76.9 / 78 = 0.99

Therefore, $EQR_{DARLEQ}$ = 0.99

## A2 Summary of changes between 1st RBMP and 2nd RBMP

The main change has centred on the revision to the status class boundaries to bring the revised method into line with methods used elsewhere in Europe. In addition changes also included the way expected references scores were predicted, as outlined above, and an update to the taxa list as detailed for the river phytobenthos method.

### Revisions to status class boundaries

Relationship between metric and nutrient pressure (TP gradient) was further explored by plotting metric scores for each lake type against the revised TP gradient. The revised expected LTDI at reference conditions were outlined earlier, and revised EQR boundaries were detailed above in Table 1. Sites are classified only as High, Good, Moderate and Poor for diatom status with the Poor/Bad boundary not applied in UK classification. Full details are provided in Environment Agency report SC070034/TR3 (https://brand.environment-agency.gov.uk/mb/DCJHIl)

Although status boundaries computed using the UK phytobenthos method were intercalibrated during the first phase of intercalibration, the changes to the method necessitated that intercalibration was repeated in order to ensure that UK boundaries were consistent with those of other countries. The revised EQRs suggest DARLEQ to be more relaxed than classification using other tools, possibly attributed to the reference benthic diatom community in low alkalinity waters being buffered against nutrient impacts, with dominant taxa being relatively tolerant to mild nutrient enrichment as detailed in palaeolecological studies of low alkalinity lakes (Bennion et at 2001). Full details are provided in Environment Agency report SC070034/TR3 (https://brand.environment-agency.gov.uk/mb/DCJHIl).

## A3 Consequences of changes

### England

**Table 2. Comparison of classifications of ecological status determined by original and revised versions of the lake phytobenthos tool, DARLEQ.**

| | | Revised | | | | | Grand Total |
|---|---|---|---|---|---|---|---|
| | | High | Good | Moderate | Poor | Bad | |
| Current | High | 2 | 1 | | | | 3 |
| | Good | 2 | 20 | | | | 22 |
| | Moderate | | | 2 | 2 | | 4 |
| | Poor | | | | 7 | 1 | 8 |
| | Bad | | | | | | |
| Grand Total | | 4 | 21 | 2 | 9 | 1 | 37 |

**Table 3. Percentage of water bodies in each class, determined using original and revised versions of the lake phytobenthos tool, DARLEQ.**

| Class | Current Method | Revised Method |
|---|---|---|
| High | 8.1% | 10.8% |
| Good | 59.5% | 56.8% |
| Moderate | 10.8% | 5.4% |
| Poor | 21.6% | 24.3% |
| Bad | 0% | 2.7% |

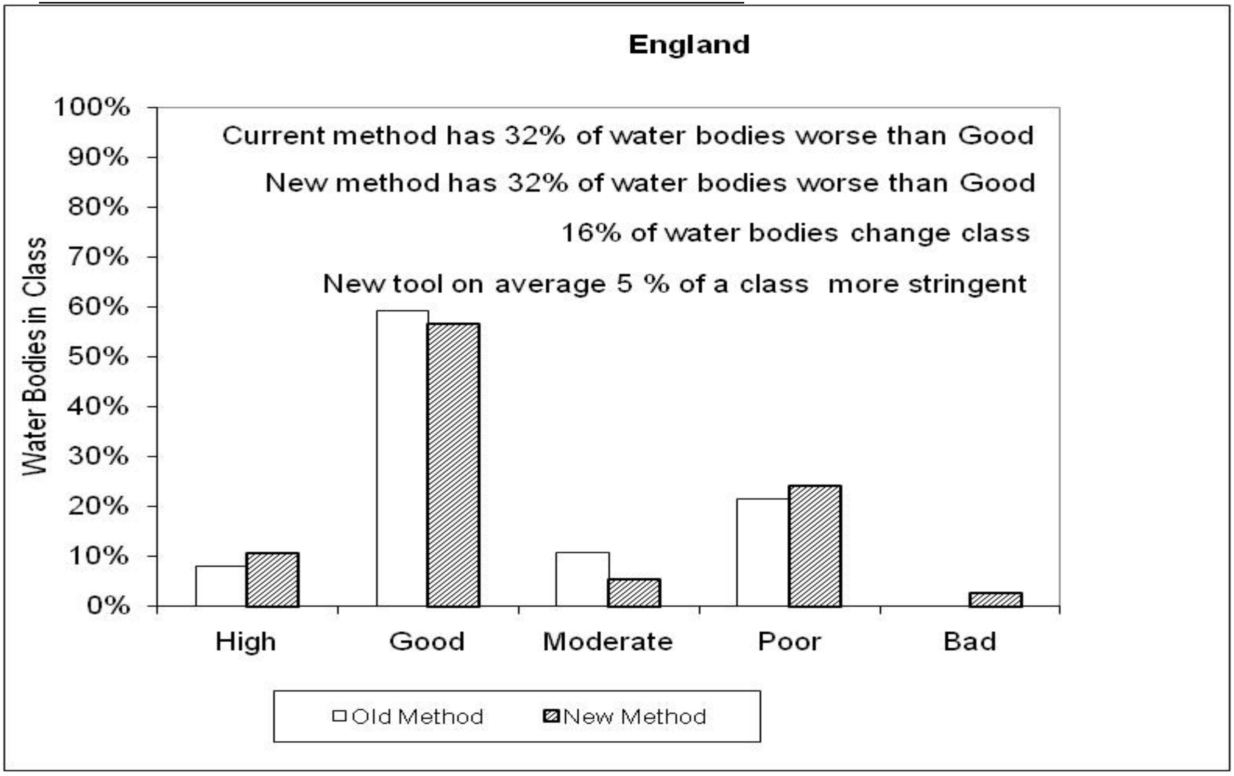


**Figure 2. Percentage of water bodies in each WFD class using the current and new lake phytobenthos assessment method.**

**Table 4. Number and percentage of water bodies that change class when using the revised version of the phytobenthos tool, DARLEQ.**

| | Number | Percentage |
|---|---|---|
| Current 4 class worse | 0 | 0.0% |
| Current 3 class worse | 0 | 0.0% |

| | | |
|---|---|---|
| Current 2 class worse | 0 | 0.0% |
| Current 1 class worse | 2 | 5.4% |
| Same class | 31 | 83.8% |
| Revised 1 class worse | 4 | 10.8% |
| Revised 2 class worse | 0 | 0.0% |
| Revised 3 class worse | 0 | 0.0% |
| Revised 4 class worse | 0 | 0.0% |

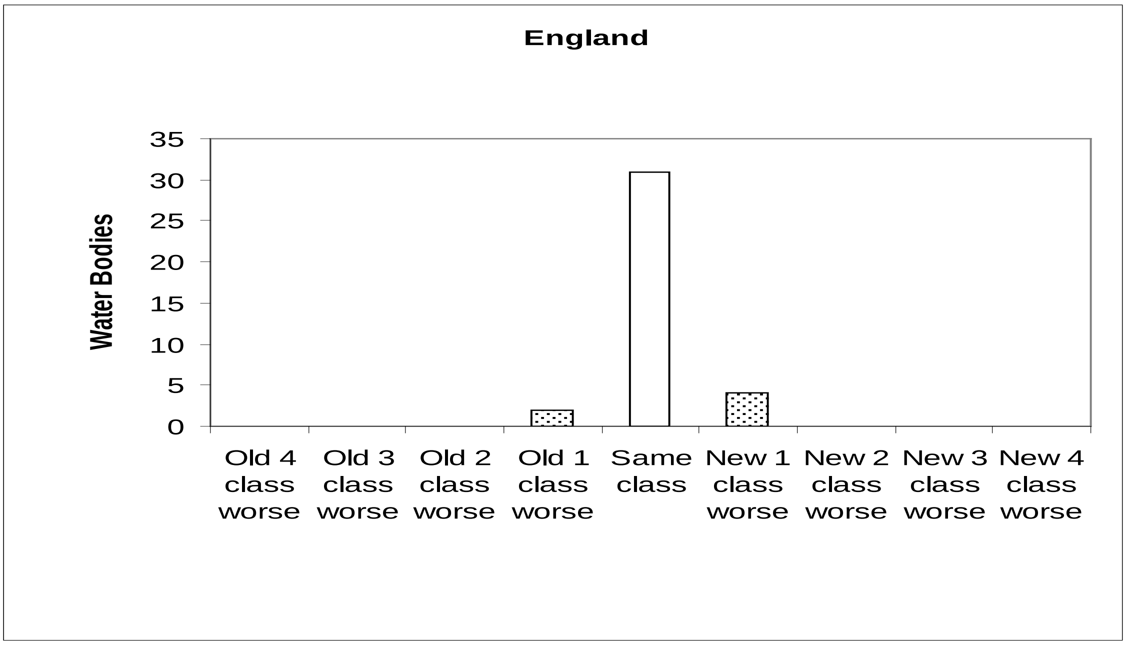


**Figure 3. Number of water bodies that change class when using the revised version of the lake phytobenthos tool, DARLEQ.**

## Wales

**Table 5. Comparison of classifications of ecological status determined by original and revised versions of the lake phytobenthos tool, DARLEQ.**

| | Revised | Grand |
|---|---|---|

| | | High | Good | Moderate | Poor | Bad | Total |
|---|---|---|---|---|---|---|---|
| Current | High | 14 | | | | | 14 |
| | Good | | 8 | | | | 8 |
| | Moderate | | | 6 | | | 6 |
| | Poor | | | | 4 | 1 | 5 |
| | Bad | | | | | | |
| Grand Total | | 14 | 8 | 6 | 4 | 1 | 33 |

**Table 6. Percentage of water bodies in each class, determined using original and revised versions of the lake phytobenthos tool, DARLEQ.**

| Class | Current Method | Revised Method |
|---|---|---|
| High | 42.4% | 42.4% |
| Good | 24.2% | 24.2% |
| Moderate | 18.2% | 18.2% |
| Poor | 15.2% | 12.1% |
| Bad | 0% | 3.0% |

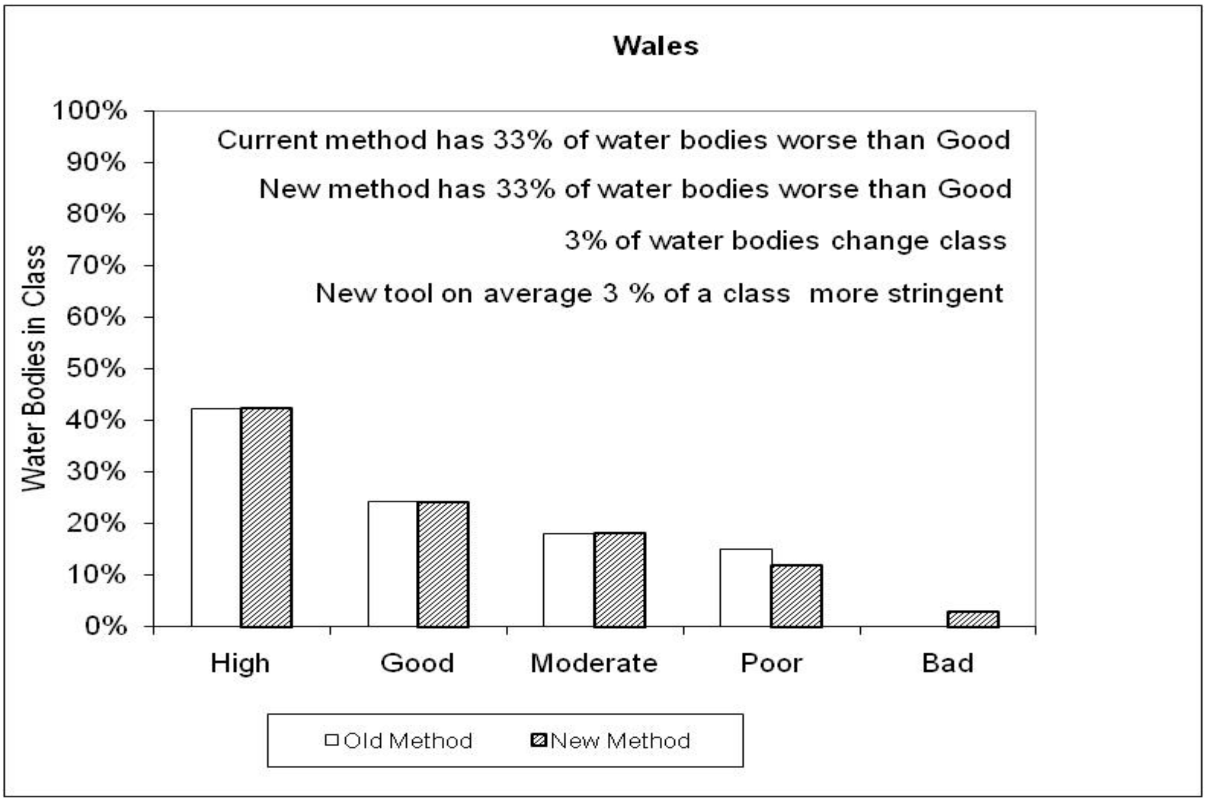


**Figure 4. Percentage of water bodies in each WFD class using the current and new lake phytobenthos assessment method.**

**Table 7. Number and percentage of water bodies that change class when using the revised version of the phytobenthos tool, DARLEQ.**

| | Number | Percentage |
|---|---|---|
| Current 4 class worse | 0 | 0.0% |
| Current 3 class worse | 0 | 0.0% |
| Current 2 class worse | 0 | 0.0% |
| Current 1 class worse | 0 | 0.0% |
| Same class | 32 | 97.0% |

| | | |
|---|---|---|
| Revised 1 class worse | 1 | 3.0% |
| Revised 2 class worse | 0 | 0.0% |
| Revised 3 class worse | 0 | 0.0% |
| Revised 4 class worse | 0 | 0.0% |

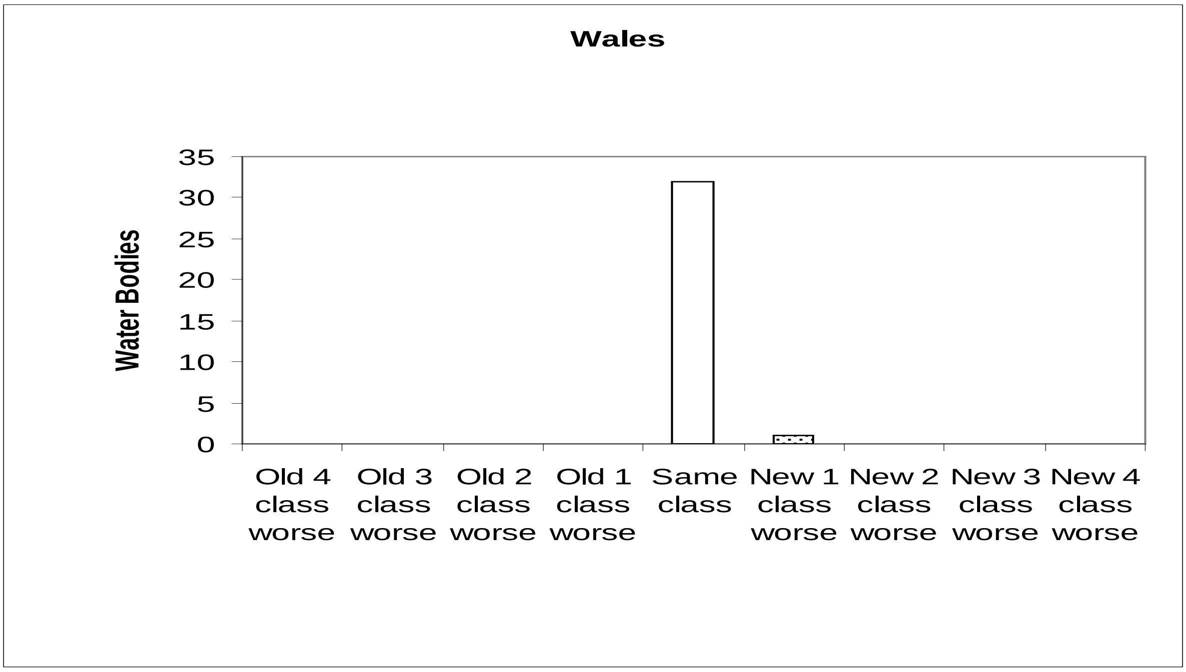


**Figure 5. Number of water bodies that change class when using the revised version of the lake phytobenthos tool, DARLEQ.**

## Scotland

**Table 8. Comparison of classifications of ecological status determined by original and revised versions of the lake phytobenthos tool, DARLEQ.**

| | | Revised | | | | | Grand Total |
|---|---|---|---|---|---|---|---|
| | | High | Good | Moderate | Poor | Bad | |
| Current | High | 18 | 12 | | | | 30 |

| | | | | |
|---|---|---|---|---|
| Good | | 10 | 4 | 14 |
| Moderate | | | 2 | 2 |
| Poor | | | | |
| Bad | | | | |
| Grand Total | 18 | 22 | 6 | 46 |

**Table 9. Percentage of water bodies in each class, determined using original and revised versions of the lake phytobenthos tool, DARLEQ.**

| Class | Current Method | Revised Method |
|---|---|---|
| High | 65.2% | 39.1% |
| Good | 30.4% | 47.8% |
| Moderate | 4.3% | 13% |
| Poor | 0.0% | 0.0% |
| Bad | 0.0% | 0.0% |

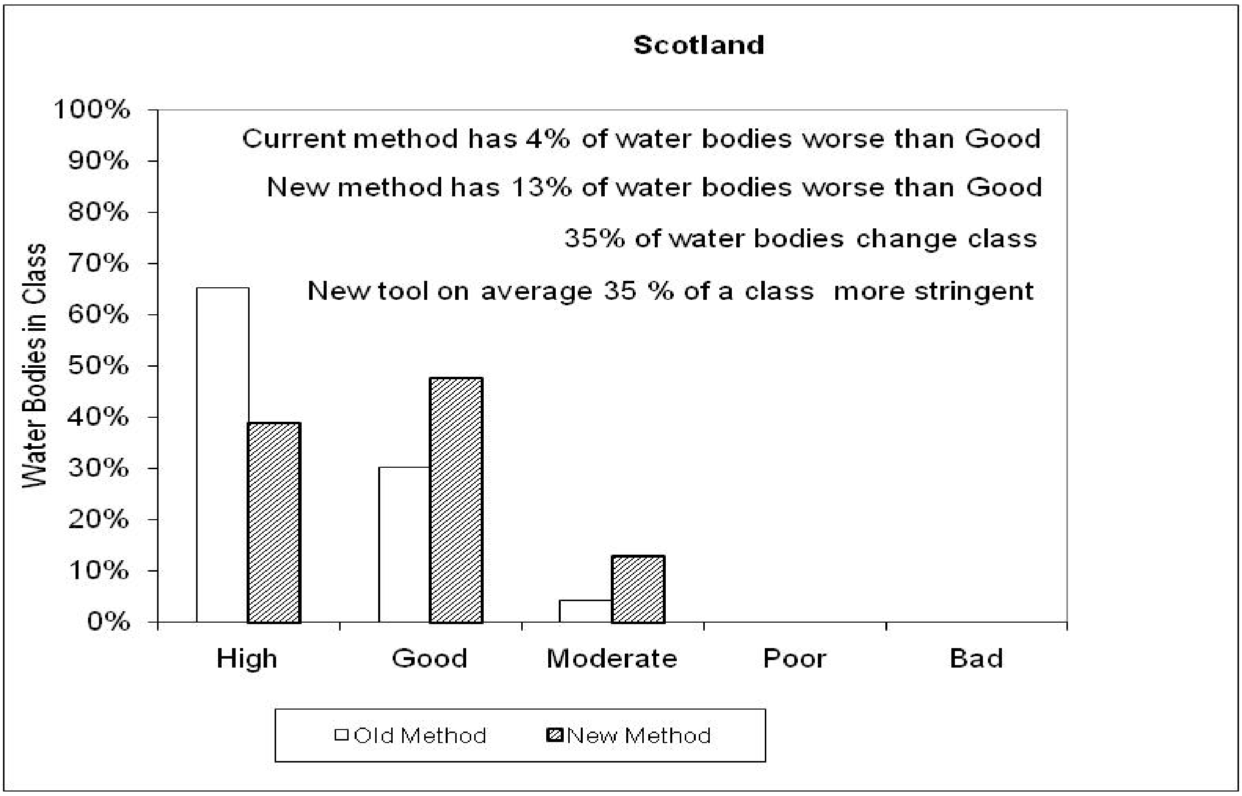

**Figure 6. Percentage of water bodies in each WFD class using the current and new lake phytobenthos assessment method.**

**Table 10. Number and percentage of water bodies that change class when using the revised version of the phytobenthos tool, DARLEQ.**

| | Number | Percentage |
|---|---|---|
| Current 4 class worse | 0 | 0.0% |
| Current 3 class worse | 0 | 0.0% |
| Current 2 class worse | 0 | 0.0% |
| Current 1 class worse | 0 | 0.0% |
| Same class | 30 | 65.2% |
| Revised 1 class worse | 16 | 34.8% |
| Revised 2 class worse | 0 | 0.0% |
| Revised 3 class worse | 0 | 0.0% |
| Revised 4 class worse | 0 | 0.0% |

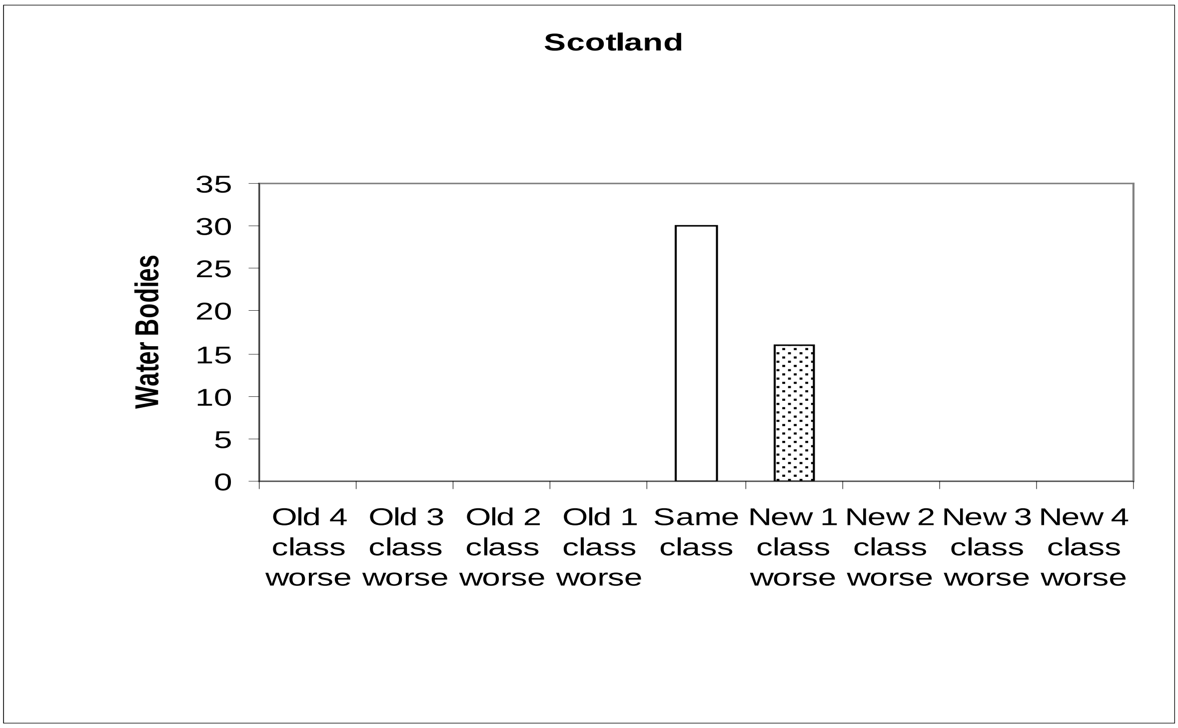

**Figure 7. Number of water bodies that change class when using the revised version of the lake phytobenthos tool, DARLEQ.**

## Northern Ireland

**Table 11. Comparison of classifications of ecological status determined by original and revised versions of the lake phytobenthos tool, DARLEQ.**

| | | Revised | | | | | Grand Total |
|---|---|---|---|---|---|---|---|
| | | High | Good | Moderate | Poor | Bad | |
| Current | High | 8 | | | | | 8 |
| | Good | 1 | 7 | | | | 8 |
| | Moderate | | | 4 | | | 4 |
| | Poor | | 1 | 2 | 4 | | 7 |
| | Bad | | | | | | |
| Grand Total | | 9 | 8 | 6 | 4 | | 27 |

**Table 12. Percentage of water bodies in each class, determined using original and revised versions of the lake phytobenthos tool, DARLEQ.**

| Class | Current Method | Revised Method |
|---|---|---|
| High | 29.6% | 33.3% |
| Good | 29.6% | 29.6% |
| Moderate | 14.8% | 22.2% |
| Poor | 25.9% | 14.8% |
| Bad | 0.0% | 0.0% |

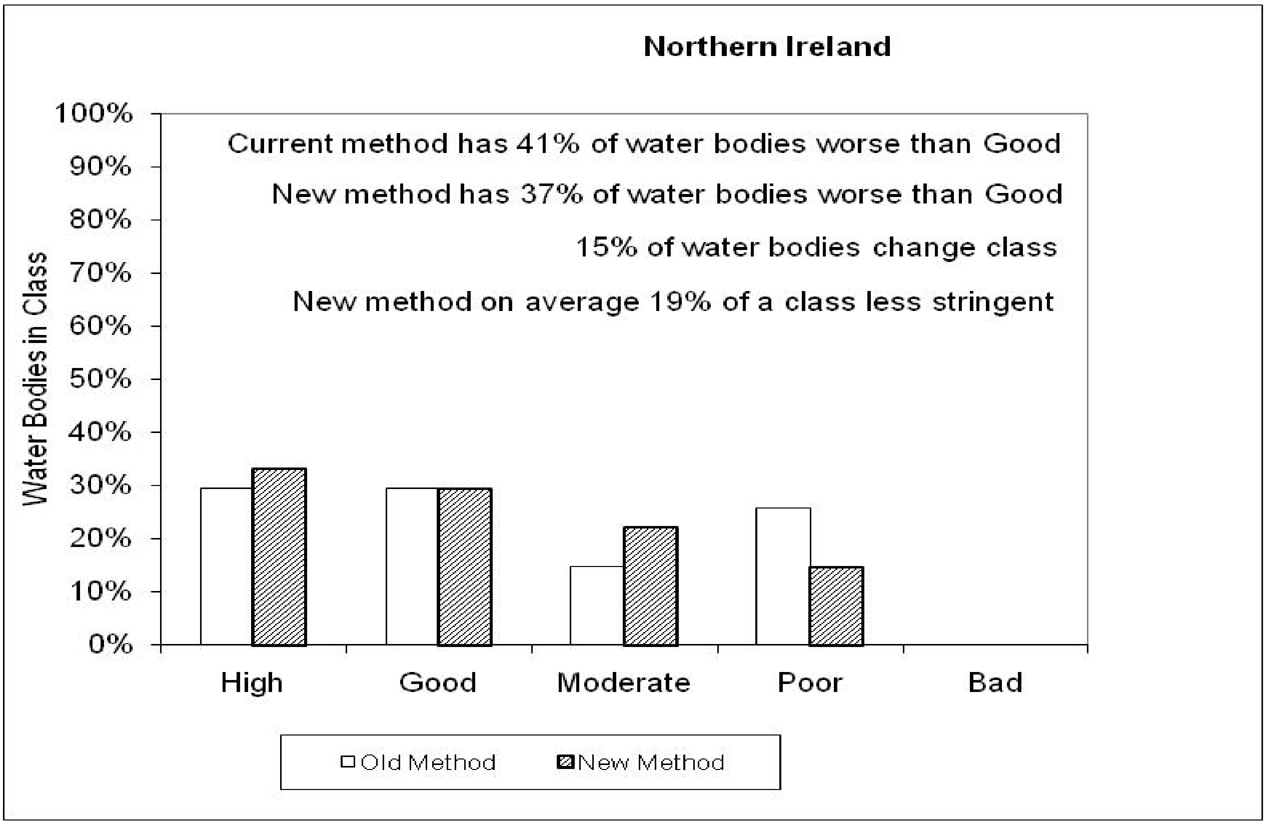


**Figure 8. Percentage of water bodies in each WFD class using the current and new lake phytobenthos assessment method.**

**Table 13. Number and percentage of water bodies that change class when using the revised version of the phytobenthos tool, DARLEQ.**

| | Number | Percentage |
|---|---|---|
| Current 4 class worse | 0 | 0.0% |
| Current 3 class worse | 0 | 0.0% |
| Current 2 class worse | 1 | 3.7% |
| Current 1 class worse | 3 | 11.1% |
| Same class | 23 | 85.2% |

| | | |
|---|---|---|
| Revised 1 class worse | 0 | 0.0% |
| Revised 2 class worse | 0 | 0.0% |
| Revised 3 class worse | 0 | 0.0% |
| Revised 4 class worse | 0 | 0.0% |

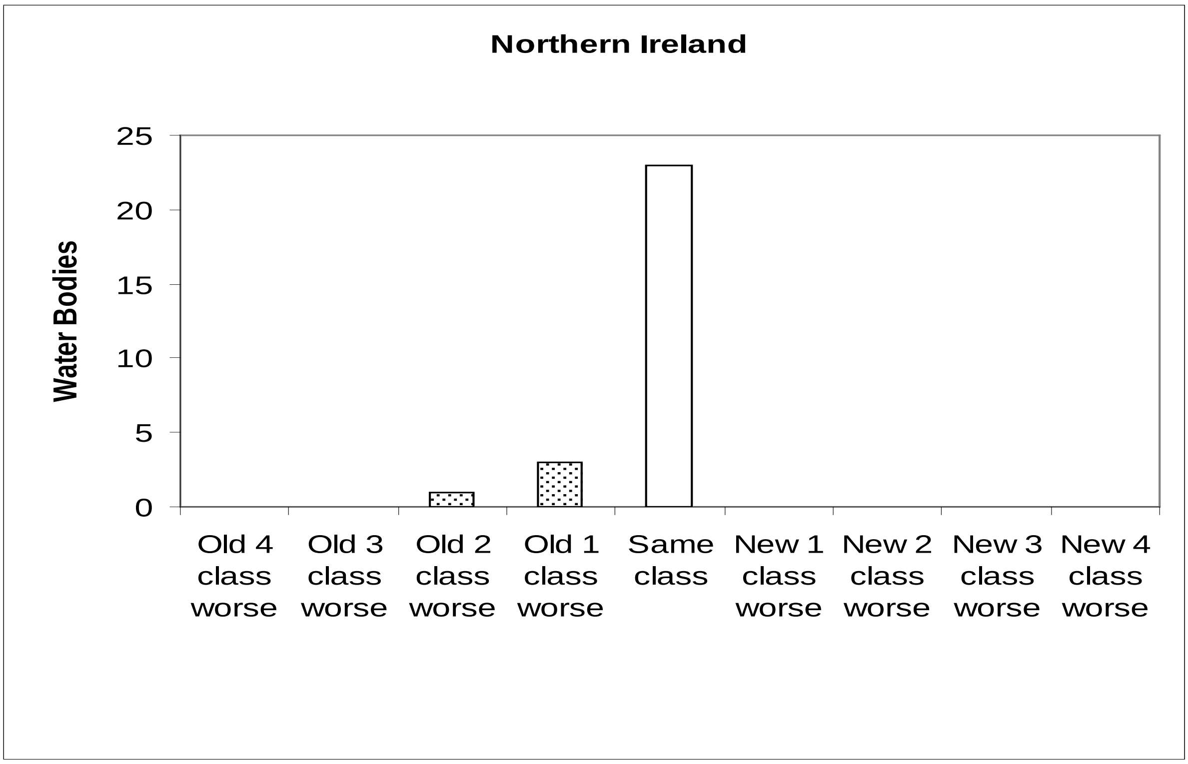


**Figure 9. Number of water bodies that change class when using the revised version of the lake phytobenthos tool, DARLEQ.**

## A4 Key documents

| | |
|---|---|
| DARLEQ method statement | Detailed description of method used for 1st RBMP (sampling and analytical methods unchanged; adjustments to calculations for 2nd RBMP): |


Bennion, H., Fluin, J., Appleby, P. and Ferrier, B. (2001). *Palaeolimnological Investigation of Scottish Freshwater Lochs*. Final report to SNIFFER No. SR(00)02 by ENSIS Ltd, University College London.

Bennion, H., Burgess, A., Juggins, S., Kelly, M., Reddihough, G. & Yallop, M. (2012) Assessment of Ecological Status in UK Lakes using Diatoms. Science Report SC070034/TR3, Environment Agency, Bristol. https://brand.environment-agency.gov.uk/mb/DCJHIl

CEN, (2003). *Water Quality – Guidance Standard for the Routine Sampling and Pretreatment of Benthic Diatoms from Rivers*. EN 13946:2003. Geneva: Comité European de Normalisation.

CEN, (2004). *Water Quality – Guidance Standard for the Identification, Enumeration and Interpretation of Benthic Diatom Samples from Running Waters*. EN 14407:2004. Geneva: Comité European de Normalisation.

Kelly, M., Juggins, S., Guthrie, R., Pritchard, S., Jamieson, J., Rippey, B., Hirst, H. and Yallop, M. (2008). Assessment of ecological status in U.K. rivers using diatoms. *Freshwater Biology*, 53, 403–422.