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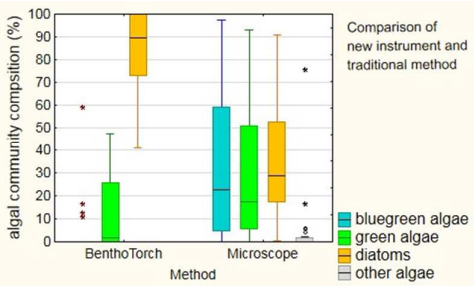

A first time comparison of in situ pigment fluorescence with conventional laboratory methods for quantifying benthic algal composition and biomass.

Environmental Impact Statement

Freshwater benthic algae are recognized as robust indicators for impacts including eutrophication, pollution and acidity, but conventional methods for assessing their abundance and diversity are costly and time consuming. This has stimulated development of new instruments, exemplified by the BenthoTorch, that use pigment fluorescence to rapidly and cost-effectively quantify algal groups in situ. The BenthoTorch is already used in both monitoring and research, despite the scarcity of published studies comparing its performance with conventional, microscope-based methods. The present study shows that the BenthoTorch has utility in quantifying total algal biomass expressed as µg chlorophyll a cm^{-2}, but its output distinguishing the biomasses of different algal groups should be interpreted with caution.

Comparing new and conventional methods to estimate benthic algal biomass and composition in freshwaters

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Abstract

We compared conventional microscope-based methods for quantifying biomass and community composition of stream benthic algae with output obtained for these parameters from a new instrument (the BenthoTorch), which measures fluorescence of algal pigments *in situ*. Benthic algae were studied in 24 subarctic oligotrophic (1.7 – 26.9, median 7.2 µg total phosphorus L^{-1}) streams in Northern Sweden. Readings for biomass of the total algal mat, quantified as chlorophyll *a*, did not differ significantly between the BenthoTorch (median 0.52 µg chlorophyll *a* $cm^{-2)}$ and the conventional method (median 0.53 µg chlorophyll *a* $cm^{-2)}$. However, quantification of community composition of the benthic algal mat obtained using the BenthoTorch did not match those obtained from conventional methods. The BenthoTorch indicated a dominance of diatoms, whereas microscope observations showed a fairly even distribution between diatoms, blue-green algae (mostly nitrogen-fixing) and green algae (mostly large filamentous), and also detected substantial biovolumes of red algae in some streams. These results most likely reflect differences in the exact parameters quantified by the two methods, as the BenthoTorch does not account for variability in cell size and the presence

of non-chlorophyll bearing biomass in estimating the proportion of different algal groups, and does not distinguish red algal chlorophyll from that of other algal groups. Our findings suggest that the BenthoTorch has utility in quantifying biomass expressed as μg chlorophyll *a* cm^{-2}, but its output for the relative contribution of different algal groups to benthic algal biomass should be used with caution.

Introduction

The assessment of benthic algal biomass and community composition in streams and lakes is essential for tracking both short and long-term changes in the dominant primary producers of most freshwater systems.[1] Such analyses are also important for basic ecological research assessing the role of benthic algae in freshwater foodwebs, but the costs of the specialised analysis involved are often beyond research budgets, limiting inference.[2] These high costs reflect not only the prolonged laboratory procedures and high degree of taxonomic expertise required, but also the high spatial and temporal variability which characterises benthic algal communities,[3, 4] necessitating extensive sampling programs.[5, 6] To address these issues, promising methods have been developed for the rapid and cost-effective estimation of both biomass and algal community composition *in situ,* based on quantification of fluorescence emitted from algae under artificial illumination with different wavelengths.[7-11] Such methods have previously been applied for phytoplankton, but new instruments for benthic algae are now also available.

The companies selling these instruments strongly advocate their utility for the "rapid quantification of green algae, cyanobacteria and diatoms on different substrates",[12] or for

providing "a quantitative estimate of the algal density and its classification",[13] or to "optimise field sampling by eliminating the need for random sample-taking and testing, and lengthy microscopic observation",[14] and to "calculate the different algae as chlorophyll-a, namely green algae, blue-green algae (cyanobacteria) and diatoms".[15] However, although these instruments are already commercially available, there is very little information about how well they perform either in the scientific literature,[but see 11] or in the specifications delivered with these products. This carries the risk that non-expert users will employ these instruments uncritically for algal biomass and community composition measurements. One such instrument, bbe Moldaenke's "BenthoTorch" is already used in different projects for monitoring of water quality, for example in Africa and China[16, 17] and has also been suggested as a standard method for rapid *in situ* bioassessment for South African rivers.[18] The BenthoTorch is under evaluation for biomonitoring in other countries[19, 20] and its use in ecological research is growing. [e.g. 21-24]

For the present project, conventional methods for quantifying benthic algal biomass as chlorophyll *a* (scraping of stones with a brush sampler followed by filtration, cold extraction of chlorophyll *a* with acetone, and spectrophotometric analysis, a standard method [25, 26]) and for determining community composition (microscopical analysis and biovolume calculation) were compared with measurements obtained from the BenthoTorch.[12] The BenthoTorch is a fully automated instrument that can be used directly in the field to analyse benthic algal biomass and community composition, albeit at a coarse level (green algae, blue-green algae (cyanobacteria) and diatoms, but not red algae). The instrument is a Pulse-Amplitude Modulated (PAM) fluorometer emitting light pulses at four different wavelengths (470, 525, 610, 700 nm), recording the response of the benthic algae at 690 nm, and calculating both

biomass (as chlorophyll *a*), and quantifying different algal groups with certain inbuilt algorithms.[8, 11, 12]

The long-term purpose of the monitoring program which provided the data for this comparison[27] is to follow changes in species composition and biomass of benthic algae in the subarctic region of Northern Sweden, where shifts in community composition and diversity are anticipated as a consequence of global climate change. Two different classes of streams can be found in the subarctic region of Sweden, arctic-alpine and boreal streams, above and below the tree line respectively, and both are included in this assessment. We hypothesised that: i) Concordance between algal biomass measurements obtained using both the conventional approach and the new BenthoTorch methods would be high; and ii) Benthic algal community composition analyses would give similar results using both the conventional and the new BenthoTorch methods.

Methods

Sites

The samples were taken in 24 streams in northern Sweden during July and August 2011 (Figure 1). The eight streams defined as arctic-alpine streams are situated in the Swedish mountain region where no month has an average temperature above 10 °C (Köppen climate classification[28]), roughly occurring above the tree line, though some minor forest areas do occur in these catchments. The sixteen streams defined as boreal are situated below the tree line, and generally drain catchments of typical boreal forests (dominated by the conifers pine and spruce, with broadleaf birch species also common), though some have a partial alpine

character, i.e. including areas not covered by forest. All boreal streams are situated north of, or very near, the Arctic Circle.

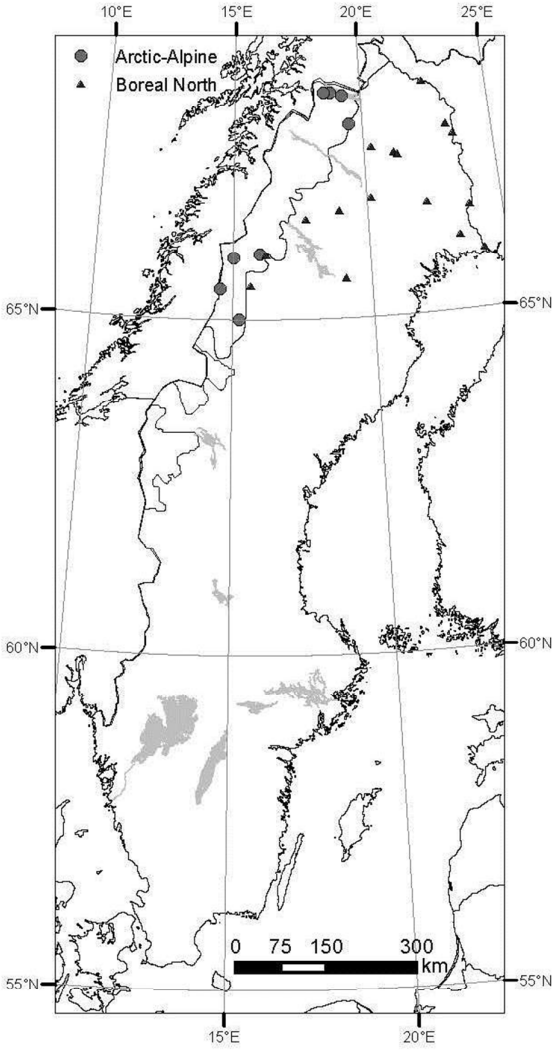


Figure 1. Sampling stations for benthic algae divided into ● arctic-alpine and ▲boreal stations north of (or near) the Arctic Circle (66° 33'N)

Sampling

Biomass of benthic algae was quantified on rocks at about 0.3 m depth in all streams, using both the new BenthoTorch and the more conventional brush sampling method. First, in each stream, four cobble stones (diameter: ~10-20 cm) separated by a distance of about 0.2 m were analysed with the BenthoTorch (surface per measurement: 0.78 cm^2, one measure per stone), with care taken to avoid physical disturbance of the biofilm. Subsequently, algae were brushed off quantitatively with a brush sampler[29] from two of the stones from exactly the same spot measured with the BenthoTorch. These samples were stored for later quantification of biomass as chlorophyll *a* (see below), enabling a direct comparison of the two methods for estimating total chlorophyll *a* from the same spot. The brush sampler improves on earlier brush samplers with syringe function being equipped with an external filtered water supply and a ball valve closing mechanism to minimise contamination and sample loss after brushing.[29] The sampler has a standardized opening with a fitted stiff bristled strip brush.[29] This standardized sampler has been shown to correctly reflect benthic algal biomass from stones sampled in streams and lakes without over- or underestimation, and with about the same magnitude of variation as control samples where all material was carefully scraped off in laboratory.[29] The other two stones were sampled in the same way for later analysis of the biovolume of the main algal groups (see below), allowing comparison of conventional methods with the BenthoTorch for estimating community composition. For chlorophyll analyses, the sampling surface area was 3.14 cm^2, for biovolume analyses the sampled area was the twice this (6.28 cm^2). The samples were stored cool and dark and sent within 24 h for analysis.

Analysis of biomass

The BenthoTorch is an instrument designed for use in the field, returning biomass and algal composition data, calculated within seconds from measured fluorescence when directed at the substrate. The instrument is delivered calibrated (on algal cultures).[11, 12] The results are given as total concentration of chlorophyll *a*, as separate measures of chlorophyll *a* concentrations for blue-green algae (cyanobacteria), green algae and diatoms, all quantified per square centimetre.

For the conventional analysis of chlorophyll in the laboratory, the sample was filtered onto GF/C filters, extracted for 12 hrs in 90% acetone and measured thereafter in a spectrophotometer at the wavelengths 664 nm, 647 nm, 630 nm and 750 nm (SS 028146[25]). Chlorophyll *a* was also calculated corrected for its degradation product phaeophytin, according to the standard method after acidification of the sample. The method of correction of the chlorophyll value has been questioned and some recommend against its use[26]. It is included here because it is required by the standard method[25], and also to test if the BenthoTorch measurements might fit better to the raw chlorophyll values or rather to the phaeophytin corrected ones.

For conventional analysis of biovolume with a microscope, the sample was shaken well and if necessary homogenized with forceps, and a subsample of 2 ml was poured into a counting chamber. All samples, except that from Lansån stream, had an optimum density of cells so neither dilution or concentration was necessary. The Lansån sample was diluted 1: 1. Algal cells were then counted at 100 x and 400 x magnification using a Hund Microscope. All taxa were identified to the lowest possible taxonomic level (mostly genus, with different size classes) and biovolume was calculated following standard geometric formulas,[30] from an

157 average of 10 cells/units. The taxa were pooled into larger algal groups to enable comparison
158 with the BenthoTorch. All biovolumes were recalculated to scraped surface.
159
160
161 *Statistical methods*
162
163 The non-parametric Sign test was used to test whether the chlorophyll values measured with
164 the BenthoTorch differed from values obtained from conventional microscope methods, using
165 *Statistica* v. 10.[31] The sign test assesses differences between the medians of paired
166 observations. The same test was used to compare differences between the BenthoTorch
167 method and the microscopical analysis in percentage of three algal groups: blue-greens
168 (cyanobacteria), greens and diatoms.
169
170 We additionally investigated the possibility that the BenthoTorch includes moss chlorophyll
171 in its measures of green algal chlorophyll, by initially analysing correlations between moss
172 abundance and green algal chlorophyll estimates obtained using both the BenthoTorch. Moss
173 cover was not available for the sampled stones directly, but was estimated according to a
174 standard field protocol on reach-level.[32] Stones from moss-rich streams typically have some
175 moss growing on them, and we assumed that, on average, stones sampled in a stream with
176 heavy moss cover have more moss cover than stones from streams with low or no moss cover.
177
178 Finally, we compared quantification of the proportional contribution of the different algal
179 groups to total chlorophyll biomass with values obtained from a conventional approach used
180 to calculate chlorophyll a, b and c concentration from spectrophotometric analysis.[33-36]

Results

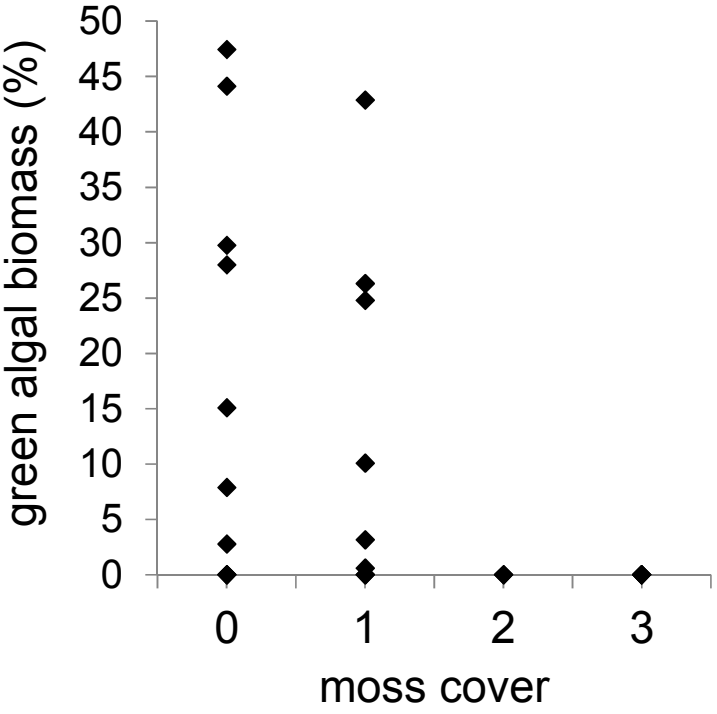
Benthic algal biomass – comparison of methods

Overall algal biomass, whether quantified as chlorophyll *a* using the BenthoTorch or conventional spectrophotometer method, or as biovolume, was low in the studied streams (Table 1). Measurements of average benthic algal biomass, quantified as chlorophyll *a,* did not significantly differ between the BenthoTorch and the conventional method, regardless of whether the phaeophytin correction was applied to the conventional method (Table 1, Sign test, $p = 0.658$) or not (Table 1, Sign test, $p = 0.302$; values were closest between the two methods when the correction was not applied).

Table 1. Benthic algal biomass (given as chlorophyll a*) and biovolume. Biomass median and interquartile range calculated of 24 studied streams in northern Sweden is the same measured with the BenthoTorch in situ or conventionally after cold extraction in acetone with a spectrophotometer. Benthic algal biovolume given for comparison.*

	BenthoTorch	Spectrophotometer	Spectrophotometer	Microscope
	Chl *a* [µg/cm^2]	Chl *a* [µg/cm^2]	Chl $a_{corr.}$ [µg/cm^2]	Biovolume [mm^3/cm^2]
Median	0.52	0.53	0.37	0.40
Interquartile range	0.58	1.04	0.83	1.21

There was no evidence that streams with a high moss cover were associated with higher measures of chlorophyll returned by the BenthoTorch. There was no correlation between green algae chlorophyll measured by the BenthoTorch and quantity of mosses (Figure 2).



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203 Figure 2. Comparison benthic green algal biomass assessed with the BenthoTorch on the
204 sampled cobble stones versus reach-wide moss cover versus moss cover (field protocol, 0 no
205 moss cover, 1: <5 %, 2: 5-50 %, 3: >50 %). ♦ sampling site

206

207 *Benthic algal taxa composition – comparison of methods*

208

209 In contrast to high concordance between methods for total algal biomass, measures for the
210 percentage composition of different algal groups did not match well between the BenthoTorch
211 and the conventional biovolume method. In most cases, large differences were found (Figure
212 3); in only a few streams did the observations match. Overall, biovolume analysis with the
213 microscope showed ~ 35% diatoms (± 26% standard deviation, average of all streams), ~ 32%
214 blue-green algae (± 33%) and ~ 27% green algae (+ 26%). Also a small amount of red algae
215 (not detected by the BenthoTorch) was found (4% ± 15%) overall, though in some streams the
216 proportion of red algae was substantially higher (20 - >70%, Fig. 3). The BenthoTorch

showed instead an overall, and very marked, dominance by diatoms (~ 85% ± 21%), followed by green algae (~ 11% ± 18%), while the blue-green algae were estimated to only ~ 4% ± 12%. The pairwise comparisons of algal groups in the studies streams were clearly significant different (Sign tests for all three algal groups: $p < 0.001$).

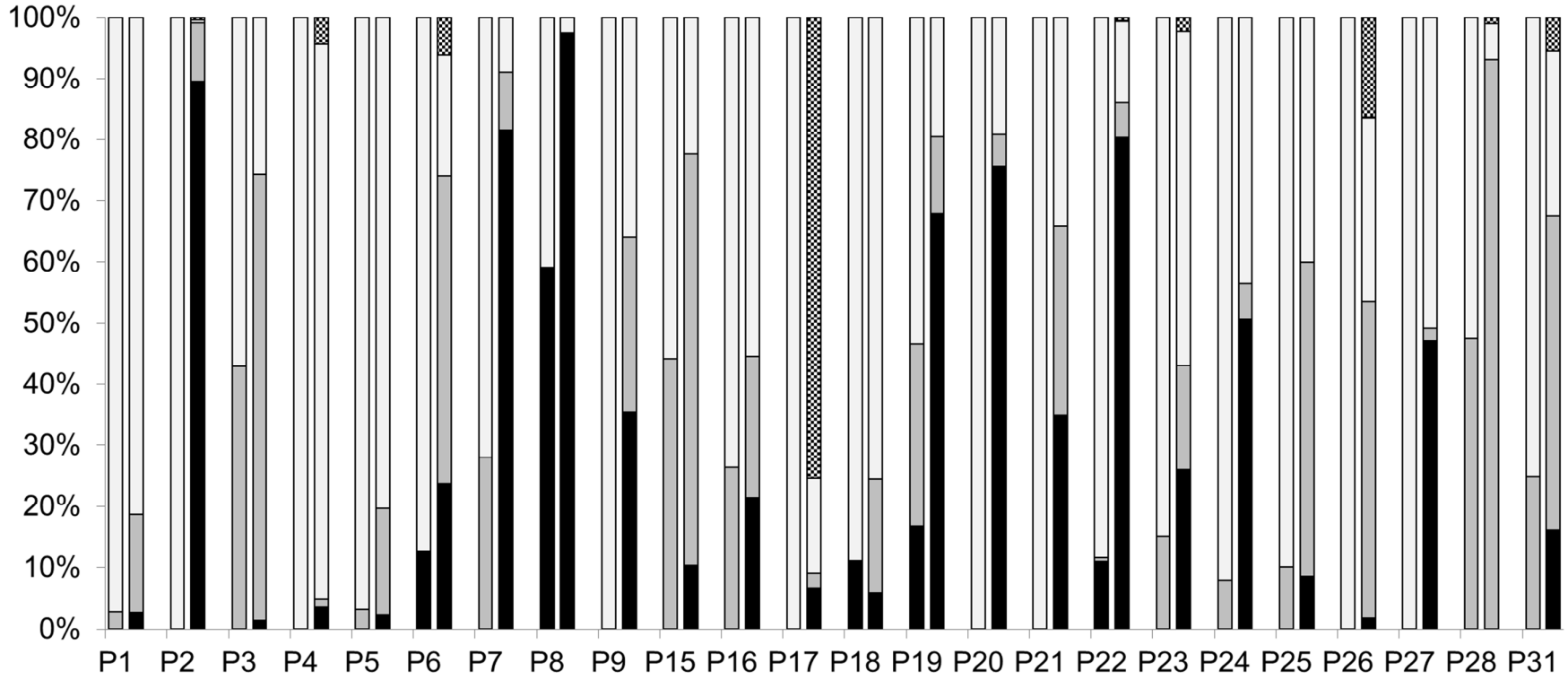

Figure 3. Comparison of benthic algal community composition assessed with the new BenthoTorch instrument (left column) and conventional microscope counting (right column) for the 24 studied streams (P1-P8: arctic-alpine streams, P9-P31: boreal streams). White: diatoms, grey: green algae, black: blue-green algae, checker pattern: other algae (mainly red algae).

In terms of algal composition, the biovolume of the diatoms was dominated by large single-celled taxa, but smaller taxa such as *Achnanthidium minutissimum* Czarnecki*, Fragilaria gracilis* Østrup and *Tabellaria flocculosa* (Roth) Kützing dominated the cell counts. The blue-green algae (cyanobacteria) were dominated by filamentous forms having heterocysts, i.e. capable of nitrogen fixation. The genus *Nostoc* dominated, followed by *Calothrix, Tolypothrix, Dichothrix* and *Rivularia*. Common in the blue-green alga group was also the genus *Aphanocapsa* in two samples. The last major group of algae was filamentous green algae, dominated by *Mougeotia* and *Spirogyra*.

A comparison of chlorophyll measurements obtained using the BenthoTorch with a conventional approach for calculating chlorophyll a, b and c concentration from spectrophotometric analysis (Table 2) indicates an agreement between those two methods, since calculated amounts of chlorophyll c, which mainly occurs in diatoms, were higher than those of chlorophyll b, only present in green algae (Table 2).

Table 2. Calculated chlorophyll a, b *and* c *from spectrophotometer analysis according to three conventional methods, given per area and as ratio per chlorophyll* a *(median for all measurements, n = 48).*

	chl *a* µg cm^{-2}	chl *b* µg cm^{-2}	chl *c* µg cm^{-2}	chl *b* : chl *a*	chl *c* : chl *a*
Strickland & Parsons 1972	0.52	0.13	0.22	0.24	0.42
Jefferey & Humphrey 1975, Mitchell and Kiefer 1984	0.53	0.10	0.11	0.20	0.21
UNESCO 1966	0.51	0.17	0.19	0.32	0.37

Discussion

In line with our first expectation, concordance between measures of total benthic algal chlorophyll obtained using the new *in situ* BenthoTorch instrument and the conventional laboratory-based method was high. On the other hand, results for the composition of algal communities often contrasted markedly between the conventional and the BenthoTorch method, rejecting our second hypothesis. A high degree of concordance between measures of chlorophyll *a* obtained using the BenthoTorch and a conventional method testing both algal cultures and field samples was also observed in a previous assessment.[11] However, this assessment did not study the proportional contribution of different algal groups relative to total algal biovolume.[11] Ours is the first assessment of the capacity of the BenthoTorch to accurately quantify the community composition of benthic algal assemblages, and suggests the BenthoTorch routinely quantifies a higher proportion of diatoms than microscopic analysis, whereas the opposite was found for the proportion of blue-greens.

In evaluating the results obtained using the two conventional methods applied here (chlorophyll *a* extraction and biovolume calculation) with those from the BenthoTorch, it is necessary to consider the specific advantages and shortcomings of each.[37] Quantification of chlorophyll *a* as a proxy for algal biomass, whether obtained from the conventional extraction method or from instruments such as the BenthoTorch, has two main advantages: it requires no taxonomic expertise and is substantially cheaper than the alternative biovolume method. Nevertheless, chlorophyll *a* is only an indirect proxy of algal biomass, as it represents just a part of the cell content, and because algal chlorophyll *a* content is known to vary substantially in both time and space (> 20-30 fold) depending on environmental conditions and algal community composition.[37] Biovolume calculation gives a more direct measure of algal quantity, since it quantifies entire cells, and not just chlorophyll content,[30] but the laboratory

analysis is expensive and time-consuming. Both conventional measures are prone to multiple sources of errors, particularly during prolonged laboratory processing of samples, and the processing time ultimately limits the number of samples that can be taken.

The great similarity in readings for total chlorophyll *a* between the BenthoTorch and extraction method are encouraging, suggesting that the BenthoTorch can be applied to further reduce costs and increase sampling effort in programs where the main aim is to quantify total algal biomass as chlorophyll *a.* Furthermore, laboratory errors are eliminated when using the BenthoTorch, since measurements are taken *in situ*, and the potential for noise in the data to arise from variability in the effectiveness of substrate scraping during field sampling is eliminated.[29] However, other sources of error may arise, including reflection of light from the substratum,[11] and the interference of other fluorescing organisms such as mosses, or algae not presently distinguished by the BenthoTorch (e.g. red algae). Indeed, the BenthoTorch potentially introduces an additional layer of complexity in the use of chlorophyll *a* as a proxy for algal biomass, since it compounds variability in the chlorophyll content of cells with potential variability in pigment fluorescence, associated with local environmental conditions and algal community composition. In our assessment, such issues evidently did not cause any divergence in the quantification of total chlorophyll *a* content between the conventional extraction method and the BenthoTorch, but might be more problematical in monitoring programs involving stronger environmental or taxonomic gradients.

In contrast with total chlorophyll *a,* findings for community composition between the BenthoTorch and the conventional biovolume method contrasted strongly. The underlying reasons for this require further investigation, but differences between the exact parameters

297 quantified by the two methods are likely to be crucial. The BenthoTorch converts pigment
298 fluorescence into chlorophyll values, whereas the microscope method in quantifying
299 biovolume focuses on entire algal cells, including large, non-chlorophyll containing organs
300 such as the vacuole or the cell wall. Implications of this are demonstrated in our findings for
301 stream P2, which was characterized by a large amount of the blue-green taxon
302 *Aphanocapsa.*[38] This alga forms colonies of tiny cells embedded in an extensive jelly matrix,
303 so that when present this taxon contributes substantially to algal biovolume seen under the
304 microscope, but their relative contribution to chlorophyll *a* readings obtained from the
305 BenthoTorch is likely to be low. Similarly, stream P8 was dominated by blue-green akinetes
306 (resting cells), which for some taxa may contain low levels of chlorophyll, reducing their
307 detectability by the BenthoTorch.[39] A further complication arises from the BenthoTorch's
308 inability to distinguish chlorophyll specifically associated with red algae, which were a
309 significant component of biovolume in some of our streams. The manual delivered with the
310 BenthoTorch gives no guidelines regarding red algae, and it is therefore unclear whether red
311 algal chlorophyll, when present, is completely excluded by the BenthoTorch, or whether it is
312 wholly or partly confounded with chlorophyll measures for one or more of the remaining
313 algal groups. Overall, these findings point to the potential high variability of chlorophyll *a* to
314 biovolume ratios in time and space, arising from differences in community composition
315 among algal assemblages. This is likely to be further compounded by the high variability of
316 chlorophyll *a* content among algal mats growing under contrasting environmental conditions
317 (e.g. ambient nutrients or light).[6]
318
319
320 The issues we identify here do not just apply to the use of the BenthoTorch. Differences in
321 what conventional microscope methods quantify relative to alternative pigment analysis

techniques (e.g. fluorescence as with the BenthoTorch, or more direct methods such as HPLC, high performance liquid chromatography), are rarely considered explicitly for benthic algae. While some studies have compared the outcome of these different techniques, it is rare that the substantially different properties of the actual variables quantified are addressed. For example, in a study comparing HPLC with microscope results in lake and stream environments,[40] the observed differences were not discussed in the light of algal biovolume. Instead, differences were attributed to causes such as variability in algal deposits, sample homogeny, and changing pigment ratios in different light conditions. Actually, the findings of this previous assessment that green algae were more frequent in the microscope-based analysis while diatom pigments dominated the HPLC results,[40] are similar to those of the present BenthoTorch assessment. It would be desirable for future studies to include parallel analyses of pigments, fluorescence and biovolume of benthic algal groups, as we need more data to draw conclusions what the observed differences among methods actually mean. Such comparisons have been more common in studies of lake phytoplankton, which have often observed reasonable correlations between HPLC and microscope-based analyses, though even these assessments have emphasised the need for more data.[41, and references therein]

The differences in the parameters quantified by the conventional method for determining algal biomass under the microscope and the BenthoTorch emphasise a need for users of both methods to frame their hypotheses around the specific parameters measured. In the case of the BenthoTorch, this is mass of chlorophyll *a* – and not cell volume or any other measure of biomass. It has not been standard within conventional methodologies to distinguish chlorophyll mass of separate algal groups, as the BenthoTorch does. Nevertheless, a comparison of the BenthoTorch measurements with an existing method estimating chlorophyll *a*, *b* and *c* concentration from spectrophotometric analysis[33-36] indicates that the

BenthoTorch is correct in identifying an overall dominance of chlorophyll biomass (and not total biomass assessed as biovolume) by diatoms across most of our streams. Specifically, chlorophyll *c*, which mainly occurs in diatoms, was estimated to be more abundant than chlorophyll *b*, only present in green algae.[33-36] However, the ecological meaning of differences in the contribution of different algal groups to chlorophyll mass, as opposed to total algal mass, is unclear. In particular, the utility of the BenthoTorch for quantifying changes in the amount of algal material available for consumption by herbivores - whether expressed as total biovolume or biovolume of "high quality" algae (e.g., diatoms) – appears limited. This is problematical since the low- and non-chlorophyll bearing components of algal biomass (cell walls, vacuole, gel-matrices, reproductive structures, stalks) are unavoidably consumed by herbivores, and may contain important energy sources, exoenzymes and trace elements[42-44], and thus are important for understanding resource flows in algae-based food chains. Actually, no methodology, whether based on conventional microscope based procedures, or fluorescence of pigments, fully accounts for the extracellular, non or low-chlorophyll bearing algal material present in an algal matrix, despite its ecological importance,[42-44] highlighting an ongoing challenge in the quantification of algal communities.

It is possible that the BenthoTorch more accurately reflects the proportion of active pigments of the different algal groups, a result which might be of significance in interpreting the photosynthetic functioning of benthic biofilms. However, we emphasise that even this requires further study, for example through parallel comparisons of fluorescence measures from the BenthoTorch as a proxy for pigment activity with more direct measures from HPLC, or of photosynthetic respiration. Further research is also required into a variety of other factors which might affect the performance of the BenthoTorch. While Carpentier *et al.* [11]

provide an extensive assessment of how variation in reflectance from substrates differing in colour and texture affects the readings obtained, the available information on the influences of factors such as algal mat thickness, the presence of mosses, and deposited sediments remains very limited. One of these factors, moss-presence, appeared unimportant in our assessment, though further research with more detailed quantification of mosses at the individual stone scale, is required to confirm this. Similarly, while the BenthoTorch offers the possibility to sample extensively *in situ,* the actual area sampled per reading is small, and guidelines on how many readings are typically required per sampling unit to overcome patchiness in algal coverage are currently lacking. It is thus presently unclear how such factors might contribute to noise in the readings obtained from the BenthoTorch, regardless of whether those readings are regarded as more indicative of biomass or pigment activity.

From this discussion, it is clear that conversion of the chlorophyll *a* values obtained from the BenthoTorch into other measures of algal quantity should be used with caution. Of particular concern are the readings the BenthoTorch returns for cell counts per taxa, in addition to the chlorophyll *a* measures. These are based on an algorithm built in within the BenthoTorch, which apparently relates chlorophyll *a* concentrations to some standard cell number per algal group (i.e. diatoms, blue-greens, and greens). This is certainly even more risky than giving pigment values, because cells sizes and chlorophyll concentrations vary substantially among different taxa.

The findings of this study emphasise the need for further testing and assessment of the BenthoTorch and similar probes, before these instruments can be generally considered as a cost-effective way to fully replace conventional analysis of benthic algae. It appears to have utility as a rapid method for assessing total algal biomass *in situ*, in the standard units of µg

chlorophyll *a* per square cm. However, we recommend against the uncritical use of the instrument for quantification of the relative contribution of diatoms, blue-greens and greens to benthic algal cover. In particular, our results highlight substantial risks in fully replacing long-term monitoring based on conventional methods of quantifying benthic algal biovolume and community composition with the output currently provided by the BenthoTorch.

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