Evidence

The integration of macrophyte and phytobenthos surveys as a single biological quality element for the Water Framework Directive

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Miranda Kavanagh

Director of Evidence

Executive summary

This report describes changes to the diatom and macrophyte assessment methods used to classify macrophytes and phytobenthos for the Water Framework Directive (WFD). It also reviews the options for combining these metrics in a single biological quality element (BQE) and provides evidence as to when each metric could be used in isolation to produce a reliable classification. Finally it describes a method of including bacterial tufts within the classification to meet the normative definition of the WFD and proposes an approach for the UK administrations to adopt.

There have been a number of changes to both the method used for assessing diatoms (Diatoms for Assessing River and Lake Ecological Quality; DARLEQ) and that for assessing macrophytes (LEAFPACS; a proper name as opposed to an acronym) since the first river basin management plans (RBMPs). These changes have reduced the differences between the two methods – in particular, the equation for calculating expected Trophic Diatom Index (TDI) was too stringent especially in high alkalinity rivers. A larger dataset has now been used to revise this equation accordingly.

Overall, the bias between diatoms and macrophytes has been reduced by these changes and 81% of sites are now classified to within one class by these methods. A rationale for incorporating bacterial tufts into status assessment is also described.

Options for combining macrophytes and phytobenthos have been evaluated and the lowest of macrophyte and diatom-based assessments is recommended. But although LEAFPACS and DARLEQ measure different aspects of river ecology and each contributes unique information to status assessments, there are situations where a reliable estimate can be obtained from just one. At low and moderate alkalinities, diatoms alone can give a reliable estimate of the combined BQE while, at very high alkalinities, macrophytes alone can do this. There is an intermediate range (>75 and <200mg/L $CaCO_3$), however, where use of both components is still recommended.

Acknowledgements

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Contents

1 Introduction

The Water Framework Directive (WFD) created a statutory obligation for EU Member States to monitor the ecological status of water bodies with the aim of achieving ‘good ecological status’ (that is, the biota is the same as or only slightly different from that expected in the absence of human activity) for all water bodies. Annex V of the WFD provides definitions of ecological status in rivers and lakes that are based on four biological quality elements (BQEs):

- ‘phytoplankton’
- ‘macrophytes and phytobenthos’
- ‘benthic invertebrate fauna’
- ‘fish fauna’

However, ‘macrophytes and phytobenthos’ comprises two groups of organisms that have traditionally been treated separately by researchers. There are a number of reasons for this, not least because of the difference in size, with six orders of magnitude between the largest rooted macrophytes and the smallest unicellular algae.

Assessment methods for rivers based on both macrophytes and algae have been developed in several European countries, leading to the development of European standards (CEN 2003a, 2003b, 2004). In the UK, two methods, the Trophic Diatom Index (TDI) (Kelly and Whitton 1995, Kelly et al. 2001) and the macrophyte-based Mean Trophic Rank (MTR) (Holmes et al. 1999) have been in use for a number of years. These methods provide a foundation for ecological status assessment but have now been refined and developed in order to provide guidance appropriate to the WFD.

The outcome of this process is two new tools:

- DARLEQ (Diatoms for Assessing River and Lake Ecological Quality), which assesses diatoms, as proxies for ‘phytobenthos’ (Kelly et al. 2008)
- LEAFPACS (a proper name, as opposed to an acronym), which assesses macrophytes (Willby et al. 2009)

These two methods are reported in the combined form of ‘macrophytes and phytobenthos’ in accordance with the requirements of the WFD.

For the first river basin management plans (RBMPs), the policy adopted by the UK was to take the lower of the two individual assessments each of which treated each as a separate quality element. However, there is no categorical distinction between macrophytes and phytobenthos. LEAFPACS includes macroalgae which are sometimes classified as phytobenthos and which interact closely with diatoms in running waters. A better distinction may be between those parts of the photosynthetic community which are **surveyed** (macrophytes) and those which are **sampled** for later analysis in the laboratory (phytobenthos). Another reason for separate assessments is that macrophytes and phytobenthos react at different time and spatial scales, although this does not take into account the inclusion of fast-growing macroalgae into macrophyte assessment systems, the interactions between phytobenthos and macrophytes (for example, the latter as a substrate for the former) and the combined effects of both on higher trophic levels.

In the event, the diatom component proved to be overly stringent and was the most sensitive of all BQEs in of river water bodies in England and Wales, classifying as moderate status, even though other BQEs suggested a healthy ecosystem. This might be due to the greater sensitivity of diatoms to pressure than macrophytes (and other BQEs), but it might also indicate a problem of calibration of one or more tools.

Another question is whether taking the worst case of macrophytes and phytobenthos assessments is the most appropriate approach for future RBMPs, or whether an alternative method of combining the two methods is more ecologically realistic and could provide better information on the likelihood of 'undesirable disturbances' occurring.

These are the problems that were addressed by a team of scientists drawn from the UK's statutory environment agencies and outside experts, reporting to UK TAG's Freshwater Task Team. This report summarises the outcome of their deliberations.

Finally, the normative definitions for macrophytes and phytobenthos also refer to 'bacterial tufts' yet neither DARLEQ nor LEAFPACS yet include these. A simple method for evaluating bacterial tufts in the field, and for incorporating this information into ecological status assessments, is also therefore considered.

2 Methods

Two adjustments were made to harmonise the response of macrophytes and diatoms to the pressure gradient:

- an additional metric was added to LEAFPACS v1.0, based on cover of filamentous algae (see Willby et al. 2009)
- the ‘expected’ TDI value in DARLEQ was recalibrated using a larger dataset

Both LEAFPACS and DARLEQ predict site-specific expected values of the component metrics by using equations based on regressions between those metrics and typological variables. The two models were derived independently and both use inverse regression models which minimise prediction errors across the entire gradient.

A limitation, particularly for DARLEQ, was the absence of data from high alkalinity lowland sites that are at or near reference state. This required a precautionary approach to be taken originally, but as more data became available, it was possible to revisit this relationship. In high alkalinity rivers diatoms consistently report a lower ecological status than macrophytes. The reference state for both diatoms and macrophytes was therefore re-examined.

A key question was whether the phosphorus threshold used when defining reference conditions for diatoms had been too stringent. It is possible that the relationship between expected TDI and alkalinity does not reach a plateau at about 100 mg/L $CaCO_3$ as the original DARLEQ model suggests, but instead values should continue to increase – as is the case in the relationship between alkalinity and the River Macrophyte Nutrient Index (RMNI) in the LEAFPACS reference model. This has implications for ecological status assessment in very high alkalinity rivers and consequently the level of agreement in the classifications based on either macrophytes or diatoms.

Analysis also highlighted differences in the way that ‘reference’ is evaluated for diatoms and macrophytes both in the UK and in the EU inter-calibration exercise (Table 2.1). For diatoms, as for invertebrates, the emphasis was on excluding impacted sites using abiotic properties (for example, land use) to remove potential sources of pressure (Pardo et al. 2012). For macrophytes, although abiotic data were considered whenever available, there was greater emphasis on biological characteristics (for example, absence/low abundance of filamentous algae) and reference to historical archive data and descriptions.

Table 2.1 Screening criteria applied in selection of reference sites for original LEAFPACS (Willby et al. 2009) and DARLEQ (Kelly et al. 2008) projects

Macrophytes	Phytobenthos (diatoms)
Filamentous algal cover <5%	Low alkalinity: 20µg/L SRP: 2mg/L
Number of macrophyte taxa >4	Nitrate-N High alkalinity: 30µg/L SRP: 4mg/L
Predictions of number of invertebrate taxa or average score per taxon > middle of good status	Nitrate-N Samples with TDI >50 removed
Total oxidised nitrogen: type specific: Low alkalinity (upland and lowland): ≤1mg/L High alkalinity: ≤ 2mg/L	
Soluble reactive phosphorus (SRP) type specific: Low alkalinity lowland: ≤20µg/L Low alkalinity upland: ≤15µg/L High alkalinity lowland: ≤40µg/L High alkalinity upland: ≤30µg/L Very high alkalinity: ≤50µg/L	

Notes: [1] Low alkalinity: <50mg/L $CaCO_3$ (based on long-term average at site); high alkalinity: ≥50mg/L $CaCO_3$; very high alkalinity: >150mg/L $CaCO_3$.

[2] Upland: >80m above sea level; lowland: ≤80m

[3] Predictions of invertebrate status were based on practice current at time of site selection, mostly based on RIVPACS (River InVertebrate Prediction And Classification System).

3 Results

3.1 Changes to the DARLEQ method

In attempting to resolve the differences between the reference concepts behind the macrophytes and diatom methods in river classifications, the 615 site reference dataset compiled for this project was screened according to the majority of the selection criteria used in LEAFPACS (except hydromorphological data). The subsequent list of 118 candidate reference sites with matched diatom and macrophyte data was then further reduced by screening out based on the expert opinions of Environment Agency staff from around the country.

Not all of the reference sites on the final list fulfil the reference criteria for the EU inter-calibration of diatoms. This does not invalidate their use as reference within the UK (see section 3.1.1), but it does suggest that extra assessment and testing may be needed to ensure a strong evidence base.

The original DARLEQ predictive model (Kelly et al. 2008) used alkalinity and season as prediction parameters; these explain approximately a third of the total variation in the expected TDI (eTDI). The relationship reaches a plateau at around 40 TDI, when alkalinity is approximately 150mg/L $CaCO_3$.

Various new options were tested using the reference sites using the revised paired macrophyte–diatom dataset. Of these, the exponential model of TDI versus alkalinity yielded the strongest fit and gave the closest match to a LOWESS model. The inclusion of other spatial predictors (distance from source, source altitude, site altitude and slope) did not result in a significantly improved fit. The original function of season (or month) of sampling was found not to be a significant explanator of variability in TDI in the reference sites in the revised dataset.

The equation derived from this new relationship is:

$$\text{eTDI} = 9.933 \times \exp(\log_{10}(\text{alkalinity}) \times 0.81)$$

where alkalinity is measured in mg/L $CaCO_3$.

The lowest eTDI calculated using this function is approximately 20 (in very low alkalinity waters). The previous model yielded lower expected values (12–16), which led to some soft water sites with apparently healthy floras being classed as impacted. At the very highest alkalinities, the eTDI can now reach 65–70. This leaves less headroom on the TDI scale at high alkalinities, but it is realistic so there is no need to artificially truncate the eTDI at a lower value. Some very high alkalinity reference sites on hard limestone still have lower TDI values (30–40), but no significant additional term to include in the eTDI function can be found to account for such sites which may therefore have a more optimistic interpretation of status than is justified. However, the proposed combination rule with macrophytes (see below) means that an impacted site with these geological characteristics should be detected via changes to the macrophyte assemblage.

This model provides a significantly better fit of the TDI values to alkalinity with an r^2 of 0.63 (Figure 3.1). This is partly due to the use of average TDIs for sites rather than individual sample TDIs. The lack of an asymptote may be partly explained by the slightly higher soluble reactive phosphorus (SRP) threshold for very high alkalinity rivers adopted for screening reference sites within LEAFPACS. The decision to exclude samples with TDI >50 in the original model also reduced the strength of the relationship. This was a reasonable judgement at the time, but the inter-calibration process favoured rigorous abiotic screening rather than relying on preconceptions about the biota of a reference site (Kelly et al. 2012, Pardo et al. 2012), and the removal of this aspect therefore also improves the outcome.

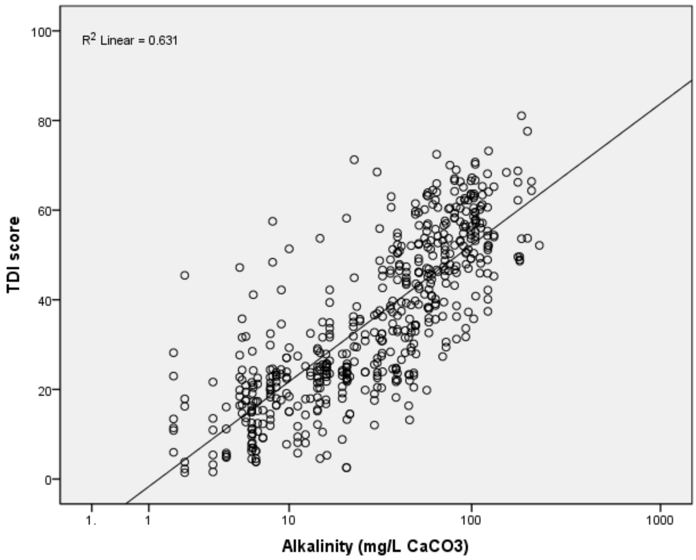

Figure 3.1 Relationship between TDI and alkalinity in putative reference sites screened from project database using LEAFPACS criteria

3.1.1 Validating reference concepts

Adopting the new equation for eTDI means that both LEAFPACS and DARLEQ are now calibrated against a common baseline. However, some differences remain between the criteria used to establish this reference dataset and those adopted by ECOSTAT for riverine reference sites (Pardo et al. 2012).

All the reference sites in the dataset provided by the Scottish Environmental Protection Agency (SEPA) have now been screened according to inter-calibration criteria. Of 48 sites, 40 passed this screening. It has not been possible to screen the Environment Agency sites (England and Wales) or those provided by the Northern Ireland Environment Agency (NIEA) in this way due to lack of resources.

Six potential high alkalinity reference sites in England and Wales were attempted, but all failed on land use criteria. This lack of ‘true’ reference sites from very high alkalinity streams is a problem throughout Europe (Kelly et al. 2012) and those used here represent the best available. However, there is no systematic trend of sites which fail the inter-calibration screening criteria (that is, having higher TDI values than other sites of a comparable alkalinity; Figure 3.2) and it is therefore likely that the predicted eTDI values at higher alkalinities are a meaningful extrapolation from the evidence.

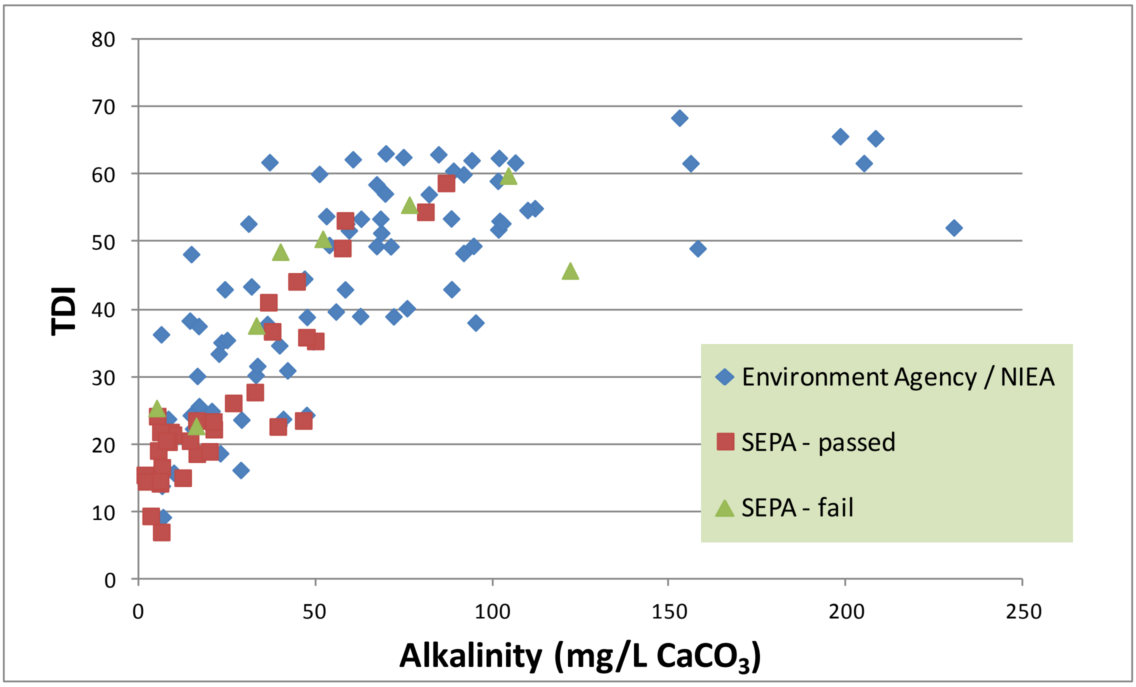

Figure 3.2 Relationship between TDI and alkalinity for UK reference samples (SEPA samples that passed or failed detailed screening are highlighted)

3.2 Changes to the LEAFPACS method

Before LEAFPACS v1.0 was finalised a metric reflecting the absolute cover of filamentous algae (ALG) was incorporated. This was done to address a gap in the specific assessment of abundance, as set out in the normative definitions of the WFD, and because it is beneficial to focus more directly on undesirable disturbances of this nature. Also, fast-responding filamentous algae should help to draw together separate the assessments based on macrophytes and diatoms.

The analysis of large datasets showed that the cover of filamentous algae increases sharply in high alkalinity rivers when SRP approaches 100µg/L but that it was rather unresponsive to increasing SRP in low alkalinity rivers (where substrate, shading, grazing and accrual time may be more critical determinants of algal cover). On this basis the ALG metric has been assigned an increasing contribution to the overall ecological quality ratio (EQR) in high alkalinity rivers, but is zero weighted in the lowest alkalinity sites.

During the inter-calibration of LEAFPACS v1.0 several small revisions were made to the method regarding the metrics included and the weight assigned to them when deriving the overall EQR for a site. The metric River Macrophyte Hydraulic Index (RMHI; based on substrate, depth and stream energy) was removed which should improve the relationship with diatom classifications as the focus of LEAFPACS is now more on eutrophication.

The weight given to two diversity metrics – the number of fully aquatic plants (N_AQUA) and the number of functional groups (N_FG) – was reduced making them less sensitive to survey effort, and the criteria for including these metrics in the final EQR was adjusted. Following these revisions LEAFPACS v2.0 was highly significantly correlated with the assessments of the other Member States for the three river types tested.

3.3 Combining macrophytes and phytobenthos

3.3.1 Agreement between macrophytes and diatoms

There is strong agreement between diatom and macrophyte assessments at low and moderate alkalinity (Table 3.1). Although the number of sites classified in the same class is lower at high and very high alkalinity, Table 3.2 indicates that 74% of these sites are still classified within ± one class and the overall bias is <0.25 class widths (originally diatoms were more stringent than macrophytes). High alkalinity lowland rivers still have the highest proportion of mismatches (that is, where one method reports a pass and the other method reports status that is two classes worse; this mismatch occurs almost three times more commonly in high alkalinity than low alkalinity rivers). Overall, 81% of sites are classified within ± one class and the bias is only 0.14 class widths (that is, macrophytes are on average now only 0.14 classes less stringent than diatoms).

Table 3.1 Agreement between the two methods on type-specific basis

		macrophytes				
HA_L		H	G	M	P	B
diatoms	H	21	34	24	5	0
	G	26	39	18	8	0
	M	38	52	33	11	1
	P	2	4	2	0	0
	B	0	0	0	0	0

		macrophytes				
LA_L		H	G	M	P	B
diatoms	H	55	17	1	0	0
	G	16	11	1	0	0
	M	11	12	5	1	0
	P	0	0	1	0	0
	B	0	0	0	0	0

		macrophytes				
HA_U		H	G	M	P	B
diatoms	H	17	9	7	3	0
	G	18	13	2	0	0
	M	10	7	4	3	0
	P	1	0	0	0	0
	B	0	0	0	0	0

		macrophytes				
LA_U		H	G	M	P	B
diatoms	H	37	10	0	0	0
	G	10	6	0	0	0
	M	4	4	1	0	0
	P	0	2	1	0	0
	B	0	0	0	0	0

Notes: Yellow cells represent exact agreement and blue cells represent problem classifications (that is, populated cells where one method indicates a pass and the other method indicates an assessment two classes lower).

Table 3.2 Summary of agreement between the two methods in the global dataset with associated comparability statistics

global		macrophytes H	G	M	P	B
diatoms	H	130	70	32	8	0
	G	70	69	21	8	0
	M	63	77	43	15	1
	P	3	6	4	0	0
	B	0	0	0	0	0

	class bias	% =class	% ±1 class	% problems
global	0.139	39.0	80.5	19.4
HA_L	0.082	29.2	74.2	25.5
HA_U	0.117	36.2	77.7	22.3
LA_L	0.229	54.2	90.8	9.2
LA_U	0.227	58.7	92.0	8.0

Notes: Yellow cells represent exact agreement and blue cells represent 'problem' classifications.
Class bias is expressed as class widths and reflects the bias of macrophytes relative to diatoms.
% = class represents the proportion of sites where both methods report the same class.
% ±1 class represents the proportion of sites where classifications according to the two methods lie within one class of each other.
% problems refers to the proportion of sites that are classified as passing according to one method but which fall two classes below this according to the second method.

3.3.2 The role of alkalinity

Figure 3.3 compares the relationships between diatom and macrophyte EQRs and SRP. Diatom EQRs show a good relationship at low and moderate alkalinity but a poor relationship at high alkalinity. Diatoms do respond to nutrient enrichment in high alkalinity streams (see Kelly et al. 2009 for an example), but when observed TDI values are converted to EQRs, the relationship is no longer apparent. This is because the TDI values from reference sites are quite variable (see Figure 3.2).

Macrophytes by contrast show significant relationship for all alkalinity classes. The prediction parameters used to derive expected values are based primarily on spatial variables and, as macrophyte EQRs use more spatial predictors (slope, source altitude and distance from source, as well as alkalinity) than diatom EQRs, it is possible that LEAFPACS better reflects these patterns. However, the only variable which had a significant effect on the eTDI function was alkalinity.

The short lifespan of diatoms may mean that incorporation of temporal variables (for example, degree days since last spate) may further improve the relationship. It is also possible that a land use variable such as percentage peat/moorland in the catchment could differentiate phytobenthos assemblages in high alkalinity systems; for example, low TDIs at reference may reflect a low alkalinity influence upstream such as in the Pennines where limestone and peat are often found in close proximity. Nonetheless diatom assessment has a role in high alkalinity stream investigations, particularly as they respond to catchment-scale changes more quickly than macrophytes.

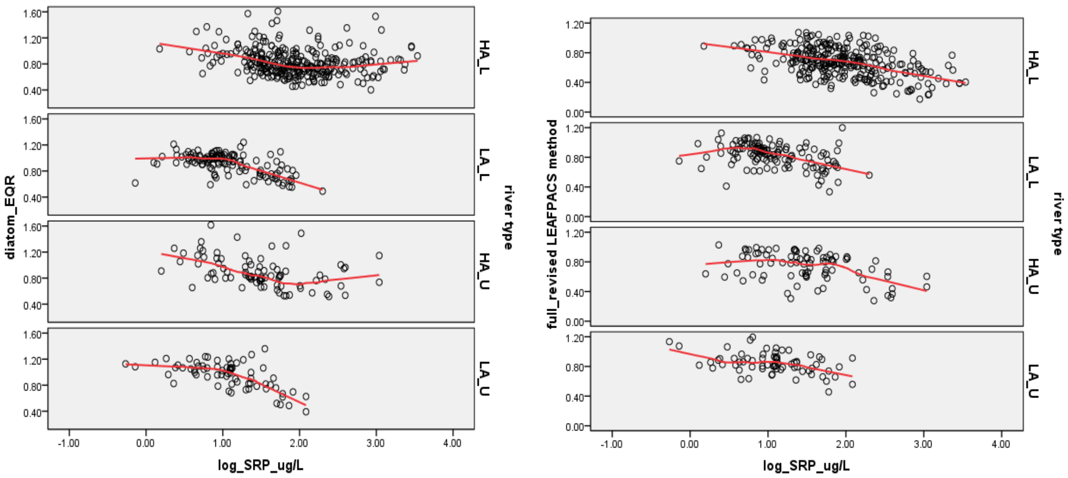

Figure 3.3 Diatom (left) and macrophyte (right) EQRs versus SRP, by river type

Notes: Note the proportionally stronger relationships between diatom EQR and SRP in low alkalinity types (equivalent to r^2 of 0.30–0.37 for diatoms versus 0.15–0.20 for macrophytes) versus stronger relationships between macrophyte EQR and SRP in high alkalinity types (equivalent to r^2 of 0.04 for diatoms versus 0.21 for macrophytes).
Lines fitted by LOWESS.

3.3.3 Developing the combination rule

The combination rule adopted for the first RBMP was to take the lowest of macrophytes and phytobenthos which, as phytobenthos were generally more stringent than macrophtyes, led to many sites being classified as moderate status or lower. The amendments described above reduce the difference between the two tools through inclusion of the filamentous algae metric into LEAFPACS plus the other revisions made during inter-calibration, and the revisions to the eTDI equation in DARLEQ. The resulting dataset can now be used to revisit the combination rule.

Two options were tested:

- TDI is incorporated into the LEAFPACS suite and combined with RMNI in assessments of ecological status.
- DARLEQ and LEAFPACS are calculated separately and then compared.

The latter option allows the two tools to be used independently and is more flexible. Within these two broad options, there are further options for combination rules, including taking the minimum of the component metrics, their average, or some weighted combination.

The implications of weighting the relative contributions of macrophytes and diatoms using either stream energy or productivity as a weighting factor were assessed. Axes 1 and 2 of the Principal Components Analysis (PCA) of River Habitat Survey (RHS) sites by Jeffers (1998) were used as proxies for productivity and stream energy respectively. In each case, various linear and logarithmic models were tested. For axis 1 (productivity) an increasing weight for macrophytes as productivity increased was assumed; for axis 2 (energy), an increasing weight for phytobenthos as energy increased was assumed.

The relationship between ecological predictors and phosphorus (based on Generalised Additive Models, GAMs) is used to evaluate these various combination rules. The revised diatom and macrophyte EQRs each display a similar correlation with SRP (r^2 = 0.31 and 0.29 respectively, based on normalised EQRs for comparative purposes) but all of the combinations of diatoms and macrophytes that were tested gave stronger relationships (r^2 =~0.4) with the pressure gradient than did either component alone (Figure 3.4). Use of the PCA axis scores to weight the combination of macrophytes and diatoms did not lead to a significant improvement in r^2 when compared with simple rules based on taking the average or minima (Figure 3.5).

Consequently, it is recommended that LEAFPACS and DARLEQ should continue to be combined using the minimum of the two values. The average is insufficiently precautionary, yielding combined EQRs typically half a class higher. Neither tool, but particularly diatoms, has a very strong relationship with SRP at high alkalinity, but in principle, as they both measure different aspects of river ecology, both are necessary.

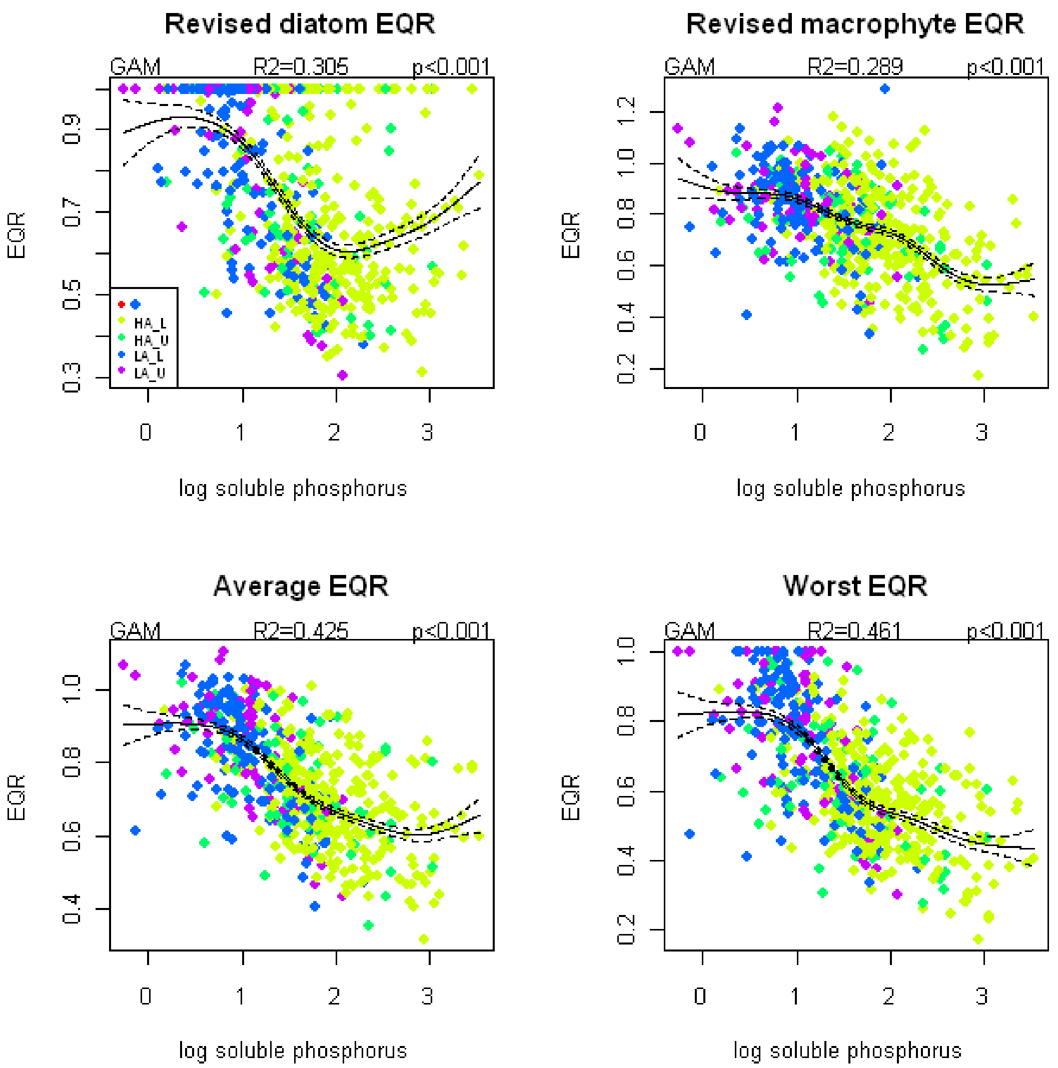

Figure 3.4 Scatterplots showing relationship between revised diatom and macrophyte EQRs alone and combined, taking either the average or the minimum of the two metrics, overlain with regression lines calculated using GAM

Key: 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.

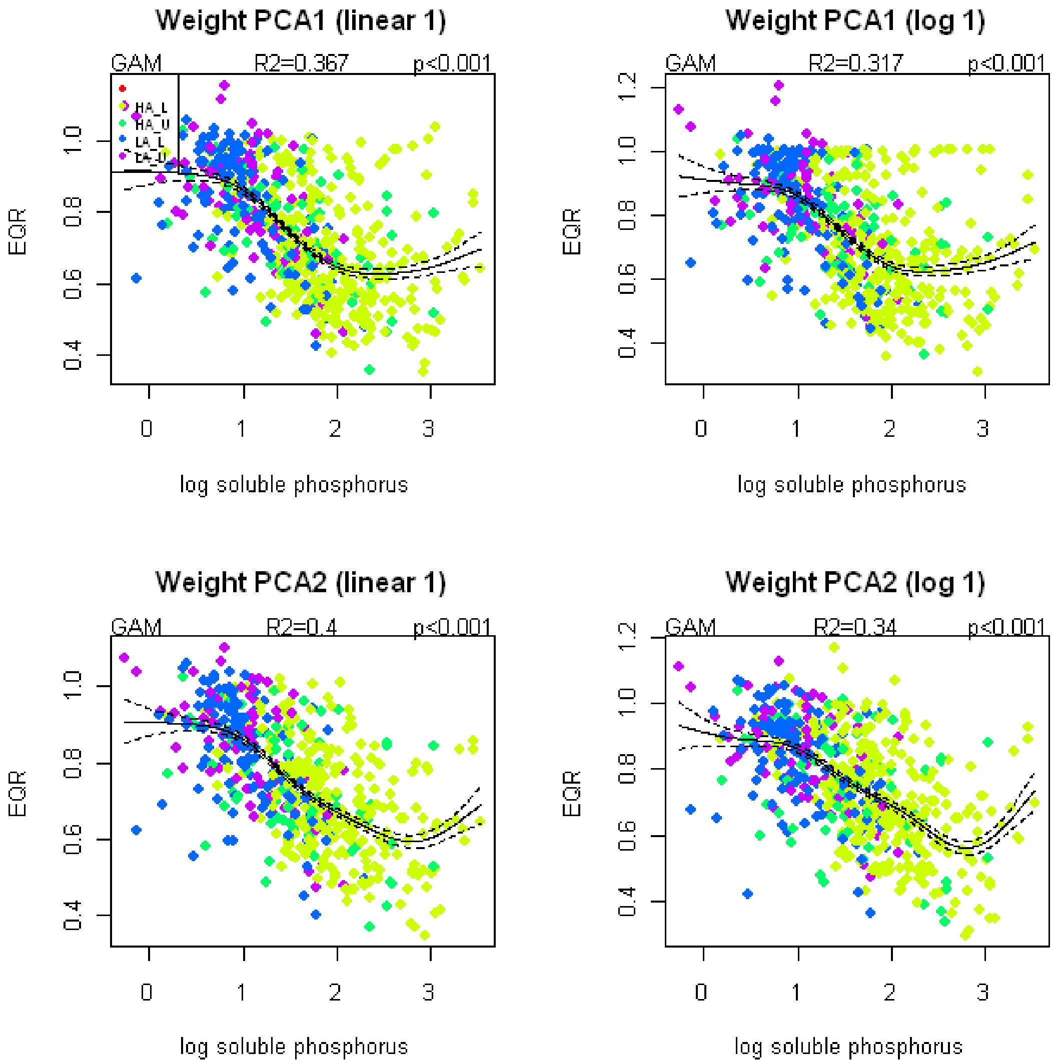

Figure 3.5 Scatterplots showing relationship between revised diatom and macrophyte EQRs combined using weights derived from axes 1 and 2 of the PCAs of Jeffers (1998)

Key: As for Figure 3.4.

3.3.4 Method selection and use in practice

The possibility that assessments could be based on just one component (for example, either macrophytes or diatoms) was explored by calculating the difference between each of the paired LEAFPACS and DARLEQ assessments and the final assessment based on the minimum of both. The product was then plotted against alkalinity (Figure 3.6) and a regression based on the 90th percentile of the data superimposed. 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). For diatoms, the quantile tracks the 0.2 threshold until alkalinity reaches about 75mg/L $CaCO_3$, confirming that below this level an assessment based on diatoms alone has high confidence of being within a class of an assessment based on both diatoms and macrophytes. Above this alkalinity the diatom quantile rises steeply. In contrast, the line for macrophytes exceeds the 0.2 EQR threshold at low alkalinity, rising to about 0.35 at moderate to high alkalinity, before declining to <0.2 EQR at very high alkalinity (>200mg/L $CaCO_3$). This confirms that above this level an assessment based on macrophytes alone has high confidence of being within a class of an assessment based on both diatoms and macrophytes.

Therefore, a diatom-based assessment alone will give a reliable classification if alkalinity is <75mg/L $CaCO_3$ while a macrophyte-based assessment alone is adequate at >200mg/L $CaCO_3$. In the middle range, both components are necessary – although, on average, an assessment based on diatoms alone will be more reliable than macrophytes alone at alkalinities up to ~120mg/L $CaCO_3$ whereas macrophytes alone will be more reliable at alkalinities above this. Using a single assessment within the range 75–200 mg L^{-1} $CaCO_3$ is not recommended; however, it is recognised that there are situations where macrophyte surveys are either not possible or are compromised (for example, rivers with heavy boat traffic, very deep rivers, some small lowland streams) and possibly situations where diatom assessments are inherently unreliable.

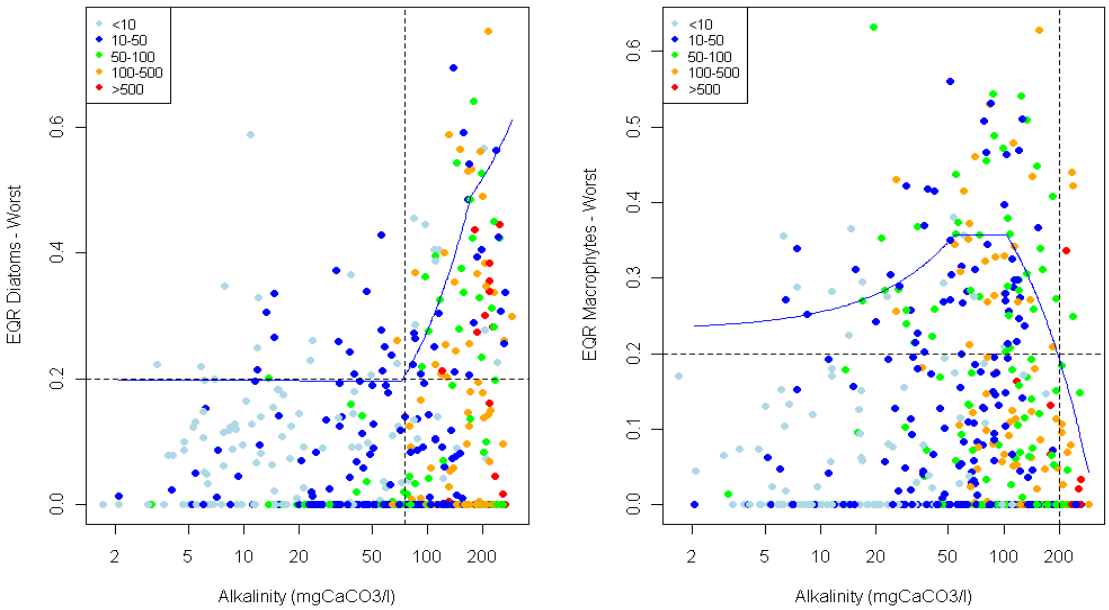

Figure 3.6 Difference between assessments based on a single component (either diatoms or macrophytes) and both diatoms and macrophytes (worst), plotted against alkalinity

Key: Blue line is the regression based on the 90th 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.

Although both tools contribute information, circumstances or resources may demand that fewer assessments are made than is ideal. Table 3.3 sets out recommendations based on the analyses made above, along with expert knowledge and data on phosphorus concentrations. As very high nutrient concentrations are unlikely to be associated with GES for either diatoms or macrophytes, these can be used for preliminary screening, after which alkalinity is the primary determinator.

Table 3.3 Recommended monitoring options for macrophytes and phytobenthos in rivers

		Alkalinity (mg/L $CaCO_3$)		
		<75	75–200	>200
Phosphorus (µg/L)	<150	Diatom	Diatoms and macrophytes	Macrophytes
	>150	Assume <GES	Macrophytes	Macrophytes
	>1000	Assume <GES	Assume <GES	Assume <GES

Notes: GES = good ecological status (WFD)

The following points should also be considered:

1. The rules developed here provide a framework for surveillance monitoring only. For site-specific investigations there are benefits in continuing an on-going temporal sequence of either or both sub-elements.

2. Nutrient concentrations are an approximation for biological responses only, and the medium-term (that is, five year) target concentrations should be considered rather than the present concentrations (that is, if improvements are likely to bring a water body into the range where macrophytes and/or diatoms are likely to respond then these data should be collected to establish a baseline against which change can be measured).

3. Where good status or better is concluded from one sub-element, a check for contradictory evidence should be made (for example, phosphorus thresholds for that water body type are not exceeded, no 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.

4. There are certain circumstances (for example, on hard limestone) where diatoms are responsive at high alkalinities and local experience could override the scheme described in the Table 3.3.

5. Diatoms may also provide additional information in situations where the macrophtye signal is compromised by other factors (for example, heavy boat traffic, very deep rivers, heavily shaded rivers, heavily channelised rivers).

3.3.5 Incorporating bacterial tufts

The framework described above provides an ecological rationale for the assessment of the ‘macrophytes and phytobenthos’ biological quality element, addressing all aspects of the normative definitions with the exception of ‘bacterial tufts and coats’.

Such growths, informally referred to as ‘sewage fungus’, have long been recognised for their role as indicators of organic pollution. Their association with macrophytes and phytobenthos in the normative definitions may seem incongruous as they are neither macrophytes nor phytobenthos. However, the normative definition refers to the displacement of macrophytes and 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 phototrophs.

Sewage fungus is recorded in the field by the Environment Agency, SEPA and NIEA but at present plays no role in the formal assessment of ecological status (in contrast to the Irish Republic, where it is included). The assessment of sewage fungus cover may be subjective and any scheme to incorporate it needs to be robust. The scheme described here provides a framework for interpreting field observations.

The term ‘bacterial tufts’ should only be used to refer to heterotrophic growths (that is, sewage fungus involving a mixture of heterotrophic bacteria, fungi and protozoans). Phototrophic bacteria (such as the cyanobacteria) and chemoautotrophic bacteria should not be included. It is proposed that bacterial tufts should only have the potential to downgrade class status from moderate or worse, because where the macrophytes and or phytobenthos classify as high or good they are not, by definition, adversely affected by bacterial tufts and coats, even if the latter are present.

Criteria have been developed to standardise the approach taken when recording bacterial tufts. This should be followed on all occasions when a macrophyte survey is undertaken or a phytobenthos sample is collected. The criteria are presented in Table 3.4.

Table 3.4 Criteria for recording bacterial tufts when undertaking a macrophyte survey or collecting a phytobenthos sample

Criterion	Details
Location	Whether the sewage fungus is observed on the upper surface (above) or on the lower surface (below) of the substrata.
Coverage	• Absent • Occasional (<30% of surface area) • Widespread (30–60% of surface area) • 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 • Massive – occupies a significant proportion of the water column

For high ecological status (HES) and good ecological status (GES), there should be no sewage fungus visible on the upper surface of substrata. At good status there may be growths on the underside of substrata (that is, the phytobenthic community is not adversely affected by bacterial tufts and coats present due to anthropogenic activity).

For moderate status, the WFD states that:

> ‘the phytobenthic community may be interfered with, and, in some areas, displaced by bacterial tufts and coats present as a result of anthropogenic activities’.

This suggests an obvious presence but not overwhelming dominance of sewage fungus-like growths. No specific criteria are given for defining poor or bad status. However, extrapolating from the definition for moderate status suggests the approach given in Table 3.5.

Table 3.5 Use of coverage and density of sewage fungus growths to distinguish between ecological classes below good status

Coverage ➔ Density 🡻	Occasional	Widespread	Extensive
Trace	Moderate	Moderate	Poor
Thin	Moderate	Poor	Poor
Thick	Poor	Poor	Bad
Massive	Poor	Bad	Bad

A visual assessment of bacterial tufts should override the combined macrophyte and phytobenthos assessments to determine final status class in situations where the status for this element is moderate or less and where the bacterial tuft assessment gives a lower class.

Although the possibility of bacterial tufts co-existing with a flora indicating good status cannot be excluded, such instances will be rare. The presence of a good status flora is itself evidence that the bacterial tufts are not having an adverse affect and therefore the site conforms to the normative definition of good status.

This system is broadly in line with the system adopted by the Irish Republic, where sewage fungus is absent at Q5 and Q4 (corresponding to high and good status), while Q3–Q4 (corresponding to moderate status) has no more than a trace of sewage fungus, and sewage fungus may be abundant at Q1–Q3 (poor status) (Toner et al. 2005, McGarrigle and Lucey 2009).

As coverage and density of sewage fungus growths will vary over time, the final classification should be the average of the records collected during the assessment period. The numerical recording used to calculate the average is Bad = 1, Poor = 2, Moderate = 3, High/Good = 4.

4 Conclusions and recommendations

The WFD sets challenging targets for developing tools for ecological assessment and this project has continued the process in the light of larger datasets that have become available due to increased monitoring activity. Much of the initial discrepancy observed between status classes derived from the original versions of DARLEQ and LEAFPACS can be explained by the stringent approach to setting expected values of metrics by DARLEQ. The outcome of this project and that of parallel activities within EU inter-calibration is a more stringent approach to defining reference conditions (along with higher values of eTDI) in high alkalinity streams and rivers, but relaxes the eTDI in soft water streams which reduces the potential for healthy flora in such sites being interpreted as impacted.

Combining macrophytes and phytobenthos gives a stronger relationship with the predominant pressure gradient than using either sub-element alone and therefore provides a better estimate of ecological status. Status class boundaries for both sub-elements are given in Table 4.1.

Table 4.1 Finalised status class boundaries for assessment of ecological status using macrophyte and phytobenthos in UK rivers [1]

	DARLEQ	LEAFPACS
High/Good	1.0	0.8
Good/Moderate	0.75	0.6
Moderate/Poor	0.5	0.4
Poor/Bad	0.25	0.2

Notes: [1] High/good and good/moderate status boundaries were successfully inter-calibrated with those of other Member States in the 2012 European inter-calibration exercise.

The combination of DARLEQ, LEAFPACS and the bacterial tuft assessment described in this report means that the UK now fulfils its obligations to assess the composition and abundance of macrophytes, the composition of phytobenthos and the abundance of bacterial tufts.

Undesirable disturbances are not assessed explicitly in any biological method, although excessive algal growth is often regarded as an undesirable disturbance and is considered implicitly within LEAFPACS. However, the precautionary approach to setting the good/moderate boundaries that were adopted during inter-calibration should mean that risk of undesirable disturbances is minimal. Decreasing EQRs from this point represent increasing hazard of undesirable disturbances and the extent to which this relates to risk depends on local factors beyond the scope of this project.

The work described here provides a basis for classifying the macrophytes and phytobenthos element in the second round of RBMPs. However, there are still some aspects that require further work. For example, the use of alkalinity as a predictor for diatom typology does not separate high energy streams flowing off hard limestone (typically with low TDIs at reference conditions) from low energy streams on softer substrata (usually high TDIs). All descriptors included in the present LEAFPACS suite have been tested (plus an additional geological term based on extent of hard limestone in the upstream catchment), but none lead to a significant improvement in the ability to predict eTDI in these conditions. The formula described can occasionally yield some very high EQRs as a result. A temporary solution has been adopted which caps individual DARLEQ values at EQR = 1.25 (before the calculation of site means) but it is beyond the scope of this project to explore this further. However the high eTDI values produced by the new equation make DARLEQ v.2.0 less responsive at high alkalinity than the original and there is therefore scope for revisiting the underlying metric, either to expand the scale or to add a separate 'saprobic' metric to complement the current 'trophic' metric in a similar approach to that used in Austria and Germany.

Overall, as a result of this research, the following recommendations are made.

- The minimum of LEAFPACS and DARLEQ EQRs should be used as the basis for classification;
- Both macrophyte and diatom results should generally be used. However, if alkalinity is <75mg/L $CaCO_3$, a reliable classification can be obtained from diatoms alone, or if it is >200mg/L $CaCO_3$, macrophytes can give a reliable classification alone. For sites with alkalinities between these values, both components should be used wherever possible. If not possible, diatoms will, on average, give a more reliable guide to overall status when alkalinity is <120mg/L $CaCO_3$ while macrophytes will be more reliable at alkalinities above this.
- If only one of LEAFPACS and DARLEQ is used and the result gives a classification of good or better, then consideration should be given to other contradictory information. For example, if phosphorus is elevated or there is a high risk of eutrophication the other sub-element should be used to confirm the class status.
- The uncertainty of the combined tool will correspond to the uncertainty of the sub-element with the lowest EQR.
- For investigations and operational monitoring, either or both components can be used (depending on local circumstances).
- If present, bacterial tufts (sewage fungus) will influence classifications at moderate, poor and bad status.

References

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

CEN, 2003b. Water Quality – Guidance Standard for the Surveying of Aquatic Macrophytes in Running Waters. EN 14184:2003. 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.

Holmes, N.T.H., Newman, J.R., Chadd, S., Rouen, K.J., Saint, L. and Dawson, F.H., 1999. Mean Trophic Rank: A User's Manual. R&D Technical Report E38. Bristol: Environment Agency.

Jeffers, J.N.R., 1998. Characterization of river habitats and prediction of habitat features using ordination techniques. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 8, 529-540.

Kelly, M.G. and Whitton, B.A. 1995. The Trophic Diatom Index: a new index for monitoring eutrophication in rivers. *Journal of Applied Phycology,* 7, 433–444.

Kelly, M.G., Adams, C., Graves, A.C., Jamieson, J., Krokowski, J., Lycett, E.B., Murray-Bligh, J., Pritchard, S. and Wilkins, C., 2001. The Trophic Diatom Index: A User's Manual. Revised Edition. R&D Technical Report E2/TR2, Bristol: Environment Agency.

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

Kelly, M.G., Haigh, A., Colette, J. and Zgrundo, A., 2009. Effect of environmental improvements on the diatoms of the River Axe, southern England. *Fottea*, 9, 343-349.

Kelly, M.G., Gómez-Rodríguez, C., Kahlert, M., Almeida, S.F.P., Bennett, C., Bottin, M., Delmas. F., Descy, J.-P., Dörflinger,G., Kennedy, B., Marvan, P., Opatrilova,L., Pardo, I., Pfister, P., Rosebery, J., Schneider, S. and Vilbaste, S., 2012. Establishing expectations for pan-European diatom based ecological status assessments. *Ecological Indicators*, 20, 177-186.

McGarrigle, M.L. and Lucey, J., 2009. Intercalibration of ecological status of rivers in Ireland for the purpose of the Water Framework Directive. *Biology and Environment: Proceedings of the Royal Irish Academy*, 109B, 237-246.

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., 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.

Toner, P., Bowman, J., Clabby, K., Lucey, J., McGarrigle, M., Concannon, C., Clenaghan, C., Cunningham, P., Delaney, J., O'Boyle, S., MacCárthaigh, M., Craig, M. and Quinn, R., 2005. *Water Quality on Ireland 2001-2003*. Wexford: Environmental Protection Agency.

Willby, N., Pitt, J.-A. and Phillips, G., 2009. *The Ecological Classification of UK Rivers using Aquatic Macrophytes.* Science Report SC010080/SR1. Bristol: Environment Agency.

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