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PRIMARY RESEARCH PAPER

The periphyton index of trophic status PIT: a new eutrophication metric based on non-diatomaceous benthic algae in Nordic rivers

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Abstract Eutrophication is one of the major problems for surface water quality in Norway, particularly in the lowlands near settlements and agricultural areas. Here, we present a new index based on non-diatomaceous benthic algae (Periphyton index of trophic status, PIT) which is developed on a dataset of >500 samples from >350 sites from the Norwegian mainland and can be used to describe trophic status at a river site. PIT indicator values for benthic algae taxa are derived from water total phosphorus concentrations and range from 1.87 for *Stigonema hormoides* to 68.91 for *Tribonema* sp. PIT site values range from 3.42 to 44.45 and cover a range from oligotrophic to eutrophic conditions. The relationship between the PIT and the total phosphorus concentration has one major threshold at 10 µg/l TP, with a slow increase below and a steep increase above 10 µg/l. We conclude that benthic algae species composition at nutrient poor sites reacts only slightly to small increases in phosphorus concentration, while it is most sensible to eutrophication in the range between 10 and 30 µg TP/l. For the genus *Oedogonium*, we found a significant positive correlation between filament width and TP concentration, making *Oedogonium* an easy to use eutrophication indicator.

Keywords Periphyton · Epilithon · Phytobenthos · Phosphorus · Indicator · Oedogonium

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Introduction

Eutrophication, manifested in excessive growth of algae and submerged macrophytes, is one of the most important pollution problems in lakes and rivers in the developed world (Hilton et al., 2006). Oligotrophy, eutrophy, and eutrophication are multifaceted terms that are defined subjectively (Hilton et al., 2006). Since they were coined in the early twentieth century, they have been applied to both autotrophic and heterotrophic processes and have been used to describe nutrient concentrations, primary production, and species composition (see, e.g., Naumann, 1929; Ohle, 1955; Rodhe, 1969; Wetzel, 1983; Dodds, 2006). Autotrophic organisms primarily rely on dissolved inorganic nutrients, while heterotrophic organisms mainly use organic material. Thus, heterotrophic processes should be separated from autotrophic processes and consequently be indicated by heterotrophic organisms (see Rodhe, 1969). There is, nonetheless, a coupling between autotrophic (primary production) and heterotrophic processes (consumers and decomposers), and organic pollution of rivers

usually also implies eutrophication processes. It is worth remembering, however, that the correlation between autotrophic and heterotrophic processes is not linear, but follows an optimum curve instead, with the highest level of primary production at an intermediate intensity of heterotrophic processes (Rodhe, 1969).

Although the concept of oligotrophy and eutrophy was applied to rivers as early as in the mid-1950s (Ohle, 1955), our understanding of eutrophication in rivers remains limited (Hilton et al., 2006). Compared to lakes, the situation in rivers is complicated by the fact that flow velocity and shading from riparian vegetation influence trophic status when it is defined as "primary production", but less when it is defined as "nutrient concentration". A river having high nutrient concentrations and flowing through a shaded area, such as a dense forest, can either be called oligotrophic because of its low primary production, which is limited by light availability, or eutrophic due to its high nutrient concentrations. Increased nutrient availability in rivers allows for a higher primary production and thus a higher biomass of benthic algae, eventually leading to a change in species composition and nuisance growth. Thus, increased nutrient availability is the cause, while changes in species composition and enhanced primary production are the effects of eutrophication. Whether or not increased primary production in nutrient-enriched rivers actually occurs, depends on additional factors such as light intensity, frequency and intensity of floods, or biomass and species composition of grazers which can significantly impair primary production (Anderson et al., 1999; Warnaars et al., 2007; Ylla et al., 2007).

Botanical indices using nutrient-based indicator values, like, e.g., the Trophic Index of Macrophytes (Schneider & Melzer, 2003) and other macrophyte indices (see Schneider, 2007) or the Trophic Diatom Index (Kelly et al., 2008), indicate eutrophic conditions in a shaded river having high nutrient concentrations (provided there is enough light that at least some indicator species occur). Accordingly, we understand river eutrophication as a change in river ecology that is mirrored in—among others—benthic algae species composition and is caused by enhanced nutrient availability. Although much of Norway has unpolluted rivers and lakes (Faafeng & Hessen, 1993), eutrophication presents one of the major water pollution problems, particularly in the lowlands near settlements and agricultural areas (Bechmann et al., 2005). Agriculture represents the main external contributor of phosphorus to lakes in Norway (Bechmann et al., 2005) and is one of the main anthropogenic sources of P input to the Norwegian coast (Selvik et al., 2006).

Within the context of the EU Water Framework Directive, a biological monitoring system is needed to detect differences in fauna and flora compared to undisturbed reference conditions (European Communities (EC), 2000), as they, e.g., may be caused by enhanced nutrient concentrations. In several European countries, benthic diatoms have successfully been used for this purpose (Kovács et al., 2006, Kelly et al., 2008). Rott et al. (1999) published indicator lists for benthic diatoms and non-diatom benthic algae that can be used for bioindication of river trophic status. Rott's indicator values for diatoms are used in the Austrian, German, and Polish methods for assessment of river benthic algae, and they were also used for intercalibration of status class boundaries of the national assessment systems in the Central and Eastern parts of Europe (Kelly et al., 2009). However, many benthic algae species common in Norwegian soft waters are not included in Rott's indicator list, thereby limiting the use of Rott's index in Norway. Schaumburg et al. (2004) used both diatoms and non-diatoms for ecological assessment of rivers in Germany. This system, however, is type specific and cannot be used in most northern European rivers, which differ from the German rivers in many respects, such as water hardness and flow regime.

Although relationships between benthic diatom species composition and surface water quality have successfully been established in Norway (Battarbee et al., 1997), it was the non-diatomaceous benthic algae rather than the diatoms which were mainly used for monitoring purposes over many decades (see, e.g., numerous reports from the Norwegian Institute of Water Research, NIVA). Among the reasons for focusing on non-diatomaceous benthic algae was a considerable increase in biomass and cover of filamentous green algae, which was observed during the 1980s and 1990s particularly in remote areas of Norway (Lindstrøm, 1993) and the fact that ultra-oligotrophic soft-water ecosystems where diatoms hardly are found are fairly common in Norway (personal observation).

Here, we present an indicator system based on non-diatomaceous benthic algae to characterize river trophic status in Norway. We analyze the response of benthic algae communities to eutrophication in a dataset of >500 samples from >350 sites, and use averaging to estimate species optima along a eutrophication gradient, reflected by total phosphorus concentration. This information is used for calculating the periphyton index of trophic status (PIT) for each sample.

Materials and methods

Benthic algae sampling method

Benthic algae, i.e., algae that live attached to the river bottom or in close contact on or within patches of attached aquatic plants, were surveyed according to the established method in Norway (Lindstrøm et al., 2004; EN, European Committee for Standardization, 2009) along an approximately 10-m length of river bottom using an aquascope. At each site, visible benthic algae were collected and stored separately in vials. Microscopic algae were collected from ten stones, with diameters ranging between 10 and 20 cm, taken from each site. An area of about 8 × 8 cm from the upper side of each stone was brushed with a toothbrush to transfer the algae into a beaker containing approximately 1 l of river water and a subsample was taken. All samples were preserved with a few drops of formaldehyde. The preserved benthic algae samples were later examined under a microscope, and identified to species level, if possible. Presence of all benthic algae was noted. Diatoms were not included, since their exact determination requires specific preparation procedures, such that in Norway not enough data on diatom species composition from river sites exist.

Dataset

In order to establish a eutrophication index, 511 samples from 387 river sites throughout Norway were used. The samples were collected in the context of numerous projects between 1976 and 2010 and are stored within the periphyton database of the Norwegian Institute of Water Research (NIVA). All sites were sampled between June and November, and no site is represented more than twice in the dataset we here use. In cases where several samples from the same year existed in the NIVA database, the one sample having maximum species number was chosen. From sites where time series exist, only the first and the last year of the time series were used for developing the PIT. Water chemistry samples were taken at the sampling sites between one and 24 times per year and the results are stored in the NIVA database. Site-specific, mean annual water chemistry data for the 1 year previous to the benthic algae sampling were used in developing the PIT. 16 sites were used as independent sites for index validation, including four sites where water chemistry was taken more than 1 year previous to the benthic algae sampling, and 10 randomly selected samples from the middle part of time series.

For calculating TP concentrations in reference rivers as a function of TOC, data from 53 samples at 53 Swedish river reference sites, 231 samples at 28 Finnish river reference sites, and 25 samples at 22 Norwegian river reference sites were collected. The Swedish data have been provided from the Swedish Environmental Agency and are compiled in the water quality criteria database of the Department of Aquatic Sciences and Assessment of the Swedish University of Agricultural Sciences. The Finnish data are from the Finnish Eurowaternet River Network (Niemi et al., 2001), and the Norwegian data are from the NIVA database. In all three countries, reference sites were defined as being largely unimpacted by human influence, thus having no major point source pollution, and agriculture and forestry in the catchment upstream of the site was only of low intensity. For more detailed information about selection of reference sites see Niemi et al. (2001), Schartau et al. (2007, 2009) and Kelly et al. (2009). Mean annual data were used and samples were taken between 4 and 26 times per year.

Quantile regression

Visual inspection of the scatterplot of the PIT and the TP concentration indicated a non-linear relationship. This relationship is visualized using quantile regression, because (a) quantiles are less affected by extreme observations, and (b) quantile regression enables estimating the minimum and maximum response in addition to the median response. Quantile regression was done using the "quantreg" package

(Koenker, 2010) in R (R Project Core Development Team, 2005).

The periphyton index of trophic status

Species optima (indicator values)

Since data on SRP (soluble reactive phosphorus) and PO_4^{3-} were not available for many of the rivers, total phosphorus concentration (TP) was used as a proxy for trophic status. TP, however, overestimates the phosphorus available to plants (Gerdes & Kunst, 1998). In initial analyses, the part of the TP that is in excess of natural background levels, and thus can be considered relevant for eutrophication was estimated by subtracting the amount of P bound in TOC from the actually measured TP concentration. Doing so, however, provided no better fit with periphyton species composition than using TP concentrations.

Each taxon's optimum was calculated by averaging log-transformed TP at the sites were the taxon occurs. This method was chosen because it is the adaptation of the weighted-averaging method (ter Braak & van Dam, 1989) to a dataset with presence-absence data, and weighted averaging usually is the most robust method for quantifying species responses to environmental parameters (Ponader et al., 2007 and literature cited therein). In initial analyses, abundances of periphyton taxa, estimated according to a 5-point scale, were used for calculating weighted optima. Including abundances, however, provided no better fit with TP concentration than presence-absence data. In the present investigation, we calculated optima for all periphyton taxa occurring at least thrice in our dataset. Each indicator taxon occurred on average in 35 samples, with individual values for taxa ranging between 3 and 194 occurrences.

A taxon's indicator value (IV) was calculated as the average log(TP) to the power of 10 at the sites were the taxon occurs. Indicator values range from 1.87 for *Stigonema hormoides* to 68.91 for *Tribonema* sp. (Table 1). Taxa whose optima were broad, such as *Stigeoclonium* sp. are not included in the PIT, nor are poorly defined pseudotaxa like "bright green Chaetophorales". Indicator values are calculated for a total of 153 taxa (Table 1). Where all species of a genus had similar ecological optima with respect to phosphorus, an indicator value for the genus is given.

Table 1 Indicator values (IV) for calculation of the PIT (periphyton index of trophic status)

Cyanophyceae	IV
Aphanocapsa sp.	7.24
Aphanothece sp.	7.83
Calothrix sp.	5.21
Capsosira brebisonii	3.98
Chamaesiphon confervicola	6.61
Chamaesiphon fuscus	5.09
Chamaesiphon incrustans	20.38
Chamaesiphon minutus	3.47
Chamaesiphon polymorphus	16.11
Chamaesiphon rostafinskii	4.37
Chlorogloea sp.	6.69
Chroococcus sp.	3.57
Clastidium setigerum	4.76
Coleodesmium sagarmathae	4.82
Cyanophanon mirabile	4.39
Dichothrix gypsophila	4.20
Dichothrix orsiniana	4.42
Dichothrix sp.	4.55
Entophysalis sp.	4.31
Geitlerinema acutissimum	24.22
Geitlerinema splendidum	43.42
Gloeocapsopsis magma/Gloeocapsa sanguinea	2.74
Gloeocapsa sp.	3.20
Hapalosiphon hibernicus	2.88
Heteroleibleinia kuetzingii	5.32
Heteroleibleinia leptonema	5.66
Heteroleibleinia sp.	7.98
Homoeothrix "grenet"	1.96
Homoeothrix batrachospermorum	3.71
Homoeothrix janthina	12.53
Homoeothrix nordstedtii	3.30
Homoeothrix varians	6.14
Hydrococcus rivularis	8.50
Leptolyngbya perelegans	4.96
Leptolyngbya crassior	3.82
Leptolyngbya sp.	7.83
Merismopedia glauca	5.33
Merismopedia punctata	3.77
Merismopedia sp.	6.28
Nostoc parmeloides	7.14
Nostoc sphaericum	5.29
Nostoc verrucosum	7.34
Nostoc sp.	7.02
Oscillatoria limosa	39.10

Table 1 continued

Oscillatoria splendida	40.99
Oscillatoria tenuis	44.24
Phormidium favosum	28.01
Phormidium hetropolare	3.40
Phormidium inundatum	35.81
Phormidium nigrum	8.22
Phormidium retzii	32.02
Phormidium tinctorum	52.77
Plectonema tomasinianum	17.60
Pleurocapsa sp.	6.66
Pseudoanabaena catenata	35.91
Pseudoanabaena frigida	3.63
Rivularia biasolettiana	4.55
Rivularia haematites	8.75
Rivularia sp.	4.99
Schizothrix lacustris	4.35
Schizothrix latierita	4.29
Schizothrix sp.	4.71
Scytonematopsis starmachii	3.08
Scytonema mirabile	3.37
Stigonema hormoides	1.87
Stigonema mamillosum	3.88
Stigonema minutum	3.30
Stigonema multipartitum	7.13
Stigonema ocellatum	3.34
Stigonema tomentosum	4.43
Stigonema sp.	3.87
Tolypothrix distorta	7.71
Tolypothrix distorta var. penicillata	5.20
Tolypothrix saviczii	4.44
Tolypothrix tenuis	6.45
Tolypothrix sp.	5.72
Others	IV
Leptomitus lacteus	22.97
Ophrydium versatile	5.36
Sphaerotilus natans	22.28
Chlorophyceae	IV
Binuclearia tectorum	3.72
Bulbochaete sp.	4.65
Chaetophora elegans	5.91
Cladophora sp.	47.00
Coleochaete sp.	4.47
Cosmarium sp.	5.14
Draparnaldia sp.	6.07
Euastrum sp.	5.47
Gongrosira sp.	6.20

Table 1 continued

Klebsormidium flaccidum	4.87
Klebsormidium rivulare	4.00
Microspora abbreviata	37.63
Microspora amoena	11.58
Microspora pachyderma	6.50
Microspora palustris	4.27
Microspora palustris var. minor	5.15
Mougeotia a2 (3–7 μ)	4.01
Mougeotia a (6–12 μ)	5.24
Mougeotia a/b (10–18 μ)	4.53
Mougeotia b (15–21 μ, short cells)	5.55
Mougeotia c (21–24)	10.71
Mougeotia d (25–30 μ)	5.87
Mougeotia d/e (27–36 μ)	4.59
Mougeotia e (30–40 μ)	4.53
Mougeotiopsis calospora	4.86
Netrium sp.	4.57
Oedogonium a1 (3–4 μ)	4.59
Oedogonium a (5–11 μ)	5.84
Oedogonium a/b (19–21 μ)	7.57
Oedogonium b (13–18 μ)	7.73
Oedogonium c (23–28 μ)	9.09
Oedogonium d (29–32 μ)	10.87
Oedogonium e (35–43 μ)	16.05
Oedogonium f (48–60 μ)	31.54
Penium sp.	3.60
Protoderma viride	3.81
Schizochlamys gelitanosa	4.61
Spirogyra a (20–42 μ,1K, L)	8.38
Spirogyra c1 (34–49 μ, 2–3K, L, l/$b > 3$)	7.11
Spirogyra d (30–50 μ, 2–3K, L)	19.18
Spirogyra sp1 (11–20 μ, 1K, R)	7.77
Spirogyra sp2 (30–38 μ, 2K, R)	19.18
Spirogyra sp5 (30–37 μ, 2K, L, l/b > 10)	7.75
Spirogyra sp6 (70–75 μ, 2K, L)	18.03
Spondylosium planum	5.76
Staurastrum sp.	3.05
Staurodesmus sp.	4.33
Stigeoclonium tenue	21.64
Teilingia excavata	4.46
Teilingia granulata	5.16
Tetraspora cylindrica	4.67
Tetraspora gelatinosa	8.66
Tetraspora sp.	5.34
Ulothrix subtilis	4.79
Ulothrix tenerrima	20.14

Table 1 continued

Ulothrix zonata	8.39
Zygnema a (16–20 μ)	4.45
Zygnema b (22–25 μ)	4.76
Zygnema c (30–40 μ)	5.07
Zygogonium sp.	3.50
Rhodophyceae	IV
Audouinella chalybea	49.42
Audouinella hermannii	21.25
Audouinella pgymaea	36.81
Batrachospermum keratophytum	3.80
Batrachospermum gelatinosum	7.06
Batrachospermum turfosum	5.37
Batrachospermum sp.	7.68
Lemanea fluviatilis	6.98
Lemanea sp.	8.88
Phaeophyceae	IV
Heribaudiella fluviatilis	4.98
Chrysophyceae	IV
Hydrurus foetidus	5.97
Xanthophyceae	IV
Tribonema sp.	68.91
Vaucheria hamata	5.84
Vaucheria sp.	42.15

The genera *Mougeotia*, *Oedogonium*, *Spirogyra*, and *Zygnema* can often only be determined to species level if they are cultured. These genera were subdivided by cell width, number and spiraling density of chloroplasts, form of end wall, and cell length/width ratio. μ = μm; 1K = one chloroplast, 2K = two chloroplasts, 3K = three and more chloroplasts or chloroplasts very densely spiraled, L = lenticular end wall, R = reticulate end wall, l/b = cell length/width ratio, pyr = pyrenoids. *Homoeothrix* "grenet" is a quite common and easily recognizable but non-described cyanobacterium. It is morphologically similar to *Homoeothrix* but has branched filaments

With the exception of *Staurastrum* sp. are the ten most oligotrophic taxa exclusively cyanobacteria, while the ten most eutrophic indicator taxa are more variable and belong to cyanophyceae, chlorophyceae, rhodophyceae, and xanthophyceae (Table 1).

Three non-autotrophic taxa are included in the list of indicators: the symbiotic ciliate *Ophrydium versatile*, the oomycete *Leptomitus lacteus*, and the filamentous bacterium *Sphaerotilus natans*. While *Ophrydium* has its optimum at oligotrophic sites, are both *Sphaerotilus* and *Leptomitus* assigned an indicator value around 22 (Table 1), corresponding to medium to high phosphorus concentrations.

Calculation of the periphyton index of trophic status PIT

The PIT, is defined as follows:

$$\mathrm{PIT} = \frac{\sum_{i=1}^{n} \mathrm{IV}i}{n}$$

where PIT is the periphyton index of trophic status, IV_i is the indicator value of species i (see Table 1) and n is the number of indicator species

According to our present experience, the following criteria need to be met in order to reliably indicate trophic status at a sampling site:

- a minimum of two indicative species (i.e., that have an indicator value according to Table 1) must be present at the sampling site
- up to now, a reliable calculation of the PIT can only be carried out in Norway as no suitable data are available that would allow trials of the PIT from elsewhere as yet. In principle, an extension of the PIT to permit an implementation in other countries is possible and desirable.

If one of the requirements is not met, the PIT should be denoted as "inconclusive".

Results

TP versus TOC concentration in reference rivers

There is a linear increase in mean annual ln TP with increasing mean annual ln TOC at reference sites in Fennoscandia, and 53% of the variability in ln TP concentration is explained by TOC ($R^2 = 0.53$, Fig. 1). The relationship between TP and TOC in reference rivers is similar for Norway, Sweden and Finland, but concentrations are generally lower in Norway than in the other two countries (Fig. 1).

The periphyton index of trophic status

The PIT was applied to 556 samples from rivers all over Norway (including 16 independent samples and 48 samples from (nearly) unimpacted reference sites) (Fig. 2). The PIT was developed based on sites with a TP concentration between 1 and 278 μg/l TP. Due to

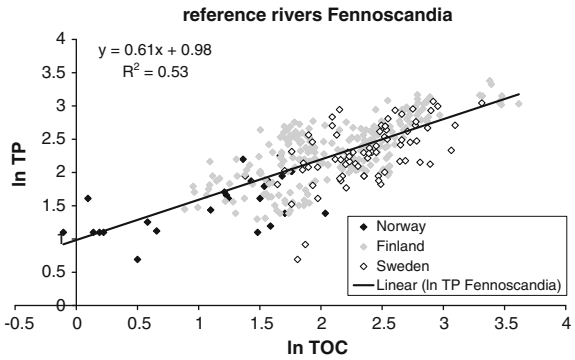

Fig. 1 Correlation between mean annual total organic carbon (TOC) and mean annual total phosphorus (TP) at (nearly) unimpacted reference rivers in Norway (25 samples from 22 sites), Sweden (53 samples from 53 sites), and Finland (231 samples from 28 sites)

averaging, the PIT is shrunken to between 3.42 and 44.45 (Fig. 2).

There is a highly non-linear relationship between the PIT and the TP concentration, with a moderate increase of the PIT up to 10 µg/l TP and a steep increase at higher TP concentrations (Fig. 2). The threshold between a moderate and a steep increase occurs between 6 µg/l TP (95% percentile) and 30 µg/l TP (5% percentile) (Fig. 2).

All independent samples lie within the variability of the other samples and thus support the general applicability of the PIT in Norway. With one exception, all reference sites exhibit low and very similar PIT values, and are found entirely within the "flat" area of the curve. The one exception is to be seen as an outlier, since this site was sampled twice in different years, and the other result fits nicely with the other reference sites.

From 12 sites, winter samples (December to May) and summer samples (June to November) taken within 1 year from each other exist. No statistical differences between winter and summer samples were detected (*t* test for dependent samples, $P = 0.54$, data not shown).

Use of morphologic categories for taxa which are difficult to identify to species

Filamentous algae of, among others, the genera *Oedogonium*, *Mougeotia*, *Spirogyra*, and *Zygnema* are difficult to identify to species without culturing. For monitoring purposes, they are therefore often

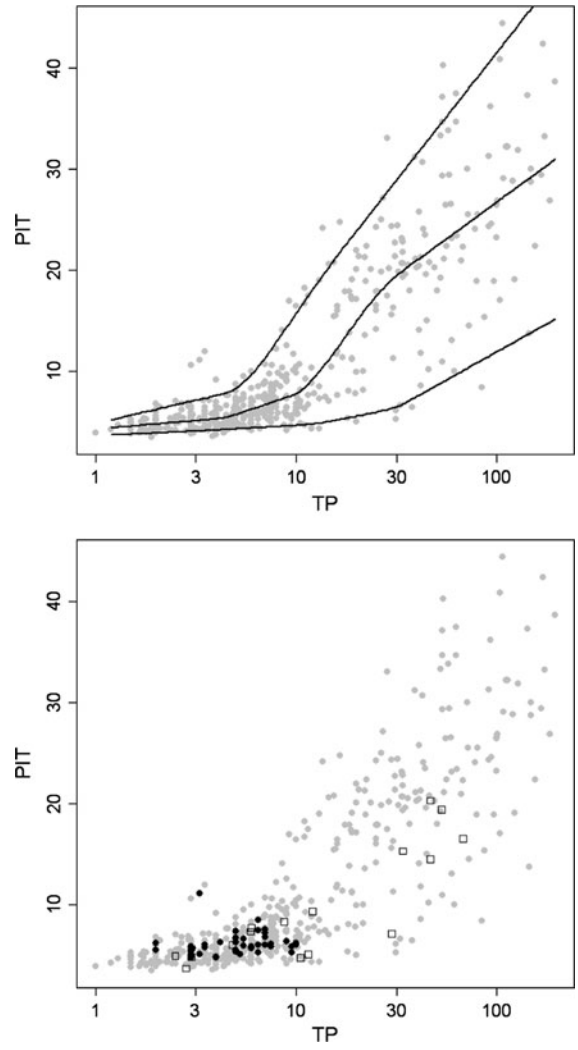

Fig. 2 Periphyton index of trophic state (PIT) as a function of total phosphorus (TP, in µg/l). *Upper panel* showing median, 95th and 5th percentile; *lower panel grey dots*, same data as *upper panel*; *black dots* unimpacted reference sites; *quadrats* independent data

categorized into morphological groups, usually based on filament width. In our results, only *Oedogonium* width categories follow a significant pattern, with wider *Oedogonium* filaments being associated with higher TP concentrations (Spearman $r = 0.32$, $P < 0.05$) (Fig. 3). *Mougeotia* width categories follow a slight and non significant optimum curve, with maximum TP concentrations for filaments between 21 and 24 µm width (Fig. 3). Neither *Spirogyra* nor *Zygnema* show a clear pattern for width categories (Table 1).

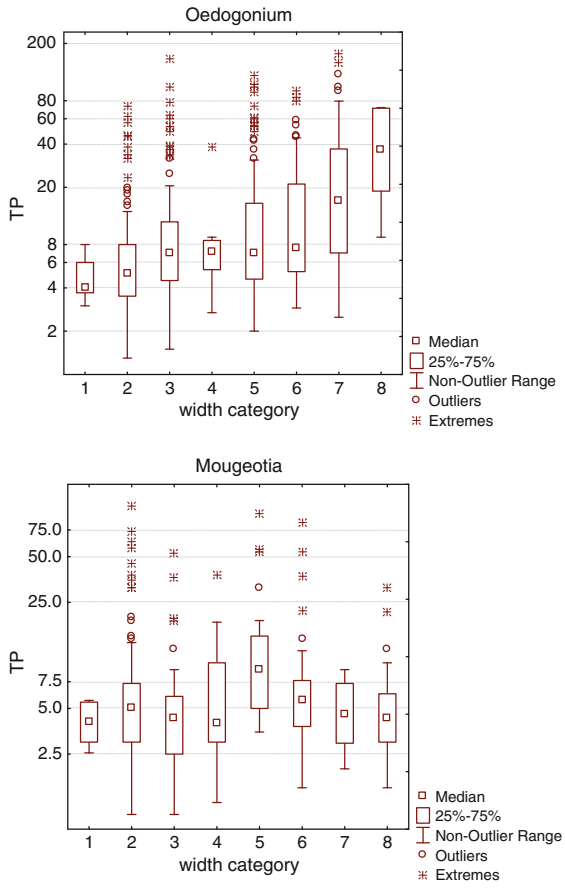

Fig. 3 Range of total phosphorus concentration at sites where *Oedogonium* (*upper panel*) and *Mougeotia* (*lower panel*) width categories are present. Width categories and number of occurrences (in *brackets*) are for *Oedogonium*: 1, 3–4 μ (7); 2, 5–11 μ (113); 3, 13–18 μ (114); 4, 19–21 (8); 5, 23–28 μ (97); 6, 29–32 μ (60); 7, 35–43 μ (45); 8, 98–60 μ (5). For *Mougeotia*: 1, 3–7 μ (7); 2, 6–12 μ (194); 3, 10–18 μ (38), 4, 15–21 μ (19); 5, 21–24 μ (21); 6, 25–30 μ (63); 7, 27–36 μ (12); 8, 30–40 μ (56)

Discussion

Factors limiting primary production

We understand river eutrophication as a change in river ecology that is mirrored in benthic algae species composition and is often caused by enhanced phosphorus availability. Several inorganic nutrients can limit primary production in rivers, the most frequent being nitrate/ammonia and phosphate (Dodds, 2006). Which nutrient is limiting primary production at a given river site depends on catchment characteristics. While many inorganic nutrients can limit primary production, there is ample evidence that the main focus for reducing eutrophication should be put on phosphorus (Withers & Haygarth, 2007, and literature cited therein). Since the indicator system presented here is designed to indicate man-made eutrophication, it is based on phosphorus concentrations. On a global scale nitrogen and phosphorus are equally important as limiting factors for stream phytobenthos (Elser et al., 2007) and nitrogen-limited lakes do exist in Norway (Lindstrøm, 1996). Nevertheless, increased phosphorus availability—mostly as a result of intensive agriculture—is generally regarded as the main reason for lake eutrophication in Norway (Ulen et al., 2007), and thus probably also is a major cause for river eutrophication. While phosphorus likely is an important driver of river eutrophication in Norway, it certainly is not limiting primary production of benthic algae at many, e.g., shaded, river sites.

Phosphorus is rarely available in excess even in nitrogen-limited systems (Elser et al., 2007). Therefore, we assume that even if our dataset probably included some nitrogen-limited sites, this did not lead to a major bias in calculating species specific indicator values. It remains to be tested how nitrogen limitation interacts with phosphorus limitation in its effects on benthic algae species composition.

Bioavailable phosphorus

Only nutrients that are available to plants can cause eutrophication. The phosphorus fraction that is readily available to plants is usually approximated by SRP (soluble reactive phosphorus; Boström et al., 1988), such that indicators for eutrophication in rivers usually rely on SRP as a basis for calculating indicator values (Schneider & Melzer, 2003; Kelly et al., 2008). The PO_4^{3-} concentration underestimates the phosphorus available to plants (Boström et al., 1988), while TP (total phosphorus) overestimates (Gerdes & Kunst, 1998).

In addition to SRP, many benthic algae species can use organic P with the help of surface-bound phosphatases (Kelly & Whitton, 1998; Mateo et al., 2010), which hydrolyze phosphorus esters into an organic moiety and orthophosphate (Siuda, 1984). Although phosphatase activity is usually associated with P-limitation (Young et al., 2010), its regulation differs between algal taxa (Hernández et al., 2002) and differences in phosphatase activity can explain

differences in benthic algae species distribution (Mateo et al., 2006).

While SRP and PO_4^{3-} concentrations usually are low in unimpacted rivers and concentrations generally increase with eutrophication, is the TP concentration of a water body not only influenced by eutrophication, but in addition by natural factors such as the amount of organic material, which in Scandinavian countries greatly varies due to natural reasons (Kortelainen, 1993) and which partly is usable for benthic algae. It has been shown that the amount of organic material in a water body correlates with its TP concentration (Meili, 1992) and the same is true for our analyses of Norwegian, Swedish and Finnish reference sites. Since neither enough SRP nor PO_4^{3-} data exist from rivers in Norway, however, TP had to be used for developing indicator values.

In initial analyses, we corrected the actually measured TP by modeling the amount of natural TP from the TOC concentration at reference sites, and then subtracting the respective amount from the measured TP concentration at all sites. In spite of the fact that TP concentration at reference sites increases significantly with TOC concentration (Fig. 1) and that parts of the P bound in TOC are not immediately bioavailable (Boyer et al., 2006), there is, however, no better fit between periphyton species composition and corrected P than with TP concentration. Several reasons might serve to explain this. (a) The relative amount and absolute values of phosphorus fractions in different wastes and sewages is variable and the part bound in organic substances can even be close to zero (Gigliotti et al., 2002). Since no data exist as to the type of TOC at our sampling sites, calculation of corrected P had to be based on the assumption that the P-content in organic matter follows the same relationship in reference and non-reference sites. The noise caused by this assumption might have counterbalanced the correction for TOC bound P. (b) Other parameters like pH (Kopacek et al., 2000) and concentration of suspended solids (Udeigwe et al., 2007) might influence bioavailability of TP, such that the effect of TOC becomes minor. (c) Especially at eutrophic sites, river TP concentration varies with river discharge (Haande et al., 2010), such that at eutrophic sites the uncertainty in TP concentration caused by the fact that only a limited number of samples was taken within a year overshadows a possibly existing bias caused by TOC. We believe that all three named reasons play a role.

The periphyton index of trophic status

Desirable features in a bioindication tool are accuracy, broad applicability, and relatively straightforward use. To keep the PIT as simple as possible, it is based on presence–absence data. Utilization of semi-quantitative data for benthic algae coverage provided no better correlation between the PIT and phosphorus concentrations (unpublished data). It is likely that this is due to the high variability of discharge that is a typical feature of most Norwegian rivers (Otnes & Ræstad, 1978). Floods can reduce the biomass of algae significantly, but have less impact on species composition (Pfister, 1993). We therefore expect the abundance of benthic algae to be mainly influenced by hydrologic conditions, while species composition mainly reflects water quality. Presence–absence data will probably be only marginally altered after floods, and therefore be less dependent on recent hydrologic conditions, reflecting water quality, as is the intention of the PIT.

Some genera of benthic algae are in their generative state problematic to identify to species, financial constraints, however, usually prohibit culturing algae for species identification within monitoring projects. Using width classes or other morphological traits to classify these taxa is a way of dealing with these practical restrictions, but it is known that these categories are of poor taxonomical value (Drummond et al., 2005 and literature cited therein). For one of these genera, *Oedogonium*, we found a significant positive correlation between filament width and TP concentration (Fig. 3), making *Oedogonium* an easy to use eutrophication indicator. Though differences in ecological optima exist for both *Mougeotia* and *Spirogyra* taxa (Table 1), the connection between indicator value and TP optimum is not linear. *Spirogyra* taxa are commonly associated with eutrophication (Drummond et al., 2005), and also in our dataset, all *Spirogyra* taxa have their optimum at slightly to moderately enhanced TP concentrations (Table 1). *Mougeotia* is largely regarded as oligo- to mesotrophic, but is known to tolerate enhanced nutrient concentrations (Gutowski & Foerster, 2009), a classification which fits nicely with our data from Norway (Table 1). *Zygnema* taxa in Norway generally have their optimum in rivers with low TP concentrations (Table 1).

We decided to include three non-autotrophic taxa as indicators in the PIT. *Ophrydium versatile* is a

freshwater ciliate with endosymbiontic zoochlorellae, which is usually characterized as oligo-mesotrophic (Sand-Jensen et al., 1994). The taxon is rarely recorded in flowing waters (Oberholster et al., 2010), but our data in Norwegian rivers give a clear picture of an oligotrophic species. The "sewage fungus" *Leptomitus lacteus* and the waste water bacterium *Sphaerotilus natans* are commonly associated with organic pollution (Friedrich, 1990). Though they both are heterotrophic organisms, and it therefore can be argued for excluding them from an indicator system for autotrophic processes, they are in our dataset both associated with generally enhanced phosphorus concentrations. The reason for this is probably, that organic pollution usually is associated with increased nutrient concentrations. It is interesting to note that, though they clearly rank among the more eutrophic taxa, 17 algae taxa have an even higher indicator value (see Table 1). This is in accordance with the long-known limnological theory (see Rodhe, 1969 and literature cited therein) that the highest intensity of autotrophic processes occurs at intermediate levels of organic pollution, and that high organic pollution, as indicated by, e.g., *Sphaerotilus natans* and *Leptomitus lacteus*, is associated with a slightly reduced trophic status.

The algal flora in Norwegian rivers is typically best developed by autumn (Lindstrøm et al., 2004). Therefore, benthic algae samples taken between December and May were excluded for calculating species optima. There is, however, no significant difference between summer and winter samples for the 12 sites in our dataset which where sampled both in summer and winter within 1 year of each other, such that assessment of a river site by benthic algae principally also can take place in winter. Since the number of species at a given site usually is lower in winter than in summer, we would nonetheless recommend sampling in summer or early autumn.

It has been shown that diatom indices can react within as little as 1 week to changing TP concentrations (Lavoie et al., 2008). Seasonal variations in river TP, as they do occur in Norway (Haande et al., 2010) might therefore be reflected in diatom-based ecological status assessment. Since the PIT is based on presence–absence rather than abundance data, as diatom indices commonly are, we do not expect it to react to short term variations in TP concentration, but rather indicate a yearly averaged situation. The PIT is thus possibly a better integrator of river ecological status, while diatom indices are more useful to indicate seasonal variations. This is supported by the fact that there are no differences in PIT between summer and winter for the 12 sites where summer and winter samples were taken within 1 year from each other. However, the hypothesis that the PIT exhibits less seasonal variation than diatom indices remains to be tested in the future.

The relationship between the PIT and the TP concentration has one major threshold around 10 µg/l TP, with a slow increase below and a steep increase above 10 µg/l (Fig. 2). Apparently benthic algae species composition at nutrient poor sites reacts only slightly to small increases in phosphorus concentration, and an increase to less than 10 µg/l P will generally not lead to major differences in benthic algae species composition. We hypothesize that a small increase in phosphorus concentration likely leads to an increase in biomass of benthic algae, rather than to major differences in species composition. However, biomass of benthic algae is additionally influenced by hydraulic regime and not enough data exist that would allow separating the effect of hydraulic regime from the effect of eutrophication. Increasing the phosphorus concentration to more than 10 µg/l will eventually lead to major changes in benthic algae species composition. The median PIT shows two additional, minor steps at around 5 and 30 µg/l TP, respectively (Fig. 2). This seems to indicate that increasing nutrient concentrations to above 5 µg/l already leads to small but noticeable changes in species composition, and that benthic algae species composition is most sensible to eutrophication in the range between 10 and 30 µg TP/l, where the slope is steepest.

In Norway, even the most humic reference sites (max = 7.6 mg TOC) do not have a TP concentration above 10 µg/l (Fig. 1). This indicates that in our dataset TP concentrations above 10 µg/l largely are caused by human impact. For nitrogen, it has been shown that a higher part of N is bioavailable from anthropogenic sources than from natural sources (Seitzinger et al., 2002), and it is reasonable to assume that the same is true for phosphorus. A higher bioavailability of TP at concentrations above 10 µg/l could explain the steeper relationship between TP and the PIT (Fig. 2). In a pristine wetland in Sweden, only 2–16% of bulk nitrogen was bioavailable

(Stepanauskas et al., 1999), and it is reasonable to assume that bioavailability of the little phosphorus that is present in pristine rivers also is low. We therefore hypothesize that low bioavailability of P in pristine ecosystems explains the very flat relationship between the PIT and TP at low concentrations (Fig. 2).

A relationship between a phytoplankton trophic index and total phosphorus concentration was established by Ptacnik et al. (2009) for lakes in Norway. The type of relation between the phytoplankton index and lake TP concentration is strikingly similar to the relation we here established for benthic algae in rivers. Both indices exhibit a "flat" part with only small changes in species composition below 10 μg/l TP, and a steeper part above that threshold. The difference between the 95 and 5% percentile gets larger at higher TP concentrations both for phytoplankton in lakes and for benthic algae in rivers, indicating that the index becomes less accurate in predicting TP concentrations at eutrophic sites. The reason for the higher uncertainty at higher TP concentrations lies probably in the fact that at high nutrient concentrations other factors like, e.g., light are likely to become limiting for primary producers, thereby influencing species composition and consequently trophic indices.

Both in Sweden and Finland exist highly humic reference sites having more than 10 μg/l TP (Fig. 1), and fresh waters in Sweden and Finland generally have a higher concentration of humic substances than in Norway (Henriksen et al., 1998). If low phosphorus bioavailability at non-impacted sites causes the flat relationship between the PIT and TP which is observed at low P concentrations, then the threshold between a steep and a flat relationship between an algae index and TP concentration can be expected to occur at higher TP concentrations in Sweden and Finland than in Norway. Unpublished data of the trophical diatom index (TDI) and total phosphorus concentration in Swedish rivers (M. Kahlert, pers comm.) indicate a threshold between a flat and a steep increase at around 20 μg/l TP, and the connection between measured TP and diatom inferred TP for Finnish rivers (Eloranta & Soininen, 2002) seems to have such a threshold at around 40 μg/l TP. We conclude that the principal reaction of benthic algae to increasing TP, with a flat relationship at low and a steeper relationship at high TP concentrations, is the same across Fennoscandia and probably also other countries, and that differences in P bioavailability cause the threshold between the flat and the steep area to occur at different TP concentrations. Measuring SRP in addition to TP would provide a tool for testing this conclusion.

It is now possible to use benthic algae species composition for assessment of both eutrophication and acidification (Schneider & Lindstrøm, 2009) of rivers in Norway. The acidification index is already further developed for assessment of ecological status according to the Water Framework Directive (Schneider, 2011) and further analyses will follow, testing the influence of river type on the PIT.

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