

## **ANNEX 3 – RIVERS – Macrophytes & Phytobenthos (combination)**

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### **A1 Description of method**

#### **Sample collection and analysis**

Samples are collected as outlined in the Technical Annexes for the two component tools: DARLEQ and LEAFPACS, Annexes 1 and 2.

#### **Calculation of the Ecological Quality Ratio**

The calculation of the individual EQRs for LEAFACS and DARLEQ are outlined in the individual Annexes.

When using the combined LEAFPACS and DARLEQ BQE it is necessary to normalise the DARLEQ EQRs to bring them in-line with LEAFPACS EQR values.

The worst of either LEAFPACS and DARLEQ is used.

#### **Status class boundaries**

The derivation of status class boundaries for the individual LEAFACS and DARLEQ BQEs are outlined in their respective Technical Annexes 1 and 2.

#### **Combining LEAFPACS and DARLEQ**

The combination rule adopted for the first RBMP was to take the lowest of LEAFPACS and DARLEQ which, as DARLEQ presented a more stringent view of ecological status than LEAFPACS, led to many sites being classified as moderate status or lower.

The same dataset was used to further develop a combination rule. DARLEQ and LEAFPACS can be calculated separately and then compared, which allows the two tools to be used independently if necessary. Within these two broad options there were further options for taking the minimum of the component metrics or the average, and included implications of “weighting” the relative contributions of LEAFPACS and DARLEQ using either stream energy or productivity as a weighting factor. All options were explored.

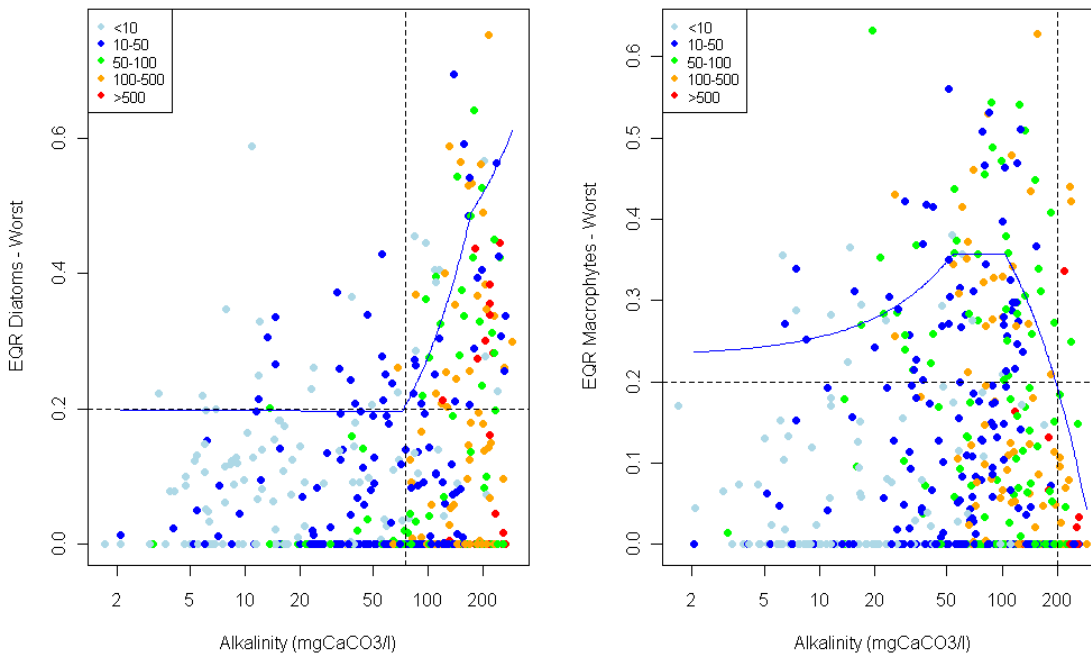
The revised DARLEQ and LEAFPACS EQRs, in combination, gave stronger relationships with the pressure gradient (TP = eutrophication) than did either component alone. Use of the PCA axis scores to weight the combination did not lead to a significant improvement in  $r^2$  when compared to simple rules based on taking the average or minima. Consequently, it was recommended that since both LEAFPACS and DARLEQ measure different aspects of river ecology, both are necessary, and therefore should be combined using the minimum of the two values. The average is insufficiently precautionary, yielding combined EQRs typically half a class higher.

A strong agreement between DARLEQ- and LEAFPACS-based assessments was evident at low and moderate alkalinity, although DARLEQ-derived EQRs showed a poor relationship at high alkalinity, by contrast, LEAFPACS showed significant relationship for all alkalinity classes.

Calculating the difference between each of LEAFPACS and DARLEQ EQRs, and the final assessment based on the minimum of both, was plotted against alkalinity, with a regression superimposed based on the 90<sup>th</sup> percentile of the data (Figure 1). Where this line exceeds 0.2 EQR units (equivalent to one class) there is a significant risk that an assessment based on one component will lead to misclassification. In summary, a DARLEQ-based assessment alone would give a reliable classification if alkalinity is  $< 75 \text{ mg L}^{-1} \text{ CaCO}_3$  whilst a LEAFPACS-based assessment alone is adequate at  $>200 \text{ mg L}^{-1} \text{ CaCO}_3$ . In the middle range, both components are necessary although, on average, an assessment based on DARLEQ alone will be more reliable than LEAFPACS alone at alkalinities up to  $\sim 120 \text{ mg L}^{-1} \text{ CaCO}_3$  (i.e. closer to the minimum of both LEAFPACS and DARLEQ), whereas LEAFPACS alone will be more reliable at alkalinities above this.

### **Operational assessments in using combined LEAFPACS and DARLEQ**

It is however recognised that there will be situations where macrophyte surveys are either not possible or are compromised, and possibly also situations where diatom assessments are inherently unreliable. A series of rules can be proposed based on these analyses, along with local knowledge and data on phosphorus concentrations, which provide a framework for Surveillance monitoring only (Table 1). As very high nutrient concentrations are unlikely to be associated with 'Good' ecological status for either DARLEQ or LEAFPACS, these can be used for preliminary screening, after which alkalinity is the primary determinant.



**Figure 1.** Difference between assessments based on a single component (either DARLEQ - diatoms or LEAFPACS-macrophytes) and both DARLEQ and LEAFPACS (worst), plotted against alkalinity. Blue line is the regression based on the 90<sup>th</sup> percentile; dashed horizontal line represents one class difference between the two methods; dashed vertical line represents the point at which the regression line exceeds 0.2 EQR.

**Table 1.** Recommended monitoring options for macrophytes and phyto-benthos in rivers.

		Alkalinity (mg L <sup>-1</sup> CaCO <sub>3</sub> )		
		< 75	75-200	>200
Phosphorus (µg L <sup>-1</sup> )	< 150	Diatom	Diatoms and macrophytes	Macrophytes
	>150	Assume < GES	Macrophytes	Macrophytes
	>1000	Assume <GES	Assume <GES	Assume <GES

Local knowledge should be used for operation decisions, since there are a range of factors such as whether sites are heavily-shaded and whether sites are heavily-channelised, which will affect diatom and macrophyte assessments. Nutrient concentrations are an approximate screening tool only and the medium-term (i.e. 5 year) target concentrations should be considered rather than the present concentrations. Where good status or better is concluded from one sub-element, there should also be a check for contradictory evidence (e.g. P thresholds for that water body type are

not exceeded, history of recent fish kills due to anoxia) before confirming the good status designation. If such contraindications exist, then the other sub-element should also be included in assessments. Moreover, macrophyte surveys may not be possible at sites with heavy boat traffic, or in very deep rivers or small lowland streams. Conversely there may be situations (e.g. on hard limestone) where diatoms are responsive at high alkalinities.

### **Operational assessments using bacterial tufts (sewage fungus)**

The normative definition refers to the “displacement” of phytobenthos by bacterial tufts and coats, implying a need to recognise a state where the organic loading is so high that heterotrophic organisms can outcompete phototrophic organisms. Bacterial tufts and coats are generally referred to as “sewage fungus” and have long been recognised for their role as indicators of organic pollution, which is currently recorded in the field but, at present, plays no role in the formal assessment of ecological status. Sewage fungus is likely to include a mixture of heterotrophic bacteria, fungi and protozoans, and it will be important not to confuse sewage fungus with phototrophic bacteria (such as Cyanobacteria), or even other filamentous algal growths.

It is recommended to record sewage fungus (bacterial tufts and growths) in the field on all occasions a macrophyte or diatom sample is collected, and that such growths should only have the potential to downgrade from Moderate or worse class (Table 2) for phytobenthos classifications. Where the DARLEQ tool classifies as High or Good status phytobenthos is, by definition, not adversely affected by bacterial tufts and coats. The recommendation is therefore that sewage fungus coverage and density (as an average over the assessment period) should over-ride the results of LEAFPACS classifications at any status and DARLEQ assessments that are moderate or worse (Table 2).

**Table 2. Use of coverage and density of sewage fungus growths to distinguish between ecological status classes below Good status for DARLEQ classifications or any status for LEAFPACS classifications.**

<b>Coverage →</b>	<b>Occasional</b>	<b>Widespread</b>	<b>Extensive</b>
<b>Density ↓</b>			
Trace	Moderate	Moderate	Poor
Thin	Moderate	Poor	Poor
Thick	Poor	Poor	Bad
Massive	Poor	Bad	Bad

where Coverage: Occasional (<30% of surface area); Widespread (30-60% of surface area) or Extensive (>61% of surface area); Density, Trace (present, but only just detectable); Thin (Obvious

presence but substrate not obscured); Thick (Thick enough to fully obscure substrate) or Massive (Occupies a significant proportion of the water column).

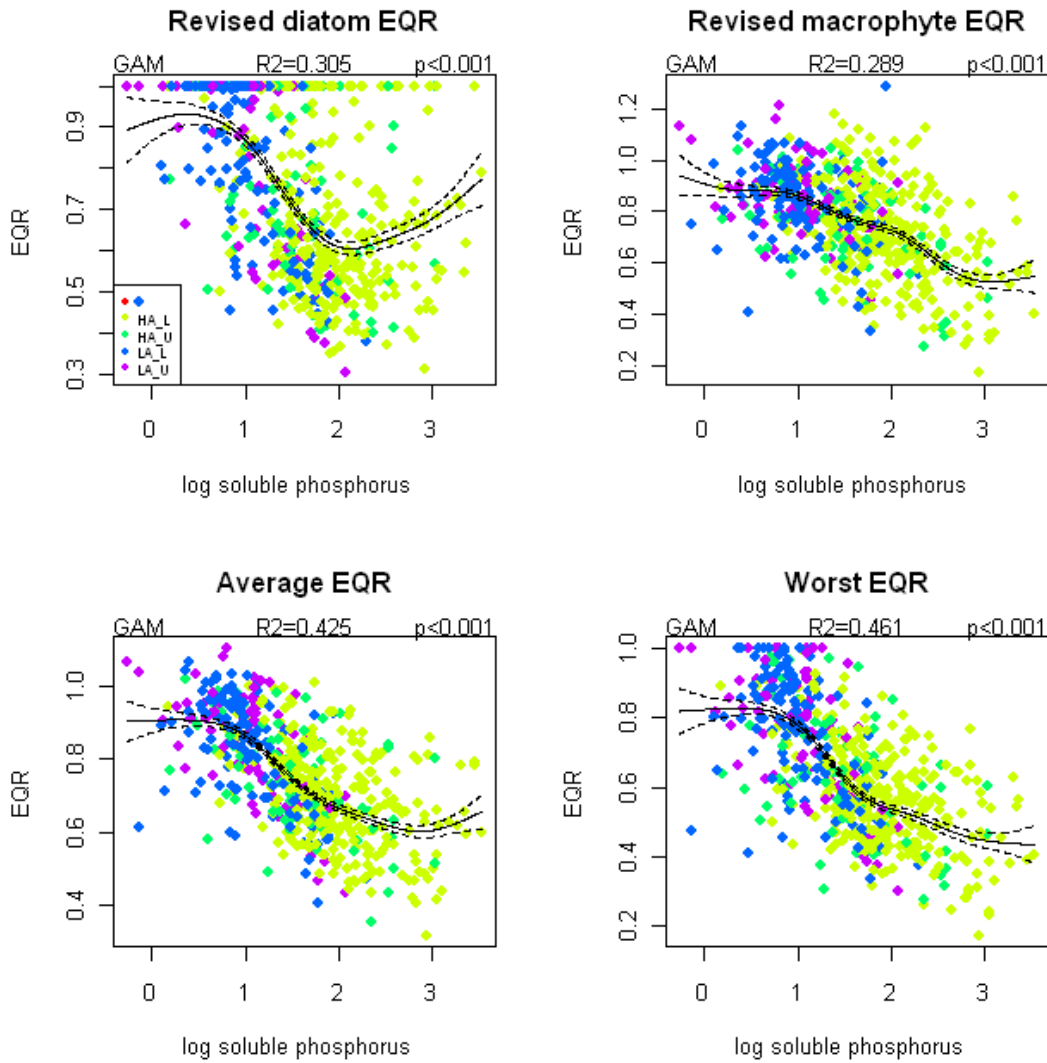
The LEAFPACS sampling period is restricted to one year therefore, if a less than good bacterial tuft assessment is made in this period, more frequent bacterial tufts surveys should be triggered which correspond to the diatom sampling frequency (2 seasons per year). This will help to confirm the temporal and spatial impact of the organic pollution.

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

Summaries for individual DARLEQ and LEAFPAC changes between the RBMPs are presented in separate Technical Annexes 1 and 2, although no account was taken of sewage fungus assessment.

A main consequence of these changes is that direct comparison with phytobenthos EQRs and status classes produced for the first RBMP was not possible. Initial assessment based on the first RBMP highlighted the most obvious consequence of combining the two tools as an increase in the proportion of sites that were classified at Good status with a great reduction in the proportion that are classified at Poor or Bad status. This change arose mainly due to revisions to the expected TDI model and was most pronounced in England and Northern Ireland where high alkalinity rivers, that are most influenced by this change, dominated.

The pressure response (inorganic P = eutrophication) is detailed in Figure 2 based on the Generalised Additive Models (GAM). The revised EQRs in combination gave stronger relationships with the pressure than either of the components alone. Using the worst of EQR gave the strongest relationship ( $r^2 = 0.46$ ).



**Figure 2. Scatterplots showing relationship between revised DARLEQ and LEAFPACS EQRs alone and combined, taking either the average or the minimum of the two metrics, overlain with regression lines calculated using GAM. Points are coloured according to a simple typology (HA\_L = high alkalinity, lowland; HA\_U = high alkalinity, upland; LA\_L: low alkalinity, lowland; LA\_U: low alkalinity, upland).**

### A3 Consequences of changes

Outlined below are consequences of changes using a combination of worst of either LEAFPACS and DARLEQ EQRs, with no account taken of sewage fungus assessment.

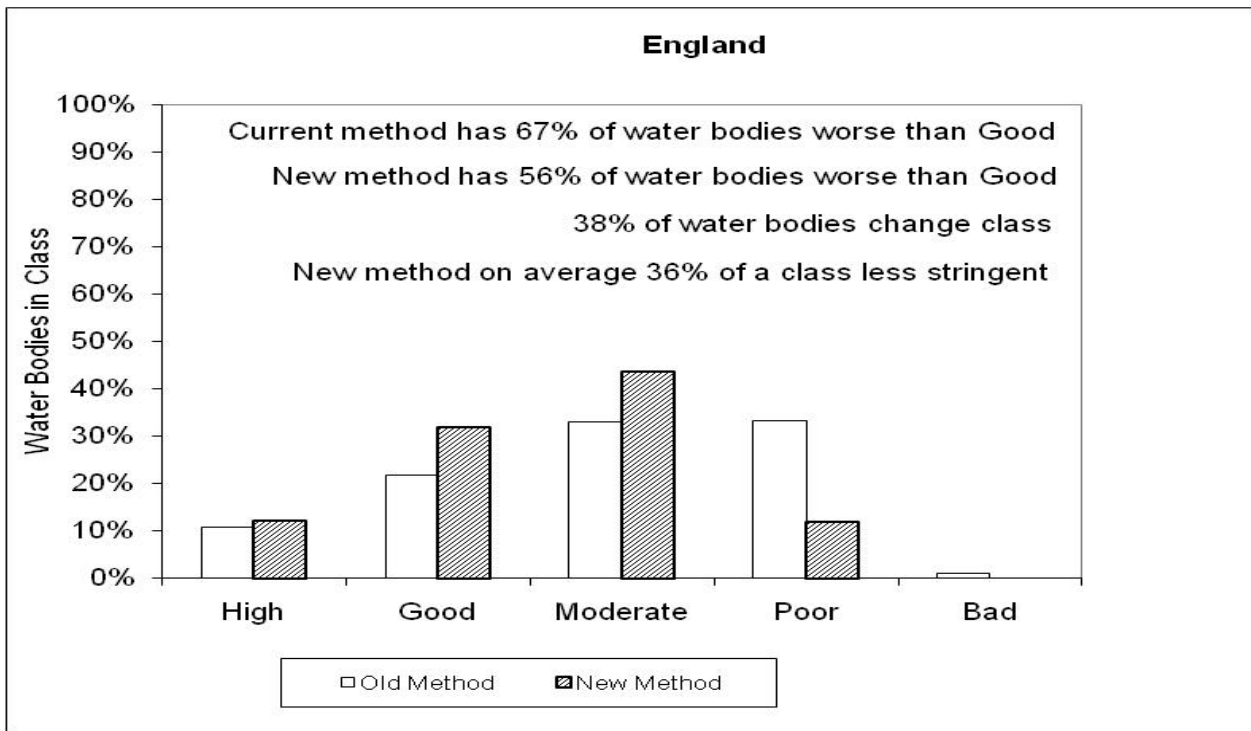
#### England

**Table 3. Comparison of classifications of ecological status determined by original and revised versions of the river phytobenthos tool, DARLEQ and river macrophyte tool, LEAFPACS.**

		Revised					Grand Total
		High	Good	Moderate	Poor	Bad	
Current	High	26	5				31
	Good	5	57				62
	Moderate	4	24	64	2		94
	Poor		5	61	29		95
	Bad				3		3
Grand Total		35	91	125	34		285

**Table 4. Percentage of water bodies in each class, determined using original and revised versions of the river phytobenthos tool, DARLEQ and river macrophyte tool, LEAFPACS.**

Class	Current Method	Revised Method
High	10.9%	12.3%
Good	21.8%	31.9%
Moderate	33%	43.9%
Poor	33.3%	11.9%
Bad	1.1%	0%



**Figure 3. Percentage of water bodies in each WFD class using the combined current and new river phytobenthos and macrophyte assessment methods.**

**Table 5. Number and percentage of water bodies that change class when using the combined phytobenthos and macrophyte assessment methods**

	Number	Percentage
Current 4 class worse	0	0.0%
Current 3 class worse	0	0.0%
Current 2 class worse	9	3.2%
Current 1 class worse	93	32.6%
Same class	176	61.8%
Revised 1 class worse	7	2.5%
Revised 2 class worse	0	0.0%
Revised 3 class worse	0	0.0%
Revised 4 class worse	0	0.0%



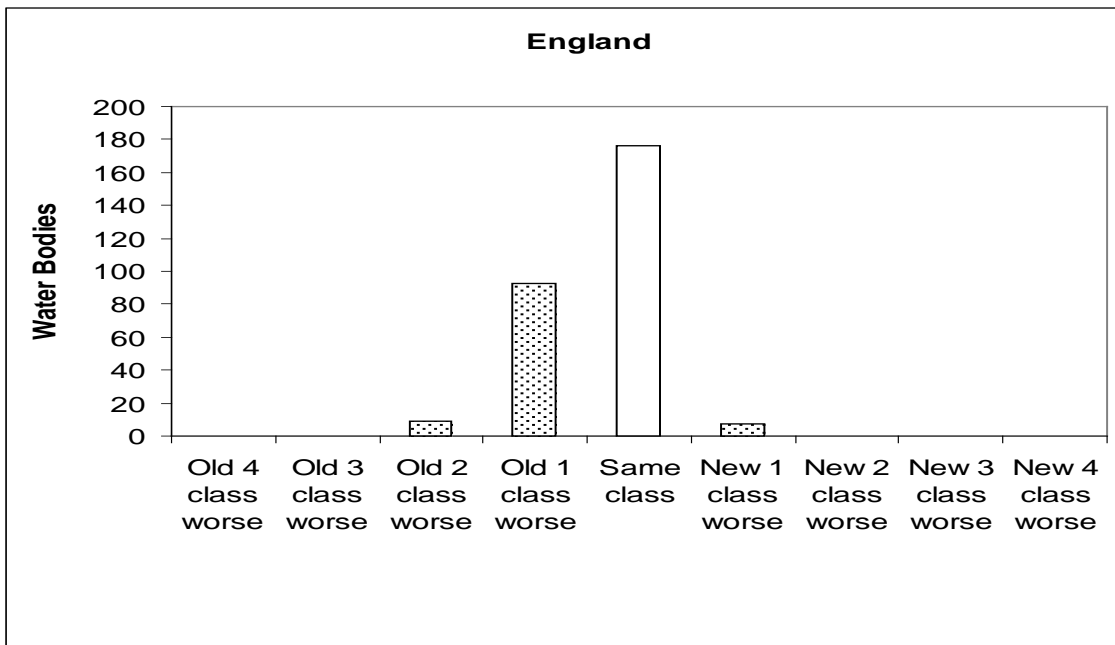


Figure 4. Number of water bodies that change class when using the revised version of the river phytobenthos tool, DARLEQ and river macrophyte tool, LEAFPACS.

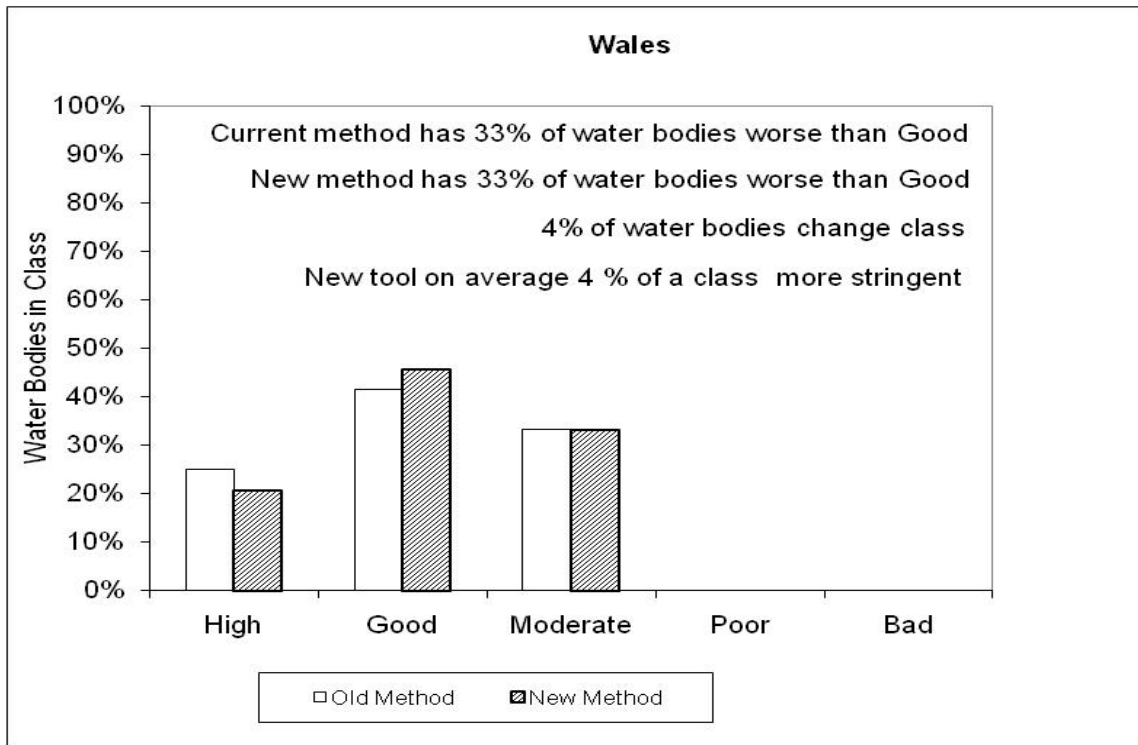
## Wales

Table 6. Comparison of classifications of ecological status determined by original and revised versions of the river phytobenthos tool, DARLEQ and river macrophyte tool, LEAFPACS.

		High	Good	Revised Moderate	Poor	Bad	Grand Total
Current	High	5	1				6
	Good		10				10
	Moderate			8			8
	Poor						
	Bad						
Grand Total		5	11	8			24

Table 7. Percentage of water bodies in each class, determined using original and revised versions of the river phytobenthos tool, DARLEQ and river macrophyte tool, LEAFPACS.

Class	Current Method	Revised Method
High	25%	20.8%
Good	41.7%	45.8%
Moderate	33.3%	33.3%
Poor	0%	0%
Bad	0%	0%



**Figure 5. Percentage of water bodies in each WFD class using the combined current and new river phytobenthos and macrophyte assessment methods.**

**Table 8. Number and percentage of water bodies that change class when using the combined phytobenthos and macrophyte assessment methods.**

	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	23	95.8%
Revised 1 class worse	1	4.2%
Revised 2 class worse	0	0.0%
Revised 3 class worse	0	0.0%
Revised 4 class worse	0	0.0%

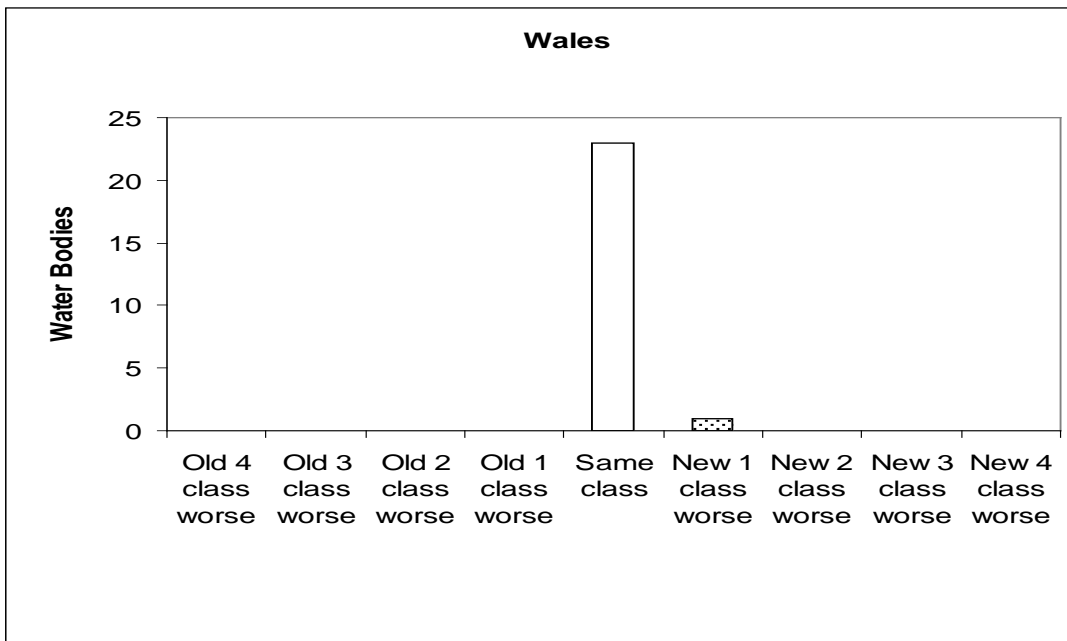


Figure 6. Number of water bodies that change class when using the revised version of the river phytobenthos tool, DARLEQ and river macrophyte tool, LEAFPACS.

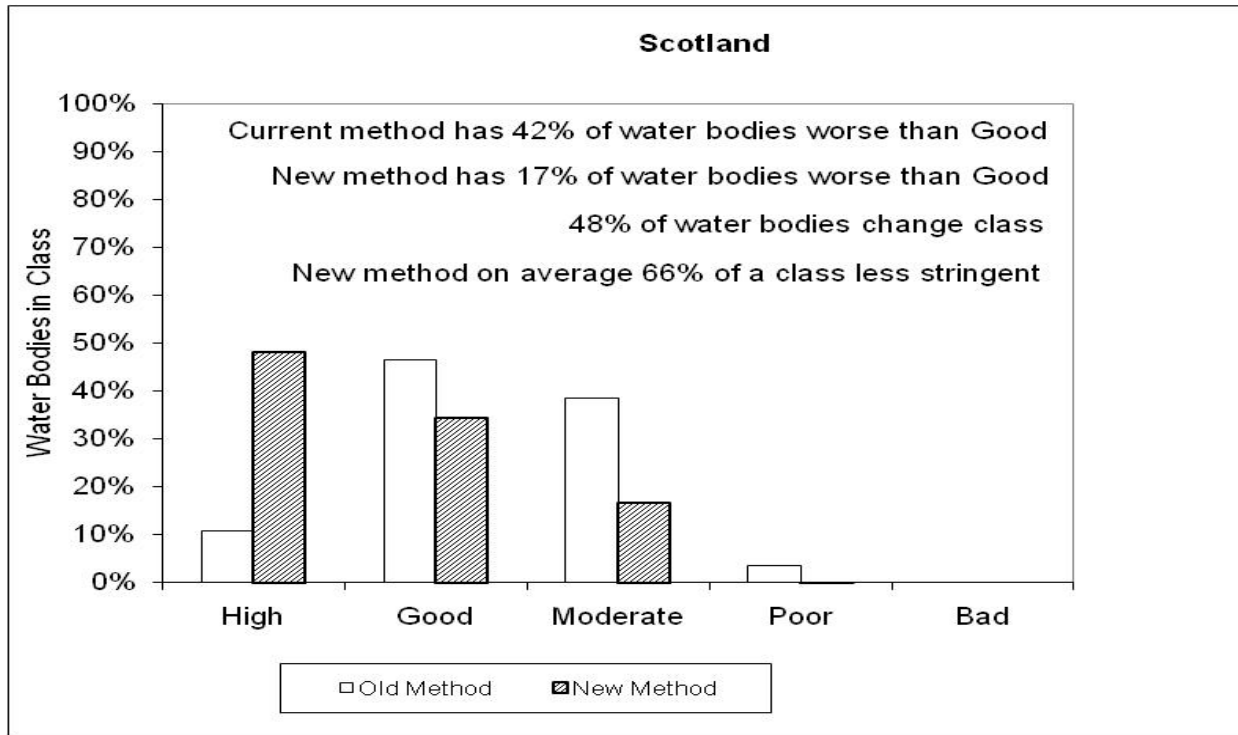
## Scotland

Table 9. Comparison of classifications of ecological status determined by original and revised versions of the river phytobenthos tool, DARLEQ and river macrophyte tool, LEAFPACS.

		Revised					Grand Total
		High	Good	Moderate	Poor	Bad	
Current	High	63	2				65
	Good	123	152	2			277
	Moderate	90	51	89			230
	Poor	11		9	2		22
	Bad						
Grand Total		287	205	100	2		594

Table 10. Percentage of water bodies in each class, determined using original and revised versions of the river phytobenthos tool, DARLEQ and river macrophyte tool, LEAFPACS.

Class	Current Method	Revised Method
High	10.9%	48.3%
Good	46.6%	34.5%
Moderate	38.7%	16.8%
Poor	3.7%	0.3%
Bad	0.0%	0.0%



**Figure 7. Percentage of water bodies in each WFD class using the combined current and new river phytoplankton and macrophyte assessment methods.**

**Table 11. Number and percentage of water bodies that change class when using the combined phytoplankton and macrophyte assessment methods.**

	Number	Percentage
Current 4 class worse	0	0.0%
Current 3 class worse	11	1.9%
Current 2 class worse	90	15.2%
Current 1 class worse	183	30.8%
Same class	306	51.5%
Revised 1 class worse	4	0.7%
Revised 2 class worse	0	0.0%
Revised 3 class worse	0	0.0%
Revised 4 class worse	0	0.0%

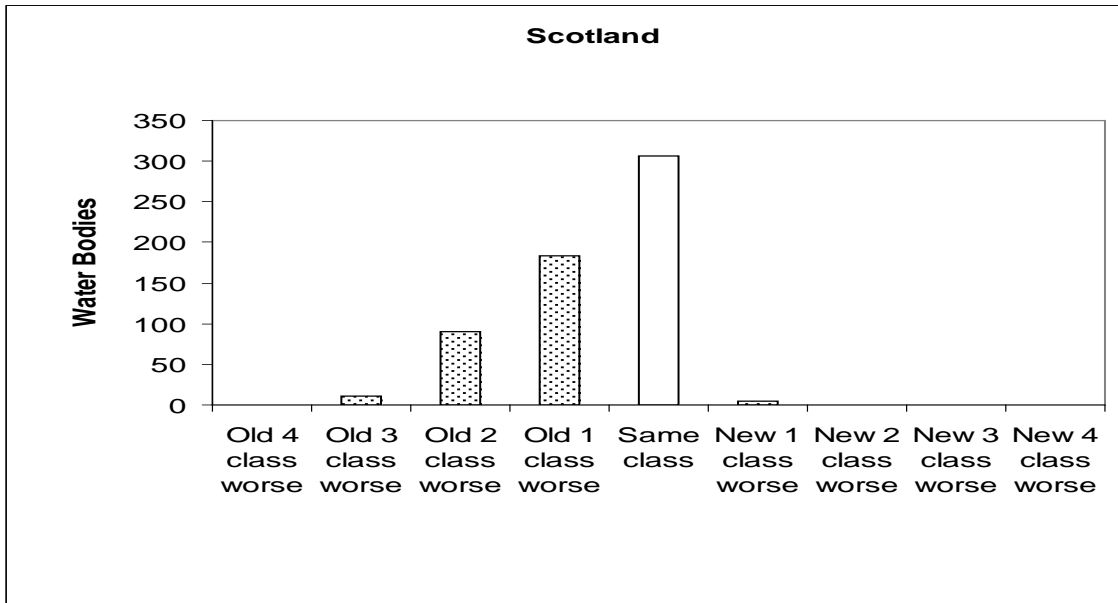


Figure 8. Number of water bodies in Scotland that change class when using the revised version of the river phytobenthos tool, DARLEQ and river macrophyte tool, LEAFPACS.

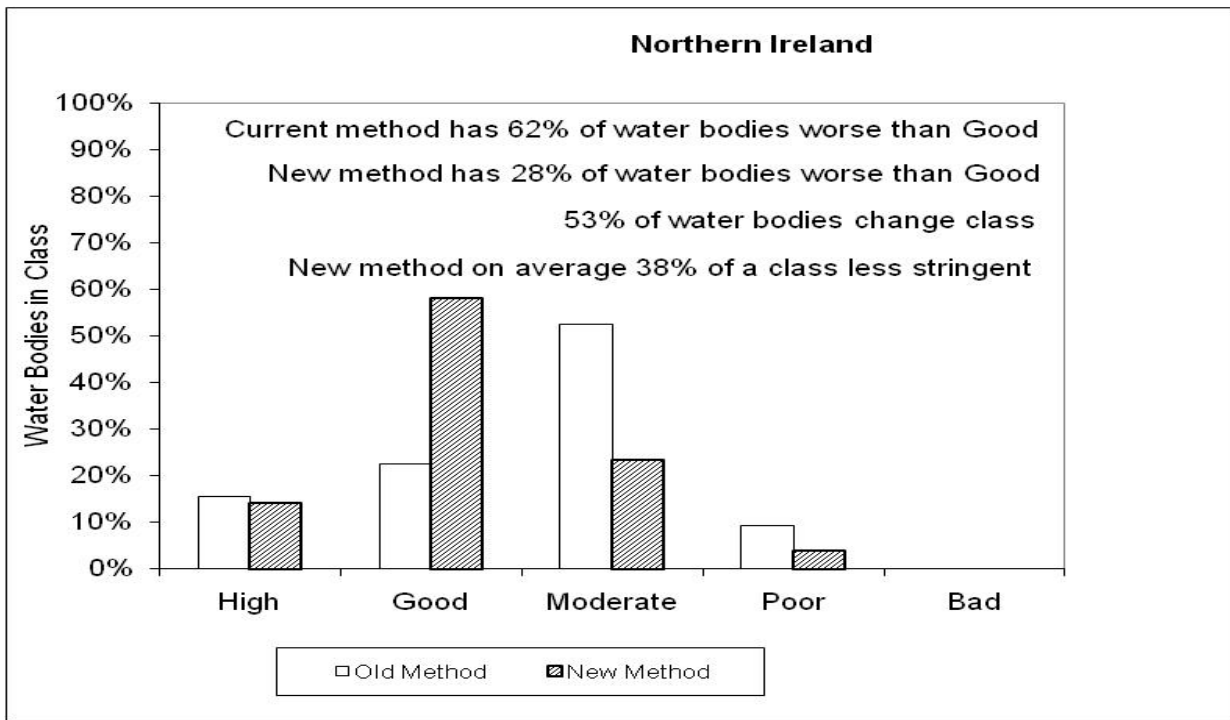
### Northern Ireland

Table 12. Comparison of classifications of ecological status determined by original and revised versions of the river phytobenthos tool, DARLEQ and river macrophyte tool, LEAFPACS.

		Revised					Grand Total
		High	Good	Moderate	Poor	Bad	
Current	High	25	22				47
	Good	15	53				68
	Moderate	3	97	54	4		158
	Poor		3	17	8		28
	Bad						
Grand Total		43	175	71	12		301

Table 13. Percentage of water bodies in each class, determined using original and revised versions of the river phytobenthos tool, DARLEQ and river macrophyte tool, LEAFPACS.

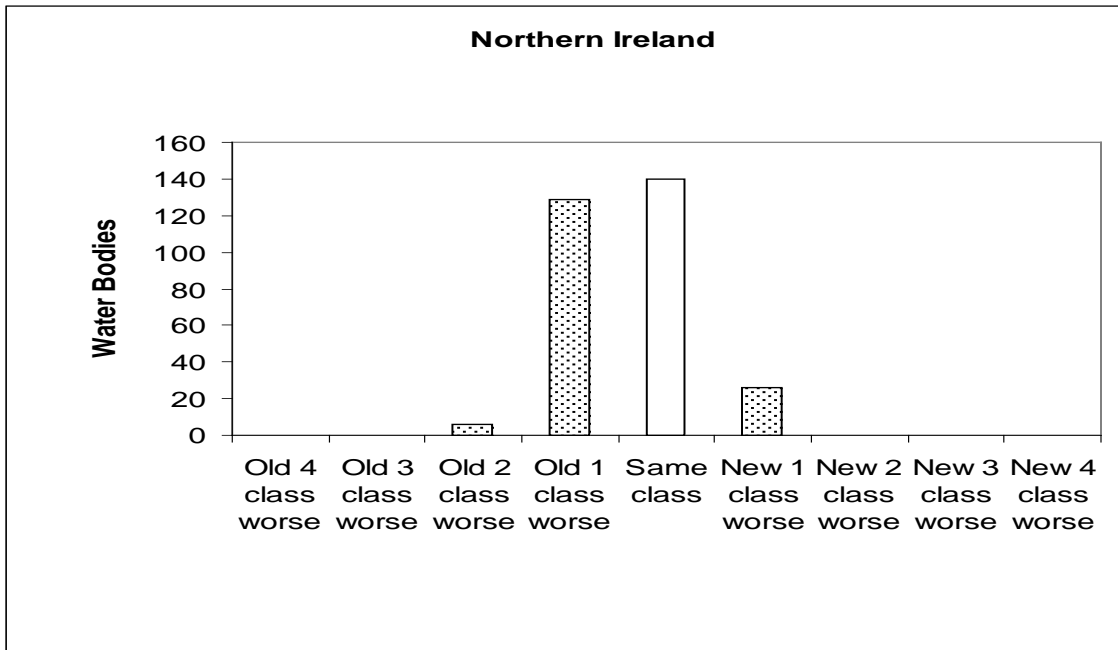
Class	Current Method	Revised Method
High	15.6%	14.3%
Good	22.6%	58.1%
Moderate	52.5%	23.6%
Poor	9.3%	4.0%
Bad	0.0%	0.0%



**Figure 9. Percentage of water bodies in each WFD class using the combined current and new river phytobenthos and macrophyte assessment methods.**

**Table 14. Number and percentage of water bodies that change class when using the combined phytobenthos and macrophyte assessment methods**

	Number	Percentage
Current 4 class worse	0	0.0%
Current 3 class worse	0	0.0%
Current 2 class worse	6	2.0%
Current 1 class worse	129	42.9%
Same class	140	46.5%
Revised 1 class worse	26	8.6%
Revised 2 class worse	0	0.0%
Revised 3 class worse	0	0.0%
Revised 4 class worse	0	0.0%



**Figure 10. Number of water bodies in Scotland that change class when using the revised version of the river phytobenthos tool, DARLEQ and river macrophyte tool, LEAFPACS.**

## A4 Key documents

Annex 1 Rivers – Macrophytes & Phytobenthos – LEAFPACS.

Annex 2 Rivers – Macrophytes & Phytobenthos – DARLEQ

Kelly, M. G, Phillips, G and Willby N (2011). Macrophytes and Phytobenthos: an ecological rationale for the combined quality element, part2. Paper submitted to Freshwater Task Team, UKTAG 2011.

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 Ofenböeck, 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.

Kelly, M.G., Juggins, S., Bennion, H., Burgess, A., Yallop, M., Hirst, H., King, L., Jamieson, J., Guthrie, R., Rippey, B. (2006). Use of diatoms for evaluating ecological status in UK freshwaters. Science Report – SC030103/SR4. Environment Agency, Bristol.