RAPPER: A new method for rapid assessment of macroalgae as a complement to diatom-based assessments of ecological status

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RAPPER: A new method for rapid assessment of macroalgae as a complement to diatom-based assessments of ecological status

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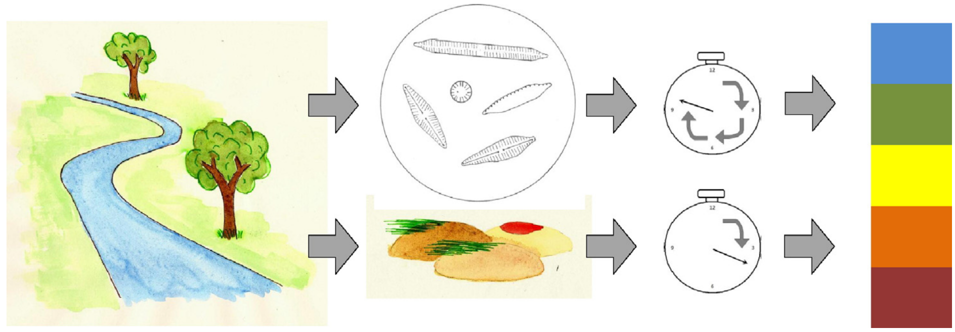
HIGHLIGHTS

- There is a need for high level ecological "triage" methods to complement traditional approaches to ecological assessment
- Identification of macroalgae, combined with assessment of cover, permits sites at risk of eutrophication to be identified
- The method has several potential applications including "citizen science".

GRAPHICAL ABSTRACT



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ABSTRACT

Most methods for ecological assessment developed since the onset of the Water Framework Directive require substantial effort by skilled analysts and are therefore expensive to use. RAPPER ("Rapid Assessment of PeriPhyton Ecology in Rivers") is a high level ecological "triage" method that enables rapid screening of sites within a water body to enable managers to identify areas subject to nutrient pressures. The method involves a survey of macroscopic algae within 10 m lengths of watercourses, taking samples for subsequent identification, and assessing cover. Genus-level identification is used to ensure rapid assessment and comparability, and that the method can be used by a wide range of users. Genera of alga that form conspicuous growths recognisable with the naked eye are designated as either "stress-tolerant" ("S-taxa") or "competitive" taxa ("C-taxa"), depending on their preference for locations with low or high nutrient concentrations. Genera whose representatives span a wide range of nutrient conditions, or for which few data are available, are placed in a third class, "unclassified". The presence of S-taxa and the relative cover of C-taxa are then used to determine whether a site is at risk from eutrophication. Field trials in Scotland demonstrated that the method discriminates between sites with low and high nutrient concentrations. Significant differences were also observed in values of the Trophic Diatom Index between RAPPER classification categories. RAPPER can be used alone (allowing greater spatial or temporal coverage within water bodies at lower cost than conventional assessment methods) or to increase confidence in assessments of the condition of the phytobenthos by incorporating algae other than diatoms. The outcomes also relate directly to the experiences of non-technical stakeholders, and will have benefits for communicating ecosystem health concepts to the wider public, for example through "citizen science".

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1. Introduction

The period since the adoption of the Water Framework Directive (WFD; European Union, 2000) has seen the countries of the European Union developing new approaches to ecological assessment in order to evaluate the state of Europe's surface waters (Birk et al., 2012). One consequence of this is a harmonised view of what constitutes “good ecological status” for any particular type of water body throughout the region (Birk et al., 2013; Pardo et al., 2012; Bennett et al., 2011). The next stage, however, requires a shift from the national and regional scale evaluations in order to target appropriate measures to restore failing water bodies to good status.

Evaluation of phytobenthos is one component of ecological status assessment in freshwaters that Member States have a duty to perform. The term ‘phytobenthos’, in this case, refers to photosynthetic algae and cyanobacteria associated with submerged surfaces. Their inclusion in the WFD reflects the important role that benthic algae play in freshwater ecosystem functioning (Allen, 1995; Stevenson et al., 1996) as well as the potentially harmful consequences of excessive algal growths on the provision of ecosystem services (Sturt et al., 2011; Everard, 2012; Camp et al., 2014). Most Member States have, subsequently, developed methods for assessing phytobenthos largely using diatoms, a diverse and sensitive group of algae, as proxies for the full phytobenthic community (Kelly, 2013).

Several studies have demonstrated strong relationships between benthic diatoms and inorganic nutrients (Potapova and Charles, 2007; Kelly et al., 2008a; Bennion et al., 2014). As a large number of water bodies throughout Europe have elevated concentrations of nitrogen and phosphorus (European Environment Agency, 2012), many are likely to exhibit phytobenthic communities different from those expected under reference conditions and, therefore, fail to achieve good ecological status. This, in turn, raises further questions, both about the management of nutrients within water bodies and about the interpretation of ecological status concepts by catchment managers. There is, in particular, a need to understand how a sample, reflecting the state of a part of the phytobenthos at a particular point in space and time, relates to broader ecosystem functioning, in order that investment in remediation measures has a high probability of success.

The UK's current WFD assessment tools fulfil the statutory obligations to produce quantitative classifications of ecological status on the basis of deviation from reference conditions. These methods are however relatively slow and resource intensive. In the case of phytobenthos assessment, sample analysis and interpretation may be performed by staff who have not visited the site and may, indeed, be based in another part of the country. The impetus for the work described here arose from discussions about reducing the inherent uncertainty associated with diatom-based assessments (Besse-Lotoskaya et al., 2006; Kelly et al., 2009a; O'Driscoll et al., 2014). In theory, confidence can be raised by increasing effort; however, as diatom analysis is a relatively resource intensive method which requires highly-skilled staff, this has significant cost implications. The discussion then considered the use of complementary evidence that could be collected at the same time as the diatom sample. Having recognised the potential for macroalgae to complement diatom analyses, the discussion quickly broadened out to consider other situations where the assessment of macroalgae may have value, independent from diatom analyses.

Formal WFD assessment tools provide information on the condition of a water body; however, the high costs means that this may be possible only at a low level of spatial detail. Against this background there is, we believe, potential for a rapid assessment method that can be performed at a large number of sites within a water body, in order to target appropriate measures for restoring ecological status. Such methods also have the potential for use by less-specialised staff and, indeed, by stakeholders. It is important to emphasise that RAPPER is not envisaged as a replacement for current diatom and macrophyte assessment methods. Rather, we believe that it could form one of a number of complementary strands of high-level evidence that will have particular value in the early stages of evidence gathering and risk assessment and which can provide rapid input to the decision-making process.

We also believe that there is a place for assessments based on visually-obvious components to communicate concerns about the condition of the stream ecosystem to non-technical staff. This has been largely overlooked in the development and evaluation of WFD assessment tools (see, for example, Hering et al., 2010); however, public participation is a core principle of the WFD (Article 16; European Union, 2000), albeit one that most member states are struggling to enact (De Stefano, 2010). There is, therefore, a strong case for new approaches to assessment which complement the formal evaluation of ecological status (Kelly, 2014).

2. Materials and methods

2.1. Site selection

RAPPER was tested through a sampling programme in Scotland during 2014. 90 sites were selected to provide good geographical coverage across Scotland, as well as encompassing a range of water quality. All sites were also sampled for benthic diatoms in previous years and results from these analyses provided an a priori estimate of ecological status for each. Benthic diatom samples were collected and analysed at the same time as the macroalgal surveys following CEN (2014a,b). Environmental data were measured in situ (width, depth, substrate composition) or obtained from the Scottish Environment Protection Agency's database (chemical variables). Substratum composition was assessed using the Krumbein phi scale, following Wright et al. (1984). Environmental data are summarised in Table 1.

The a priori estimate of ecological status based on benthic diatom results indicated that the majority of sites were at moderate ecological status (71%), 26% at high status and only 3% at poor ecological status. No sites classified as bad ecological status were available.

2.2. Assessment of macroalgae composition

An assessment of the composition and abundance of macroalgae was performed at the same time as diatom samples were collected. This was based on Holmes and Whitton (1981) and the macroscopic phytobenthos survey described in CEN (2009). It involves a survey of an approximately 10 m length of the stream, located at the same point from which the diatom samples were collected. The abundance of all algal growths that were visible with the naked eye was recorded and either identified in the field or a small subsample was returned to the laboratory for identification. These surveys were performed between May and October 2014 by biologists who were either experienced macrophyte surveyors or who had attended a specialist course on macroalgal identification. Cover was assessed using a 9-point cover scale (Table 2). These were amalgamated into three categories for data analysis; the threshold between categories corresponds to the cover associated with good/moderate and moderate/poor boundaries based on the filamentous algal metric within the UK's macrophyte assessment tool, LEAFPACS (Willby et al., 2009). The good/moderate boundary in LEAFPACS is placed at 7.5% cover; however, as cover is assessed using a semi-quantitative scale in both LEAFPACS and RAPPER (Table 2), the boundary has been placed at the lower limit of the class within which this threshold falls (5%). Similarly, the moderate/poor boundary occurs at 17.5% cover in LEAFPACS, so has been placed at the upper limit of the class in which this threshold falls (25%).

When identification was not possible in the field, samples were brought back to the laboratory for confirmation. Periphyton taxa were identified using Gutowski and Foerster (2009); John et al. (2011) and drafts of an as yet unpublished key written specifically for UK rivers.

Table 1
Summary environmental data for 58 sites where biological samples were collected from sites close to routine chemical sampling points. All chemistry is expressed as the average of 12 monthly readings for the period preceding sample collection. TON = total oxidised nitrogen; SRP = soluble reactive phosphorus; TP = total phosphorus. "Consistently H" = consistently high ecological status; "Consistently M" = consistently moderate ecological status; "Consistently P" = consistently poor ecological status.

A priori status classification		Width(m)	Depth(m)	Substratum phi	Cond μS cm^{-1}	pH	Alkalinity mg $CaCO_3$ L^{-1}	NH_4N mg L^{-1}	TON mg L^{-1}	SRP mg L^{-1}	TP mg L^{-1}
Consistently H (n = 15)	Minimum	1	0.1	−216.25	55	5.16	0.59	0.017	0.148	0.002	0.0105
	25th %ile	11.25	0.19	−155.625	59.53	6.41375	9.1705	0.017	0.235	0.008	0.016875
	Median	20	0.26	−137.5	62.05	6.685	16.91	0.022	0.282	0.008	0.02425
	75th %ile	27.5	0.45	−113.844	113.225	7.21375	22.98	0.024	0.353	0.009	0.033875
	Maximum	57	0.7	−32.5	202	8.065	73.77	0.027	0.4305	0.009	0.05
Consistently M (n = 41)	Minimum	1.2	0.09	−174.688	153.9	6.565	3.411	0.0195	0.643	0.016	0.011
	25th %ile	3.375	0.1675	−140.938	253.305	7	39.5	0.0305	1.395	0.024	0.051
	Median	6	0.235	−118.438	350.5	7.82	71.97	0.042	3.07	0.041	0.081
	75th %ile	12.375	0.3	−99.375	576.96	8.115	120.05	0.079	5.4825	0.07	0.10775
	Maximum	47	1.5	−16.875	912	8.43	307.1	0.51	8.885	0.29	0.2985
Consistently P (n = 2)	Minimum	8	0.18	−160	328	8.17	97.85	0.0435	0.742	0.037	0.072
	25th %ile	14.75	0.205	−150.469	348.875	8.175	104.4025	0.0455	2.10275	0.052125	0.0755
	Median	21.5	0.23	−140.938	369.75	8.18	110.955	0.0475	3.4635	0.06725	0.079
	75th %ile	28.25	0.255	−131.406	390.625	8.185	117.5075	0.0495	4.82425	0.082375	0.0825
	Maximum	35	0.28	−121.875	411.5	8.19	124.06	0.0515	6.185	0.0975	0.086

2.3. Development of RAPPER

The method for evaluating macroalgae, termed "RAPPER" ("Rapid Assessment of PeriPhyton Ecology in Rivers") evolved from a rapid survey method of Marsden et al. (1997) and the weightings applied to filamentous algae in the River Macrophyte Nutrient Index (RMNI), a constituent metric of LEAFPACS (Willby et al., 2009). Marsden et al. (1997) just examined cover of filamentous "green" algae with no taxonomic characterisation; however practical experience showed that, whilst this was quite effective at confirming impacts where these occurred, resolution was relatively poor in cleaner water (J. Krokowski, pers. comm.).

RAPPER is based on the assumption that taxa that are well-adapted to survival in nutrient-stressed conditions will be out-competed when limiting nutrients are abundant (Biggs et al., 1998). Such taxa are referred to as "stress-adapted" ("S") taxa within RAPPER. As a low concentration of inorganic nutrients is the typical state of natural systems in the absence of anthropogenic pressures, these taxa are mostly associated with high and good status. A second group, "competitive" ("C") taxa, are widespread, but typically only dominate when resources that constrained population growth are abundant (Biggs et al., 1998). Increased availability of inorganic nutrients, therefore, leads to C taxa out-competing S taxa, leading to a shift in community structure away from the natural ("expected" or "reference") state. Abundance of C taxa at a site is, therefore, a good indication that the site is no longer at high or good status, leading to potential impairment of ecological services. A few genera, whose representatives span a wide range of nutrient conditions, or for which few data were available, were placed in a third class, "uncertain" ("U").

PIT ("Periphyton Index Trophic"), a method for evaluating the ecological status of Norwegian rivers and streams based on non-diatoms (Schneider and Lindstrøm, 2011), has been shown to give consistent results when compared to the current UK diatom-based assessment method (Schneider et al., 2013). The Norwegian method, however, is based on a species list that includes many that are not recorded in John et al. (2011) and, for this reason, we decided, as a first step, to evaluate the potential for using genus-level information from this method. In order to test this, the indicator value (IV) assigned by Schneider and Lindstrøm (2011) to the 150 taxa included in PIT (two sewage fungus organisms plus the protozoan *Ophrydium* are also included in PIT but have been omitted here) was compared with the average IV for all representatives of the genus. Overall, there was a good relationship between species- and genus-level IVs (Fig. 1; Spearman's rank correlation, $r = 0.77$, $P < 0.001$). 38 genera were represented either by a single species or are not differentiated beyond genus due to identification difficulties (typically, the absence of reproductive organs in most field populations). A few genera are differentiated into "operational taxonomic units" (OTUs) based on filament width, with each OTU assigned a different IV in the PIT. In some cases (e.g. *Mougeotia*) all indicate a similar level of status; in others (e.g. *Oedogonium, Spirogyra*), the OTUs span several status classes. Heterocystous Cyanobacteria all have IVs typical of high status, whereas non-heterocystous genera either span several classes (e.g. *Phormidium*) or are consistent indicators of enrichment in PIT (e.g. *Oscillatoria*).

The classifications in PIT are in broad agreement with experience of the distribution of these algae in the UK (authors, unpublished observations). *Cladophora* has a relatively lower score in PIT than in the River Macrophyte Nutrient Index (RMNI, a component metric within LEAFPACS), whilst *Audouinella* has a higher score. *Audouinella* is most evident in relatively clean UK streams whereas all three species in PIT have IVs indicative of enrichment. The high score for *Audouinella* in PIT may reflect the more detailed microscopical analysis of samples and the lack of quantification used in PIT. *Audouinella* is present in small quantities even in enriched waters in the UK. However, the RAPPER method is based on the presence of visually-obvious growths of organisms and such growths are only associated with low levels of nutrient pressure.

The indicator potential of three taxa was changed during this study. Of these, the change in the status of *Hildenbrandia* is of particular note. This was not recorded by Schneider and Lindstrøm (2011) and the provisional status as a S taxon within RAPPER was based on an RMNI score of 6.03, which may be rather relaxed for a species that can be found across a wide range of nutrient concentrations. *Hildenbrandia* was, therefore, reclassified as "U".

A provisional taxon list for RAPPER was established (Appendix 1) based on the indicator values of taxa within existing phytobenthos classification systems. Taxa with a RMNI score <7 and an indicator value in PIT <16 (indicating the approximate position of the good/moderate

Table 2
The nine-point scale for assessing cover of macroalgae for RAPPER assessments.

Cover value	Percent cover of stream bed	Category
1	<0.1	Low cover
2	0.1 < 1	
3	1 < 2.5	
4	2.5 < 5	
5	5 < 10	Moderate cover
6	10 < 25	
7	25 < 50	High cover
8	50 < 75	
9	≥75	

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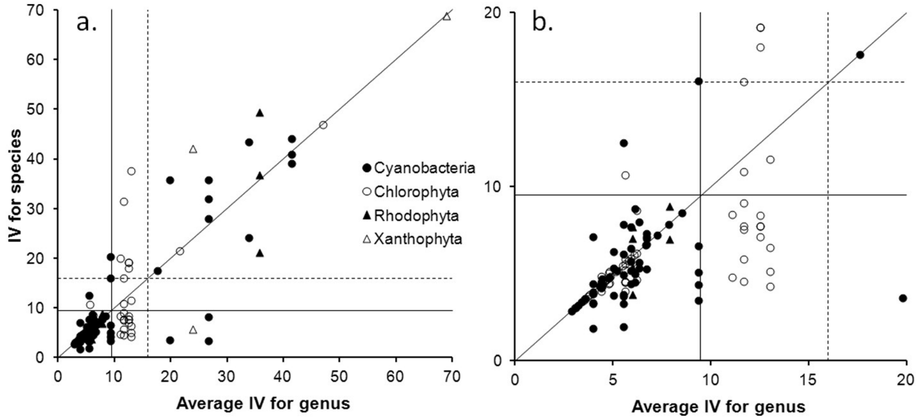

Fig. 1. Relationship between species-level indicator values (IV) assigned by Schneider and Lindstrøm (2011) and the average IV for all taxa within a genus. a. shows distribution along the whole scale; b. shows the scale from 0 to 20 in more detail. Diagonal line: slope = 1. Horizontal and vertical lines indicate the position of the high (solid) and good (dashed) status boundaries adopted in Norway for rivers with >1 mg L^{-1} Ca. Two genera, *Heribaudiella* (Phaeophyta) and *Hydrurus* (Chrysophyta) have been removed, for clarity; both are represented by a single species, and have IVs of 4.98 and 5.97 respectively.

status boundary in all but the very softest waters in Norway: Schneider and Lindstrøm, 2011) are assumed to be "S" taxa; those with higher values were assigned to the "C" category. In cases where there was a discrepancy between RMNI and PIT scores, the assignment of the taxon in the "Phytobenthos ohne Diatom" (PoD), the German non-diatom assessment system (which is, like RAPPER, semi-quantitative) was considered. This also regards *Audouinella* (except *A. pygmaea*) primarily as indicators of high and good status (Gutowski and Foerster, 2009; Schaumburg et al., 2012).

Genera whose PIT indicator values straddled the good status boundary were classified as "uncertain" and not used, at this stage, to classify sites. In some cases (e.g. *Hildenbrandia rivularis*), we have evidence of the species thriving across a wide range of nutrient concentrations in UK rivers; in other cases (e.g. *Aegagropila*), we do not have enough data, at this stage, to make an assignment with confidence. It may be possible, in the future, to assign width-based OTUs to filamentous genera such as *Oedogonium*; however, we were reluctant to import the OTUs used in the PIT due to the differences in stream types between Norway and the UK.

The results of RAPPER surveys were interpreted using the matrix in Table 3. There are two clear-cut scenarios: presence of "S" taxa with "C" taxa absent or covering a small area of the streambed, indicating a low risk of eutrophication, and absence of "S" taxa coupled with high cover of "C" taxa, indicating that the water body is at risk of eutrophication. However, a number of sites had intermediate properties: in a few cases both "S" and "C" taxa were present, and there were also a few sites where neither indicator was present (or C indicators were only present in small quantities). Both of these were designated as "maybe at risk" of eutrophication. The former instance represents genuinely borderline cases where "S" taxa had not been out-competed by "C" taxa whilst the latter state cannot be classified due an absence of strong evidence either for or against eutrophication. In both instances, more information would be required before a judgement could be reached. Classification is performed regardless of the number of taxa present as, in our experience, many enriched lowland streams only have a single conspicuous macroalga present.

2.4. Data analysis

Statistical analyses were performed using the R software package (R Core Team, 2012) using the vegan package (Oksanen et al., 2007) for multivariate analyses. The structure of the data was examined using non-metric multidimensional scaling (NMDS: McCune and Grace, 2002) and related to the environmental data by Spearman's rank correlation. Analysis of similarity (ANOSIM: Clarke, 1993) was used to explore differences between sites based on their status, as determined from diatom-based assessments. Non-parametric tests (Wilcoxon or Kruskal–Wallis test, as appropriate; Dalgaard, 2002) were used to test for differences between groups. A confidence threshold of $P < 0.05$ was used throughout the study.

3. Results

3.1. Distribution of macroalgae in Scottish rivers

Surveys from 90 reaches in Scottish rivers were available for analysis. 33 taxa were recorded from these samples, including distinctive macroscopic growths of diatoms (e.g. *Didymosphenia geminata*, *Melosira varians*), where these were conspicuous (Appendix 1). Four records of "blue-green algal mats", one of "filamentous green" and a "diatom-desmid film" were deleted prior to further analyses. A single record of "*Cladophora*/*Rhizoclonium* agg" was included with *Cladophora glomerata*.

No macroalgae were recorded at three of the sites visited, whilst just over half (53%) had just one or two taxa present. Those sites designated as "consistently high status" tended to have more taxa (median: 3; maximum: 8) than those designated "consistently moderate status" or "consistently poor status" (median: 2). Only one site designated as "consistently moderate status" had more than four taxa whilst no site designated as "consistently poor status" had more than three taxa. *C. glomerata* was the most commonly recorded taxon (43 records), followed by *Oedogonium* and *Vaucheria* (Appendix 1).

Table 3
Matrix of periphyton taxa indicative of risk of eutrophication and likely ecological status. "S taxa": taxa associated predominately with high and good status; "C taxa": taxa associated predominately with moderate, poor and bad status.

Condition	Not at risk	Maybe at risk		At risk
Likely status	High/good	← Moderate →		Poor/Bad
S taxa	Present	Present	Absent	Absent
C taxa	Low abundance ≤5%	Moderate or high cover >5 ≤ 25%	Low abundance ≤5%	Moderate or high cover >25%

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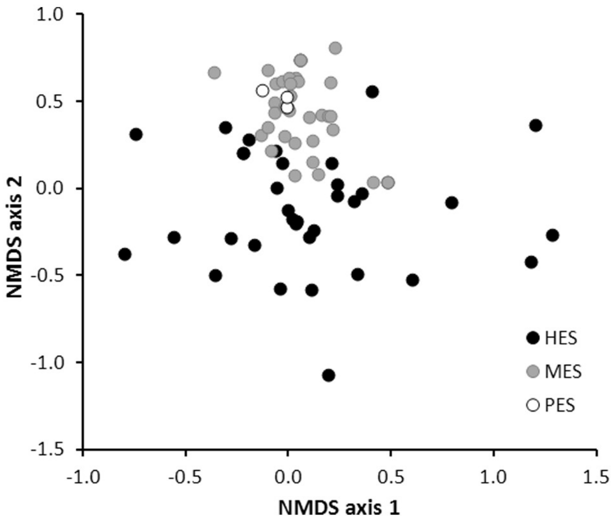

Fig. 2. Ordination results for the SEPA macroalgal dataset using NMDS, showing positions of sites, classified by their most recent diatom results: HES: high ecological status; MES: moderate ecological status; PES: poor ecological status.

Though absent from sites with very low alkalinity (i.e. <15 mg L^{-1} $CaCO_3$), *C. glomerata* was found in abundance across the rest of the alkalinity gradient, as were *Vaucheria* and *Oedogonium* sp. Clear preferences were harder to recognise for the less-frequent genera due to the relatively small number of records of each; however, *Microspora* sp. was only abundant when alkalinity was <100 mg L^{-1} $CaCO_3$ and pH < 7.

The structure of the data was investigated using Non-metric Multi Dimensional Scaling (NMDS). The stress (which measures the robustness of the ordination) was relatively high (0.28) but some patterns can be discerned within the dataset. In particular, there was a clear separation on Axis 2 between sites classified a priori as "high status" from those classified as "moderate status" or lower. (Fig. 2: ANOSIM R = 0.1195; P = 0.002). It is not clear which factors controlled distribution along Axis 1: neither alkalinity nor substrate composition (as phi) was correlated with Axis 1 scores although both showed a relationship with Axis 2 (r = 0.58 and 0.67 respectively).

3.2. Relationship of macroalgal taxa to diatom indices and phosphorus concentrations

Sites where at least one S taxon was found were generally associated with low TDI values and low concentrations of soluble P (Fig. 3; Wilcoxon test: P < 0.001 for both variables); however, the relationship between these variables and the cover of C taxa was less straightforward. Although sites with low cover of C taxa were generally associated with low TDI values and soluble P concentrations, there was no relationship between either of these variables and the abundance of C taxa above the threshold of 5% used to define the "low cover" category (Fig. 4). Having established the lack of differentiation between enriched sites, based on cover of C taxa, these categories were merged for subsequent analyses.

3.3. Application of RAPPER to the dataset

The first objective of this study was to see how well the RAPPER method could differentiate between sites that are in high or good status from those that are at moderate status or lower. Based on the initial taxon list, 70% were correctly assigned (i.e. the "likely status" given in Table 3 corresponded to the classification based on diatoms). Of the remainder, 18% fell into the "maybe at risk" category due to absence of "S" taxa and low cover of "C" taxa.

Average TDI scores for the sites were generally low where "S" taxa were present and high where "C" taxa were abundant (Fig. 5). Those sites with both "S" taxa and abundant "C" taxa generally had intermediate scores, suggesting that this category may be a good indicator of "moderate status" or sites that "maybe at risk" of eutrophication. Sites where "S" taxa were absent and cover of "C" taxa was low had a wide range of TDI values. The difference in TDI values between these categories was highly significant (Kruskal–Wallis test = 39.8, P < 0.001).

Strong differences between these categories were also observed when median chemistry for the year of the study was compared (Fig. 6), with significant differences (Kruskal Wallis test) for soluble P (25.8, P < 0.001), total P (19.9, P < 0.001); total oxidised nitrogen (24.0, P < 0.001) and ammonia-N (24.9, P < 0.001).

4. Discussion

4.1. Overview

There is growing recognition that eutrophication in rivers presents a significant challenge to catchment managers (Dodds, 2006; Hilton et al., 2006). Clear cause–effect relationships with nutrient inputs are not always obvious and, therefore, that the success of any particular intervention is not guaranteed (Page et al., 2012; Harris and Heathwaite, 2012). Faced with such a complex situation, decision making should be improved if there are several complementary strands of evidence available (Wimsatt, 1994) and, in particular, if evidence is gathered in a hierarchical manner that allows managers to "home in" on appropriate locations within water bodies, and to diagnose pressures (and appropriate

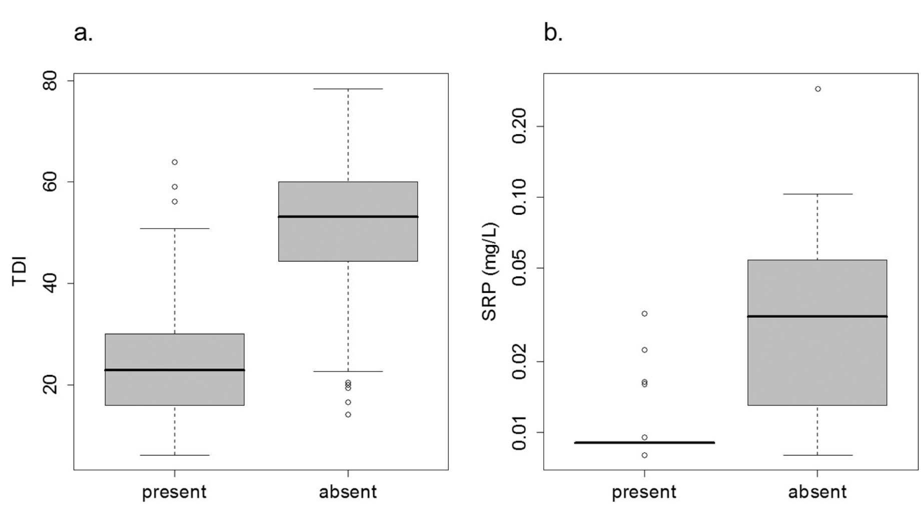

Fig. 3. TDI values (left) and soluble P concentrations (right) associated with sites where "S" taxa are present or absent.

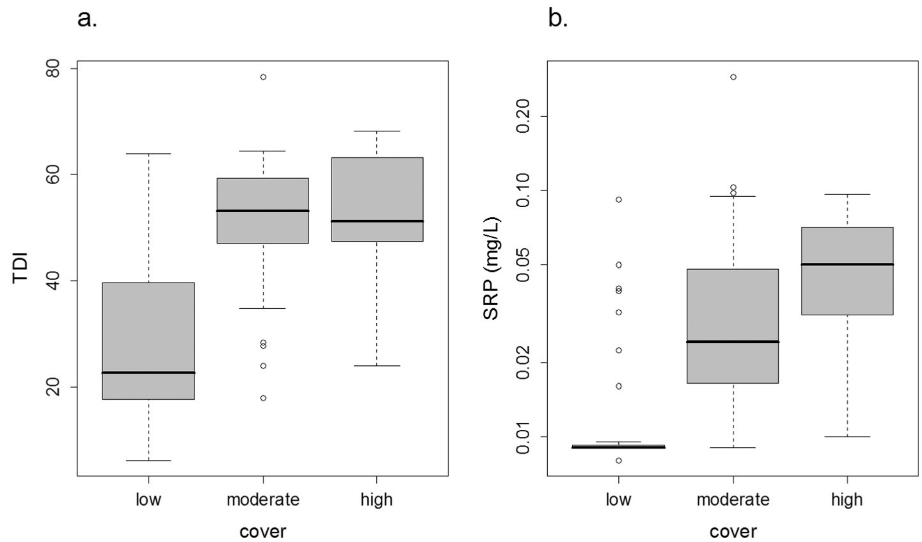

Fig. 4. TDI values (left) and soluble P concentrations (right) associated with sites with different levels of cover of "C" taxa.

solutions) correctly (Kelly, 2014; DeNicola and Kelly, 2014). This, in turn, creates a need for high level "triage" methods that allow a rapid screening. Agreement of 70% between RAPPER and DARLEQ is in accordance with other studies where diatoms and non-diatoms have been compared (Kelly, 2006; Kelly et al., 2008b; Schneider et al., 2013) suggesting that the RAPPER method is as robust as other more labour-intensive methods for assessing non-diatoms. Indeed, if sites where both S taxa are present and C taxa are abundant are regarded as correct portrayals of situations where there is likely to be a risk of eutrophication (Figs. 5, 6), then agreement rises to 88%. Those sites where neither S taxa are present nor C taxa are abundant could, perhaps, be assumed to be sites where algal growths are unlikely to constitute an "undesirable disturbance" (i.e. where secondary effects of eutrophication are evident) even if they cannot be shown to be at high or good status.

The association between axis 2 of the NMDS and alkalinity raises the possibility that changes in the macroalgal composition are at least partially driven by local geology rather than pressure. There is a tendency for high status sites in this study to be associated with low conductivity and alkalinity (Table 1), supporting this suggestion. In reality, low alkalinity sites are associated with hard rocks and, therefore, mountainous areas with low population density, leading to a low risk of nutrient enrichment. There is sufficient overlap in conductivity and alkalinity

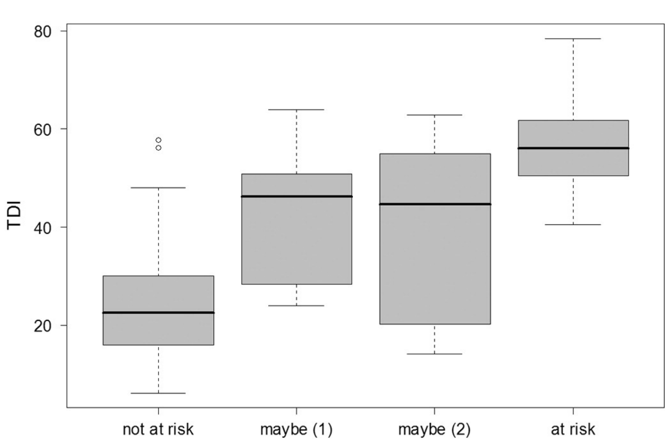

Fig. 5. Box-and-whisker plot showing difference in average TDI values for sites classified as not at risk of eutrophication (equates to high or good status; "S" taxa present, "C" taxa absent or present with low cover), maybe at risk of eutrophication (moderate status; category 1: "S" species present; "C"-taxa moderate abundance), maybe at risk of eutrophication (category 2: "S" taxa absent; "C" taxa present but not abundant) and at risk of eutrophication (poor or bad status; "S" taxa absent; "C" taxa abundant).

between a priori status classes to suggest that geology cannot be the main driver and, in any case, ecological status classifications are based on observed/expected calculations and geology is taken into account in the calculation of expected values of metrics (Kelly et al., 2008a).

The method follows the practise of several other macrophyte (e.g. Haury et al., 2006; Willby et al., 2009) and benthic algae (Schneider, 2015) methods in how cover is recorded. A nine-point assessment scale was adopted for this study as this scale is used routinely by the macrophytes surveyors who performed the RAPPER surveys although Figs. 3 and 4 suggest that this could be reduced to a simpler scale in the future. However, such cover estimates are two-dimensional simplifications of three-dimensional relationships that some have attempted to estimate for macrophytes (e.g. Kohler, 1978) or macroalgae (Rott et al., 1999). These are, themselves, approximations of the quantity of photsynthetically-active biomass through which ecosystem processes are mediated. There are certainly instances where short tufts of algae cover a large area of the river bed, and cover-based systems will not differentiate these from situations where the river bed is smothered by thick wefts of algae. There is no ready answer to this issue: perhaps a simple cover based approach will lead to risks of occasional "false positives"; however, this needs to be balanced by the risk that a site will not be assessed at all because a method that is more labour intensive will inevitably result in fewer surveys within a set budget. Overall, we recognise that all survey-based methods involve approximations and estimates and, indeed, that some non-diatom phytobenthos methods (Holmes and Whitton, 1981; Schaumburg et al., 2012) bypass issues of biomass versus cover entirely, restricting themselves to estimates of relative abundance.

Preliminary indications are that RAPPER is relatively insensitive to season. A potential complication is that a few good indicators of low nutrient conditions (e.g. *Lemanea*) are more abundant in late winter and early spring whilst some indicators of enrichment (e.g. *Cladophora*, *Ulva*) are most abundant in late Summer (e.g. Holmes and Whitton, 1981).

4.2. Functional basis for RAPPER

Adoption of the terminology of Biggs et al. (1998) to differentiate between "stress-selected" and "competitive" taxa provides a bridge between ecological assessment and theoretical studies (see Kelly et al., 2009b). In particular, the shift between sites where the phytobenthos assemblage was dominated by S taxa and those with abundant C taxa may reflect differences in the availability of nutrients, particularly phosphorus. High status, near-pristine streams typically have low levels

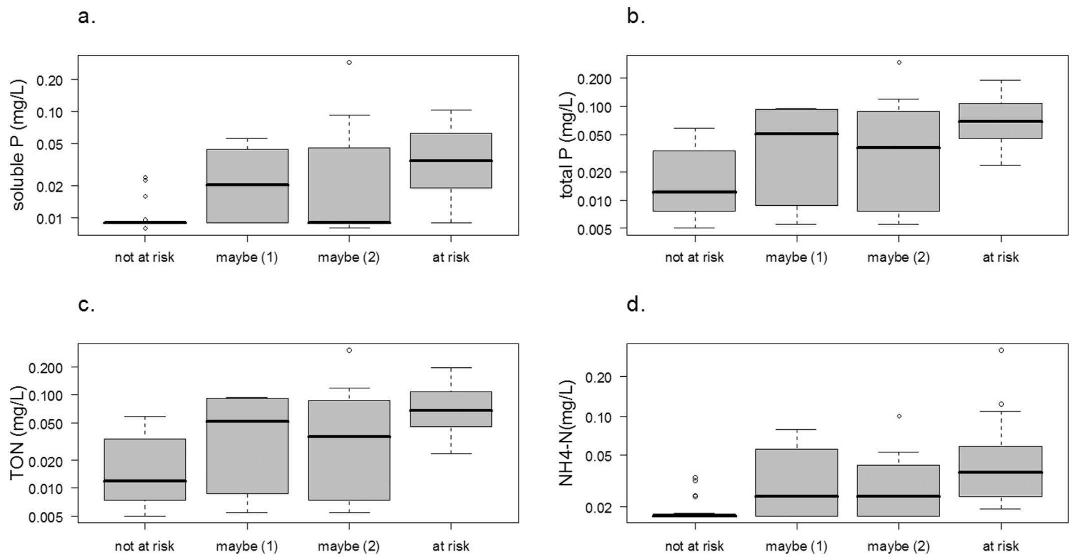

Fig. 6. Box-and-whisker plot showing difference in median for soluble P (top left), total P (TP, top right), total oxidised nitrogen (TON, bottom left) and ammonia-N (NH4-N, bottom right). See Fig. 5 for explanation of categories.

of readily-available nutrients; however, they may be exposed to occasional pulses of nutrients, both dissolved and bound to particulates and organic complexes. Many of the taxa recorded from such streams have the capability to hydrolyse such phosphorus, via phosphatase enzymes (Whitton and Harding, 1978; Livingstone and Whitton, 1984; Gibson and Whitton, 1987; Whitton, 1988). Several cyanobacteria genera associated with high/good status are heterocystous, offering a competitive advantage when concentrations of dissolved nitrogen are low (Scott and Macarelli, 2012). By contrast, streams subject to point-source enrichment typically have higher concentrations of dissolved nutrients, which offer a competitive advantage to algae such as *Cladophora glomerata* and *Vaucheria* spp. (Kelly et al., 2009b).

It is, however, simplistic to interpret all variations in macroalgal composition and abundance in terms of nutrients. There will be situations where changes in the phytobenthos assemblage at a site may be the result of changes in grazing pressure (Jones and Sayer, 2003; Sturt et al., 2011), hydrological regime (Mebane et al., 2014; Schneider, 2015) or light (Bowes et al., 2012). The cover of macroalgae may, indeed, represent a more direct measure of the risk that elevated nutrients present to ecosystem services, rather than a proxy measurement of hazard (Kelly, 2013). It is also possible that the poor relationship between cover of C taxa and pressure (Fig. 4)_reflects reach-level factors (i.e. substratum type and stability limiting the area available for macroalgal colonisation).

Conversely, there may be situations where the algae respond to changes in nutrients that are missed by conventional coarse-scaled water chemistry measurements (Whitton and Neal, 2010). Indeed, there is circumstantial evidence that the biomass of some S taxa are higher now than in the past even in some relatively pristine streams (e.g. River Ehen, Cumbria, I. Killeen, pers. Comm.) for reasons that are not yet clear. Interpretation of results from RAPPER using the matrix in Table 3 should, therefore, be restricted to testing a priori hypotheses (e.g. changes around suspected sources of nutrients). Nonetheless, the RAPPER methodology does provide a means of collecting data in a systematic manner from which such trends could be deduced in the future (e.g. Schneider, 2015).

4.3. Conclusion: future development of rapid algal assessment methods

There is, we believe, scope for using RAPPER both alongside diatom assessments to give broader perspectives of the condition of the phytobenthos, as well as possibilities for using this technique for "walkover" surveys to identify "hotspots" at a higher resolution than is possible using full diatom or macrophyte assessment methods. It may even be possible for a biologist equipped with a field microscope to make several assessments in the course of a day and be in a position to feed these straight to decision-makers. On the other hand, rapid assessment has limitations and, for this reason, RAPPER has a rather broad "maybe at risk" category that should stimulate more detailed investigations. Again, it is important to emphasise the complexity of stream ecosystems (see above) and to recognise that a low cover of algae may reflect a low level of hazard (i.e. low nutrient concentrations) or an elevated hazard in the presence of factors that reduce the risk of a high biomass developing. Low biomass can also be interpreted as an indication that the risk of secondary effects caused by excessive algal growth was unlikely to occur.

The intention throughout has been to develop a method that is within the capabilities of a generalist freshwater biologist, rather than a specialist phycologist. Other methods based on non-diatoms (Schaumburg et al., 2004; Schneider and Lindstrøm, 2011) exist; however, these mostly require species-level identification. An early observation in this study was that, in many cases, species within the same genus have similar ecological responses and it was possible, therefore, to work at a higher level of taxonomy whilst retaining much of the sensitivity. There are, however, exceptions; in particular, several genera of filamentous green algae are large and are sufficiently widely distributed that it is not possible to make precise ecological diagnoses from genus alone. Species-level identification requires reproductive organs that are rarely present in field populations. These have been tackled, in the past, by considering cell width as an operational taxonomic unit (Whitton et al., 1979; Kinross et al., 1993; Schneider and Lindstrøm, 2011) and it may be possible to develop this in the future.

A strength of RAPPER over more detailed approaches is that the outcomes correspond more directly to public perceptions of healthy rivers. Low cover of filamentous algae, irrespective of species, is regarded as "desirable", with cover values of <5–10% being regarded as "desirable" by most participants in surveys conducted in Montana (Suplee et al., 2009). Whilst public agreement on the quantity of algae considered "desirable" should not be confused with an ecological evaluation of a well-functioning ecosystem, it does, at least, mean that there is broader understanding of the reasons why remediation is required.

Though RAPPER has a number of advantages, there are also a number of challenges still facing the method. This includes the need for a better understanding of the relationships between these algae

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and their chemical and physical environments which, in turn, will provide a more secure foundation for decision making. Issues such as survey timing may also be pertinent, particularly as some taxa (e.g. *Lemanea*) are much more likely to be encountered in the spring than summer. Training is also important; though the level of identification should not create a substantial hurdle, there is a general need for awareness of these taxa and an ability to spot them when out in the field. We have noted, in particular, instances where filamentous green algae assumed to be *Cladophora* by macrophyte surveyors were actually *Oedogonium* when inspected under the microscope. In addition, several key indicators, particularly the Cyanobacteria, are easily overlooked. Set against these challenges, however, are several opportunities, not least of which is the potential for using visually-obvious organisms that are not affected by short-term changes in environmental factors as a more direct means of communicating ecosystem functioning to stakeholders.

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Appendix 1. List of macroalgal taxa and indicator values for RAPPER. Algal genera are classified as either indicators of resource-stressed conditions ("S"), as "competitive" taxa ("C") or uncertain ("U"). In a few cases genera that are designated as "uncertain" may be assigned to a category once more data are available. The maximum cover value recorded in the study is based on the scale given in Table 2. A few broad categories ("filamentous green", "blue green algal mat", "benthic diatoms") have been omitted from this table. The diatoms *Didymosphenia geminata* and *Melosira varians* have been included, although they are not used for classification

Taxon	This study		RMNI		PIT		PoD category	Final RAPPER category	C/S (Biggs et al., 1998)
	Records	Maximum cover value	Mean	Max	Mean	Max			
Cyanobacteria									
Calothrix			–	–	5.21	5.21	A	S	S
Chamaesiphon			–	–	9.34	16.11	A/B*	S	
Dichothrix			–	–	4.33	4.55	A	S	
Homoeothrix			–	–	5.53	12.53	A/B/C	S	
Lyngbya			–	–	5.54	7.83	A–D	S	
Nostoc			4.66	4.71	6.7	7.34	A	S	S
Oscillatoria	3	5			41.4	44.24	C/D	U	
Phormidium	3	2			26.7	32.02	A–D	U	C–S
Rivularia			4.77	4.77	6.1	8.75	–	S	
Schizothrix			–	–	4.45	4.71	A/B	S	C–S
Scytonema			–	–	3.37	3.37	–	S	
Stigonema	2	1	4.32	4.32	3.97	7.13	–	S	
Tolypothrix	1	1	2.96	2.96	5.91	7.71	A	S	S
Rhodophyta									
Audouinella	4	3	–	–	35.8	49.42	A/B*	S	S
Batrachospermum	5	3	5.46	5.46	5.98	7.68	A/B	S	S
Hildenbrandia	9	6	6.03	–	–	–	B	U	
Lemanea/Paralemanea	18	6	4.53	4.53	7.90	8.88	A/B	S	
Chlorophyta									
Aegagropila	2	1	5.66	5.66	–	–	–	U	
Bulbochaete	2	1	–	–	4.65	4.65	B	S	
Chaetophora			–	–	5.91	5.91	B	S	
Chara			3.85	3.85	–	–	(A)	S	
Cladophora	43	9	7.5	7.5	47	47	B/C	C	C
Draparnaldia	2	1	3.04	3.04	6.07	6.07	A	S	S
Gongrosira			7.46	7.46	6.2	6.2	B/C	S	
Hydrodictyon	1	4	8.79	8.79	–	–	B	C	
Klebsormidium	1	1	–	–	4.4	4.87	B/C	S	
Microspora	10	8	–	–	13	13.63	A/B/C	U	
Mougeotia	1	6	(6.45)		5.63	10.71	A/B	S	C–S
Nitella			4.59				(A/B)	S	
Oedogonium	29	9	7.61		11.66	31.54	C	U	C–S
Rhizoclonium			8.66				B/C	C	C
Spirogyra	16	7	(6.45)		12.5	19.18	B	S	C–S
Stigeoclonium	4	4	–	–	21.64	21.64	D	U	C
Tetraspora	1	2	6.72		6.2	8.66	A	S	
Ulothrix	8	6	7.61		11.1	20.14	B/C	S	
Ulva	2	2	9.52		–	–	C/D	C	
Zygnema	6	6	(6.45)		4.76	5.07	B	S	
Zygogonium			(6.45)		3.05	3.05	–	S	
Xanthophyta									
Tribonema			8.41		24.0	42.13	B/C	U	
Vaucheria	29	8	–		68.91		A/B/C	C	C
Chrysophyta									
Hydrurus			–		5.97	5.97	A/B	S	

(*continued*)

Taxon	This study		RMNI		PIT		PoD category	Final RAPPER category	C/S (Biggs et al., 1998)
	Records	Maximum cover value	Mean	Max	Mean	Max			
Bacillariphyta/diatoms									
Didymosphenia	2	1						(S)	
Melosira	3	8						(C)	C

Notes:

1. Biggs et al. (1998) assign 35 species to categories on a resource supply/disturbance matrix; 16 correspond to taxa included in this study (the remainder exhibit microscopic, rather than macroscopic, growth forms. In addition to "C" and "S" taxa, Biggs et al. (1998) also recognise an intermediate "C–S" category plus "R" (="ruderal") species. The latter are all diatoms with the exception of *Ulothrix zonata*.
2. PoD categories are: A: sensitive species, characteristic of particular types of water body; B: less sensitive species, more widely distributed, indicating good conditions; C: tolerant species, indicating eutrophication when present in high abundance; D: species preferring strongly eutrophicated conditions (Schaumburg et al., 2004). Categories for *Chara* and *Nitella* are derived from the German macrophyte assessment system: A: taxa with high abundance at reference sites and low or no abundance under non-reference conditions; B: taxa which show no preference for reference or non-reference conditions; C: taxa rarely found under reference conditions which usually have high abundance at sites with low or no abundance of Group A taxa (Schaumburg et al., 2004).
3. Predominately epiphytic taxa are excluded when determining the category to which a genus belonged (so, for example, *Chamaesiphon incrustans* was not considered when deciding the category to which *Chamaesiphon* belongs).
4. *Chamaesiphon polymorphus* is assigned to classification category "C" in PoD.
5. The name "*Lyngbya*" is retained, following John et al. (2011); PIT preferences are based on those for *Leptolyngbya*.
6. *Audouinella pygmaea* extends into PoD classification category C but is rare in the UK.
7. *Batrachospermum* includes *Sheathia* spp.
8. *Mougeotia, Spirogyra, Zygnema* and *Zygogonium* are included in a single category, Zygnemetacae, in RMNI.

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