ANNEX 2 – RIVERS – Macrophytes & Phytobenthos – DARLEQ

Contents

A1 Description of method

Sample collection and analysis

Samples of the biofilm which covers the upper surfaces of cobbles or small boulders in rivers are collected by brushing or scraping with a toothbrush. If there are no cobbles or small boulders present at the sampling site, the submerged stems of emergent macrophytes, such as *Phragmites australis*, *Sparganium erectum*, *Glyceria maxima* or *Typha* species, or leaves and stems of submerged macrophytes such as *Ranunculus* species and *Potamogeton* 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.

Permanent slides are then prepared from each sample, and these 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.

Calculation of the Ecological Quality Ratio

The TDI was originally an expert-derived 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. The resulting index was highly correlated with the first axis of a Canonical Correspondence Analysis constrained by soluble P and nitrate-N, suggesting that the TDI faithfully reflects the underlying inorganic nutrient pressure gradient (Kelly et al., 2008).

The TDI 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 in column 2 of Table 1 corresponding to the taxon in column 1 of that Table represented by j.

The expected value of the TDI (eTDI) is predicted from a regression equation derived from a subset of reference sites characterized by very low levels of human pressure. The model uses alkalinity, an environmental variable that reflects background geology and fertility.

The value for the expected TDI at reference conditions applicable to the river should be calculated using the following equation:

$$eTDi = 9.933 * \text{Exp}(\text{Log}_{10}(\text{alkalinity})*0.81) \qquad \text{Equation 3}$$

where:

"alkalinity" is the observed annual mean alkalinity of the river in mg/l $CaCO_3$ unless the observed annual mean alkalinity is < 5 mg L^{-1} $CaCO_3$, in which case the value is set at "5"; or ≥250 mg L^{-1} $CaCO_3$, in which case the value is set at "250".

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

$$EQR_{DARES} = (100 - \text{observed value of river trophic diatom index}) \div (100 - \text{reference value for river trophic diatom index}) \qquad \text{Equation 4}$$

Where the calculated EQR is > 1.25, its value should be set to "1.25".

Status class boundaries

The boundary between high and good status in the original DARLEQ tool was the 10^{th} percentile of the EQRs of samples from reference sites. The boundary between good and moderate status was determined by plotting the relative abundance of “sensitive” taxa (i.e.

those where "s_j" ≤ 2) and "tolerant" taxa (i.e. those where "s_j" ≥4) against EQR. The point at which the two curves crossed reflects the point at which the composition of the sample ceases to be dominated by taxa associated with reference conditions, and taxa generally associated with nutrient enrichment become more common. Boundaries between moderate and poor status and poor and bad status were then determined by division of the remaining scale. These boundary values were subsequently adjusted during the second phase of intercalibration, to give final values as follows:

High/good status	1.0
Good/moderate status	0.75
Moderate/poor status	0.50
Poor/bad status	0.25

Worked example

The following taxon list was obtained from an analysis of a sample collected from a site in the English midlands:

The first column has the taxon name, the second column shows the abundance (= number of valves) of that taxon in the sample; the third column has the nutrient sensitivity score for that taxon and the final column shows the product of a × s.

Taxon	**a**	**s**	**as**
Achnanthidium minutissimum type	12	2	24
Amphora montana	1	5	5
Amphora pediculus	1	5	5
Cocconeis pediculus	5	4	20
Cocconeis placentula	61	3.1	189.1
Cyclotella atomus	1	0	0
Cyclotella meneghiniana	3	0	0
Diatoma vulgare	1	5	5
Encyonema minutum (E. "ventricosum")	1	2.6	2.6
Encyonema silesiacum (E. "ventricosum")	14	2.6	36.4
Fistulifera / Mayamaea	1	3.9	3.9

Taxon	**a**	**s**	**as**
Fragilaria vaucheriae	1	2	2
Gomphonema "intricatum" type	3	3.6	3
Gomphonema clavatum	1	2	2
Gomphonema parvulum	3	3	9
Melosira varians	3	4	12
Navicula capitatoradiata	54	4	216
Navicula cryptotenella	107	4	428
Navicula gregaria	2	4	8
Navicula lanceolata	1	4	4
Navicula menisculus	1	4	4
Navicula minima	1	3	3
Navicula reichardtiana	1	4	4
Navicula tripunctata	2	5	10
Nitzschia fonticola	2	4	8
Nitzschia gracilis	1	3	3
Nitzschia palea	7	4	28
Nitzschia paleacea	3	3	9
Nitzschia sp.	1	3	3
Planothidium frequentissimum	2	3	6
Reimeria sinuata ("Reimeria sp.")	6	3	18
Reimeria uniseriata ("Reimeria sp.")	4	3	12
Rhoicosphenia abbreviata	7	4	28
Unidentified pennate diatom	1	3	3
Σa =	315	Σas =	1121.8

The observed value for the river trophic diatom index for this sample is calculated as follows:

sum **as** for all taxa in sample = 1121.8

sum **a** for all taxa in sample = 315

Calculate W = sum **as** / sum **a** = 3.56

Calculate the observed value of TDI using equations 1 and 2:

$(W \times 25) - 25 = (3.74 \times 25) - 25 = 64.0$

The mean total alkalinity for this sample in 2003 was 88.5 mg/l $CaCO_3$.

Using the equation 3, the reference value for river trophic diatom index for the sample is:

$-25.36 + [56.83 \times \log_{10}88.5] - [12.96 \times \log_{10}(88.5)^2] + [3.21 \times 1] = 38.03$
$9.933 * Exp(Log_{10}(88.5)*0.81) = 48.1$

Applying equation 4, the ecological quality ratio for the sample is:

$(100 - 64.0) / (100 - 48.1) = 36 / 51.9 = 0.69$

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

There have been three changes to the phytobenthos tool since the first RBMP:

1. Modifications to the taxa list

The original version of DARLEQ required analysts to recognize over 500 diatom taxa. These included a number of groups of morphologically-similar taxa whose taxonomy and ecology have still not been fully resolved, along many taxa that were only found in a few samples in low numbers. Merging the "difficult" taxa into aggregates and removing the need to identify rare taxa makes the analytical task more straightforward and comparisons between outcomes based on the full taxa list and a streamlined list consisting of 265 taxa are minor. In 96% of cases the difference was lower than the variability encountered between replicate analyses of the same sample. See Environment Agency report SC070034/TR1 for more details (https://brand.environment-agency.gov.uk/mb/BwvKeX).

2. Modifications to calculation of expected TDI

Comparisons between the original versions of DARLEQ and LEAFPACS revealed a large difference in outcomes, with DARLEQ consistently delivering more stringent assessments of ecological status. Close examination of both tools suggested that DARLEQ was underestimating the value of the TDI at reference sites, particularly when alkalinity was high (Fig. 1). Monitoring during the 1st RBMP allowed a larger dataset of samples to be compiled than was available for the original development of DARLEQ. This, along with a clearer understanding of how reference sites should be defined, gained from the EU's intercalibration exercise (Pardo et al., 2012), allowed expected values of the TDI to be recalculated.

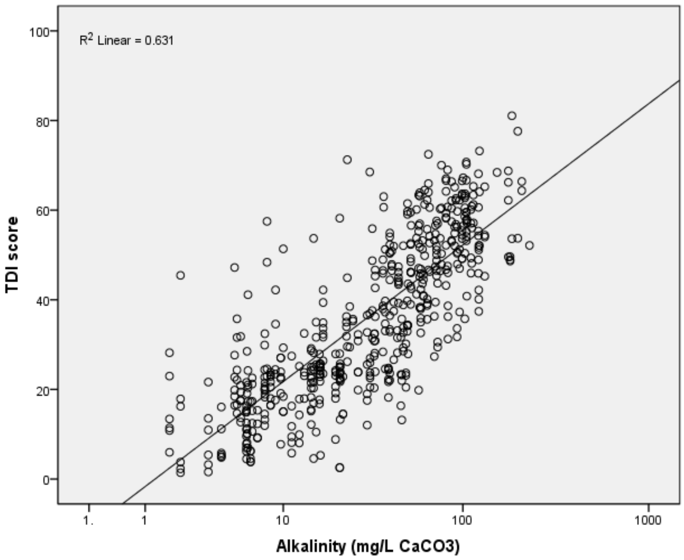

Figure 1. Relationship between TDI and alkalinity in putative reference sites screened using LEAFPACS criteria.

3. Revisions to status class boundaries

Although high/good and good/moderate 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. This led to the high/good boundary being increased from 0.93 to 1.00 and the good/moderate status boundary being lowered from 0.78 to 0.75. See Water Framework Directive Intercalibration Technical Report Part 1: Rivers for more details.

A3 Consequences of changes

England

Table 1. Comparison of classifications of ecological status determined by original and revised versions of the phytobenthos tool, DARLEQ .

		Revised					
		High	Good	Moderate	Poor	Bad	Grand Total
Current	High	246	29				275
	Good	94	114				208
	Moderate	265	269	219			753
	Poor	18	321	666	204		1209
	Bad			1	136	43	180
	Grand Total	623	733	886	340	43	2625

Table 2. Percentage of water bodies in each class, determined using original and revised versions of the phytobenthos tool, DARLEQ.

Class	Current Method	Revised Method
High	10.5%	23.7%
Good	7.9%	27.9%
Moderate	28.7%	33.8%
Poor	46.1%	13.0%
Bad	6.9%	1.6%

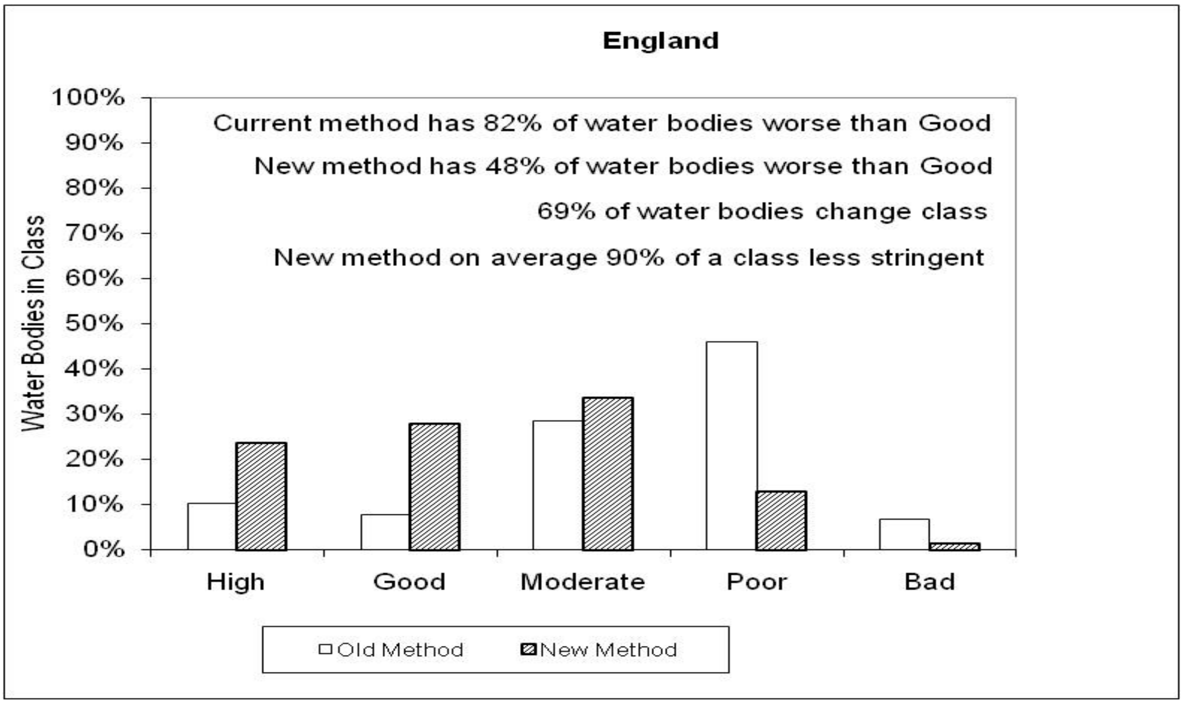

Figure 2. Percentage of water bodies in each WFD class using the current and new river phytobenthos assessment method.

Table 3. 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	18	0.7%
Current 2 class worse	587	22.4%
Current 1 class worse	1165	44.4%
Same class	826	31.5%
Revised 1 class worse	29	1.1%
Revised 2 class worse	0	0.0%
Revised 3 class worse	0	0.0%
Revised 4 class worse	0	0.0%

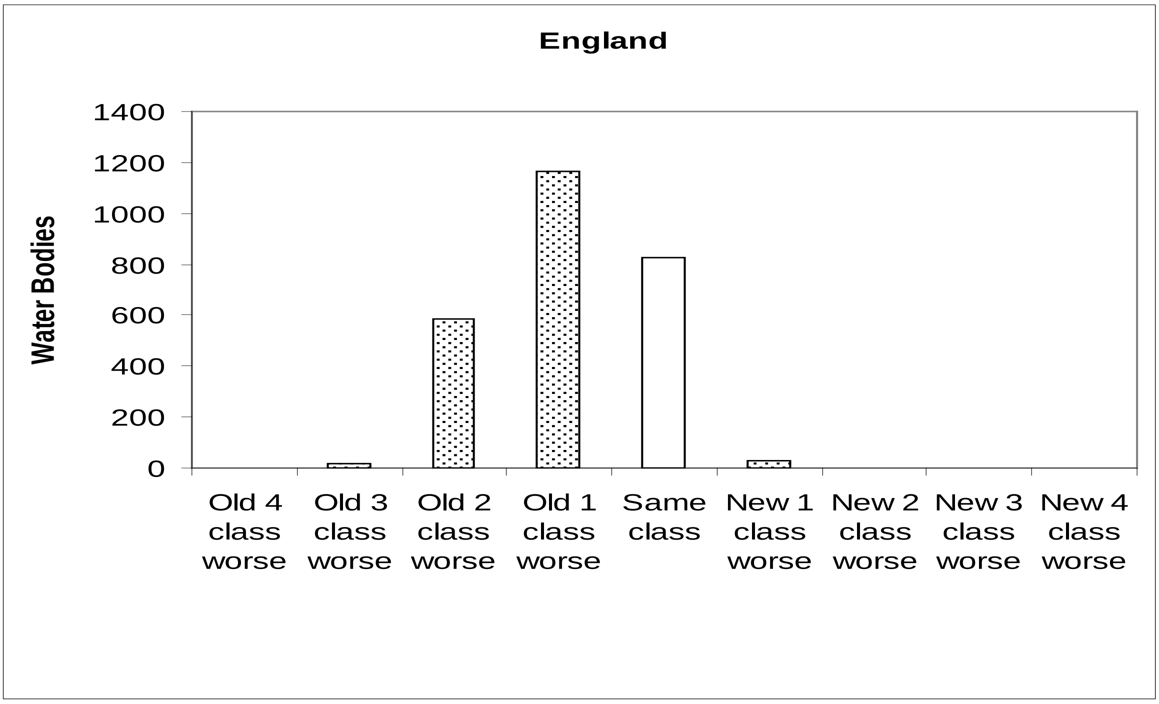

Figure 3. Number of water bodies that change class when using the revised version of the phytobenthos tool, DARLEQ.

Wales

Table 4. Comparison of classifications of ecological status determined by original and revised versions of the phytobenthos tool, DARLEQ.

		Revised					
		High	Good	Moderate	Poor	Bad	Grand Total
Current	High	57	10				67
	Good	13	72				85
	Moderate	23	52	52			127
	Poor	1	20	31	15		67
	Bad					1	1
	Grand Total	94	154	83	15	1	347

Table 5. Percentage of water bodies in each class, determined using original and revised versions of the phytobenthos tool, DARLEQ.

Class	Current Method	Revised Method
High	19.3%	27.1%
Good	24.5%	44.4%
Moderate	36.6%	23.9%
Poor	19.3%	4.3%
Bad	0.3%	0.3%

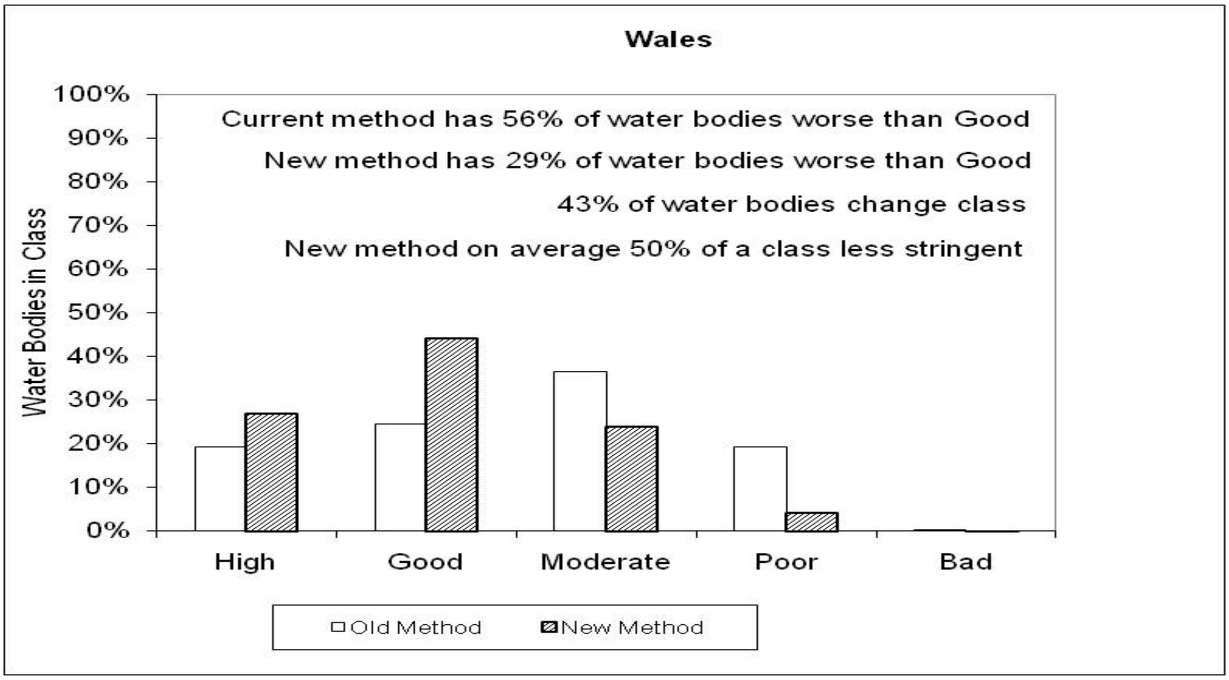

Figure 4. Percentage of water bodies in each WFD class using the current and new river phytobenthos assessment methods.

Table 6. 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%
Current 3 class worse	1	0.3%
Current 2 class worse	43	12.4%
Current 1 class worse	96	27.7%
Same class	197	56.8%
Revised 1 class worse	10	2.9%
Revised 2 class worse	0	0%
Revised 3 class worse	0	0%
Revised 4 class worse	0	0%

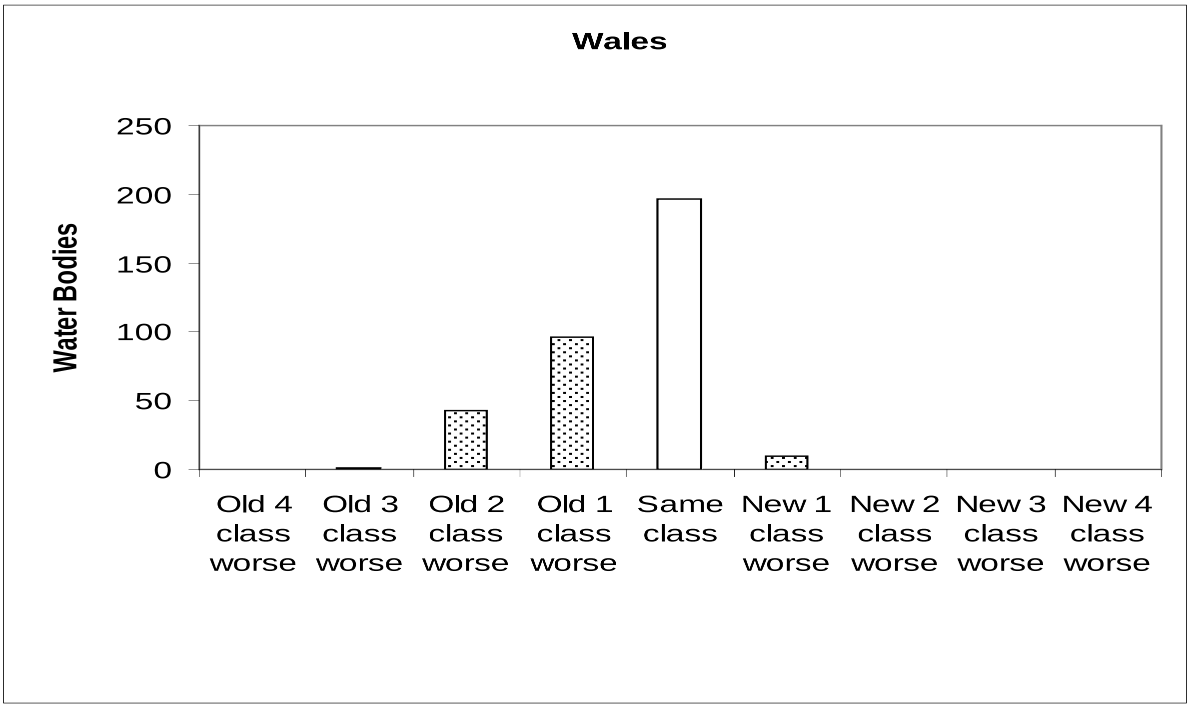

Figure 5. Number of water bodies that change class when using the revised version of the phytobenthos tool, DARLEQ.

Scotland

Table 7. Comparison of classifications of ecological status determined by original and revised versions of the phytobenthos tool, DARLEQ.

		Revised					
		High	Good	Moderate	Poor	Bad	Grand Total
Current	High	178	48				226
	Good	48	114	3			165
	Moderate	8	183	115			306
	Poor		5	30	4		39
	Bad						
	Grand Total	234	350	148	4		736

Table 8. Percentage of water bodies in each class, determined using original and revised versions of the phytobenthos tool, DARLEQ.

Class	Current Method	Revised Method
High	30.7%	31.8%
Good	22.4%	47.6%
Moderate	41.6%	20.1%
Poor	5.3%	0.5%
Bad	0%	0%

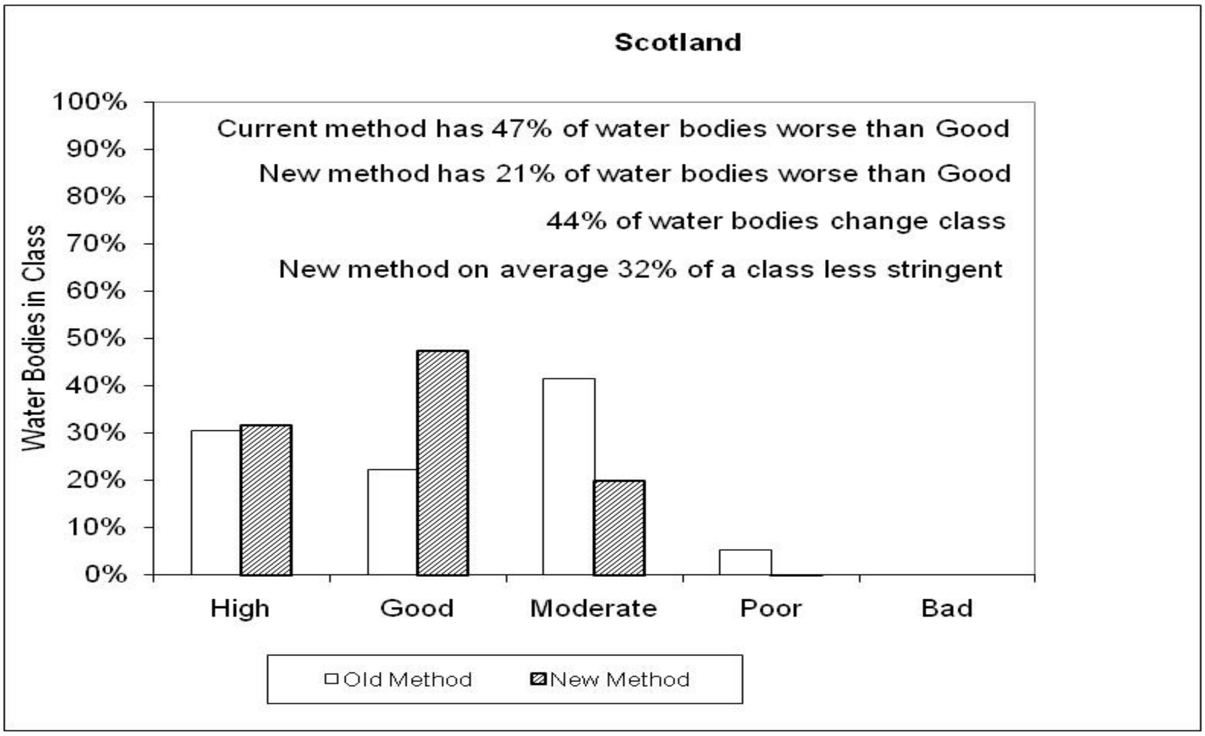

Figure 6. Percentage of water bodies in each WFD class using the current and new river phytobenthos assessment methods.

Table 9. 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%
Current 3 class worse	0%	0%
Current 2 class worse	13	1.8%
Current 1 class worse	261	35.5%
Same class	411	55.8%
Revised 1 class worse	51	6.9%
Revised 2 class worse	0%	0%
Revised 3 class worse	0%	0%
Revised 4 class worse	0%	0%

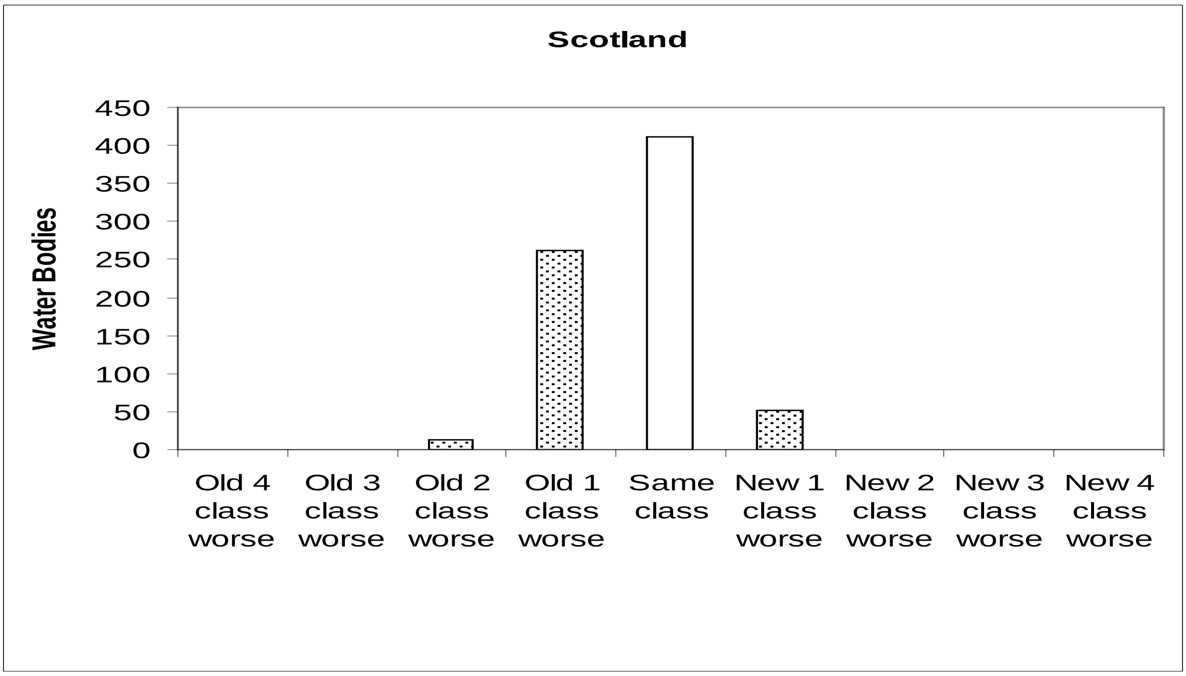

Figure 7. Number of water bodies that change class when using the revised version of the phytobenthos tool, DARLEQ.

Northern Ireland

Table 10. Comparison of classifications of ecological status determined by original and revised versions of the phytobenthos tool, DARLEQ.

		Revised					
		High	Good	Moderate	Poor	Bad	Grand Total
Current	High	38	19				57
	Good	23	42				65
	Moderate	15	109	42			166
	Poor		5	15			20
	Bad						
	Grand Total	76	175	57			308

Table 11. Percentage of water bodies in each class, determined using original and revised versions of the phytobenthos tool, DARLEQ.

Class	Current Method	Revised Method
High	18.5%	24.7%
Good	21.1%	56.8%
Moderate	53.9%	18.5%
Poor	6.5%	0%
Bad	0%	0%

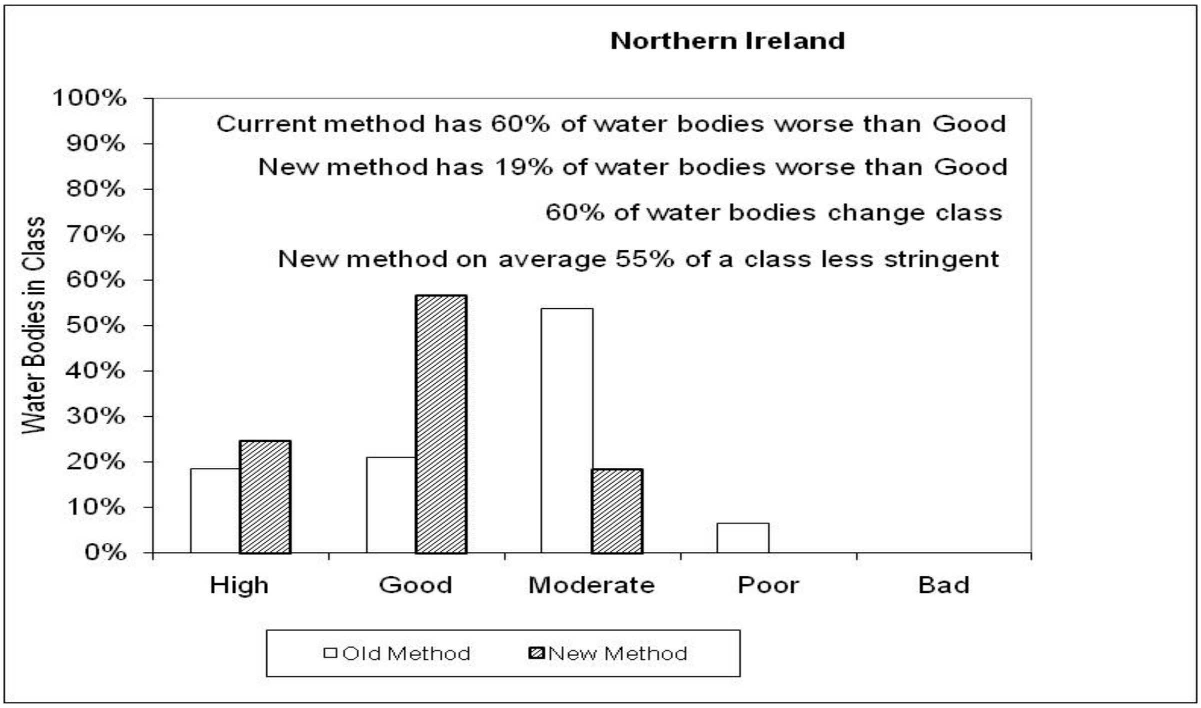

Figure 8. Percentage of water bodies in each WFD class using the current and new river phytobenthos assessment methods.

Table 12. 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%
Current 3 class worse	0%	0%
Current 2 class worse	20%	6.5%
Current 1 class worse	147%	47.7%
Same class	122%	39.6%
Revised 1 class worse	19%	6.2%
Revised 2 class worse	0%	0%
Revised 3 class worse	0%	0%
Revised 4 class worse	0%	0%

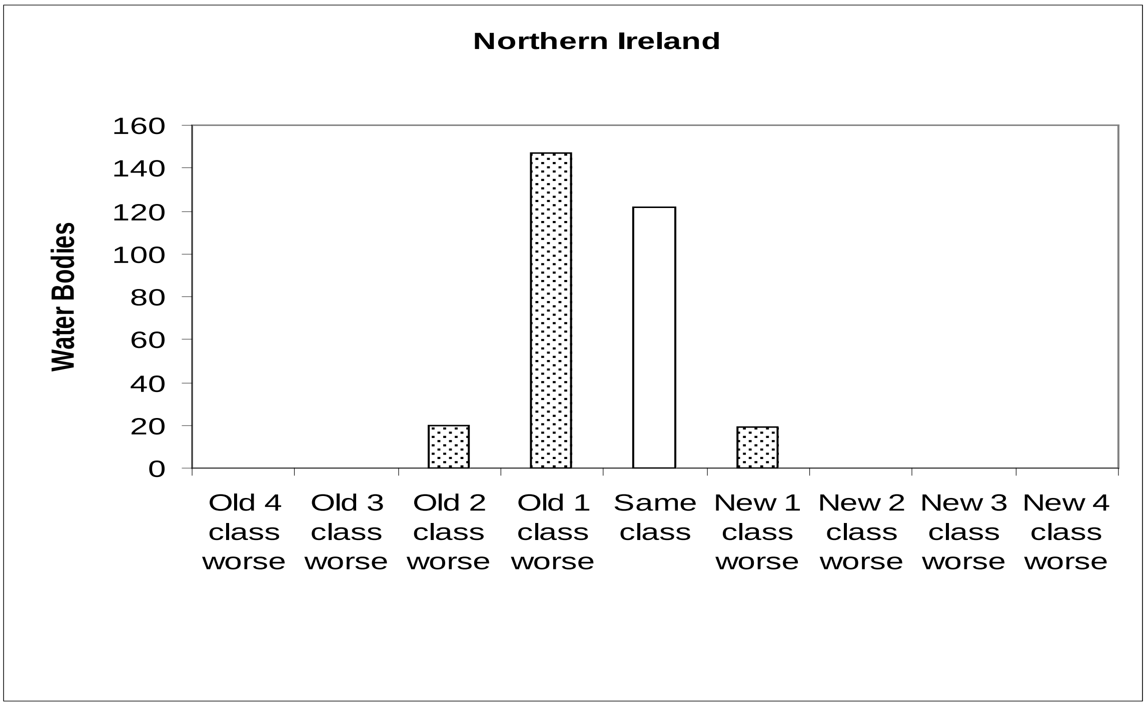

Figure 9. Number of water bodies that change class when using the revised version of the phytobenthos tool, DARLEQ.

A4 Key documents

DARLEQ method statement	Detailed description of method used for 1st RBMP (sampling and analytical method unchanged; revised taxa list and changes to calculations for 2nd RBMP),

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.

Environment Agency (2012). A streamlined taxonomy for the Trophic Diatom Index. Environment Agency Report – SC070034/TR1. ISBN: 978-1-84911-280-2.

Joint Research Centre (2009). Water Framework Directive Intercalibration Technical Report. Part 1: Rivers. (http://publications.jrc.ec.europa.eu/repository/handle/111111111/294).

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

Pardo, I., Gómez-Rodríguez, C., Wasson, J-G., Owen, R., van de Bund, W., Kelly, M., Bennett, C., Birk, S., Buffagni, A., Erba, S., Mengin, N., Murray-Bligh, J., and Ofenböck, G.,(2012). The European reference condition concept: A scientific and technical approach to identify minimally-impacted river ecosystems. *Science of the Total Environment* 420: 33-42.