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Numerical simulations suggest that counting sums and taxonomic resolution of diatom analyses to determine IPS pollution and ACID acidity indices can be reduced

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Abstract Implementation of the European Union Water Framework Directive and associated national guidelines has emphasized the value of using biota, such as epilithic diatoms in streams, as indicators of water quality. However, guidelines for evaluating diatom samples have been established without explicitly evaluating their statistical robustness. We used epilithic diatom samples from 73 streams in northern Sweden and simulated the effects of variations in the counting sum size and taxonomic resolution of classifications for two indices indicating pollution (Indice de Polluo-sensibilité Spécifique, IPS) and acidity (acidity index for diatoms, ACID). Instead of the stipulated 400, we found that a count sum of 40 diatom valves for 50 streams, and 80 valves for 60 streams, would have been sufficient to obtain the same IPS index classification. The ACID index is more sensitive to count sum reductions, since the same classification would only have been obtained for 12 streams with 40 counted diatom valves or 24 streams with a count of 80 valves. Excluding rare taxa had negligible effects on the IPS and ACID indices. Excluding taxa occurring with less than 1.0% frequency affected the IPS classification of only one stream, and excluding taxa with less than 2.5% and 5.0% frequencies affected those of just one and no streams, respectively. The ACID index was affected for none, five, and 12 streams, respectively. At least in relatively unpolluted regions such as northern Sweden, our simulations suggest that a simplified methodological approach with site-specific counting sum sizes and reduced taxonomical resolution could be adopted, taking into account the way sites are classified in relation to established class boundaries. The simplified method is a step forward in improving the cost efficiency for stream monitoring, as costs of diatom analysis to obtain identical IPS and ACID classifications of our streams could be reduced considerably. Before the simplified method can be widely adopted, further simulations including regions with a higher proportion of polluted streams are required.

Keywords Epilithic diatoms · Index · Monitoring · Counting sum size · Numerical simulations

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Introduction

Implementation of the European Union (EU) Water Framework Directive (European Council 2000) has initiated intensive efforts to survey water bodies in all EU member countries, with the ultimate goal of achieving "good status" for all surface waters by 2016. Whereas chemical and physical properties have traditionally been important quality criteria, biological indicators have become more important under the most recent directive. The advantage of biological indicators is their ability to record and integrate water quality, both temporally and spatially. Among other biological indicators, diatom communities are promising for water quality assessment, mainly due to their wide distribution, species richness, and sensitivity to pollution and acidity.

To transform relative abundances of different diatom taxa into water quality terms, various index systems are often used, such as the so-called Indice de Polluo-sensibilité Spécifique (IPS index; Cemagref 1982). In addition, regional indices have been developed to address specific environmental problems, for example, the acidity index for diatoms (ACID) characterizing acidity using epilithic diatoms in Swedish streams (Kahlert et al. 2007; Andrén and Jarlman 2008). The index approach has shown to be an efficient tool to assess water quality across the globe (Jüttner et al. 2003; Chessman et al. 2007; Feio et al. 2009). However, there are also disadvantages associated with index approaches, such as the arbitrary predetermination of environmental requirements for each diatom taxon and the high degree of taxonomical harmonization required between the developed reference database and individual investigators (Kahlert et al. 2009). Moreover, diatom analysis is time-consuming and can only be done by analysts who have had intensive taxonomical training. Due to economic constraints, water authorities are seeking simplifications of the established methods that would reduce costs, without compromising the reliability of water quality assessments.

The main objective of our study was to evaluate the effects of two methodological simplifications on the water quality classification of streams, based specifically on the IPS and ACID indices. For this purpose, we used an epilithic diatom dataset from northern Sweden, including samples from 73 streams (Gälman et al. 2009). In a first step, we assessed the effects of reducing the stipulated counting sum size of 400 diatom valves on the streams' IPS and ACID indices (and hence the classification of the streams' water quality as high, good, moderate, poor, or bad according to the established European scheme). The main question addressed was whether the same stream classifications could be obtained by counting fewer diatom valves (i.e., 90%, 80%, etc. down to 10% of the stipulated number) per sample, which would considerably reduce processing time and thus analysis costs. In a second step, we investigated the effects of excluding rare taxa on the IPS and ACID indices and stream classification. We excluded rare taxa from each sample in a stepwise process and evaluated the degree to which this affected the stream classifications. Again, the taxonomic determination of rare species is time-consuming and affects analytical costs. The ultimate aim of the study was to develop statistically sound and robust recommendations for a simplified method, giving identical or nearly identical stream classifications with considerable reductions in count sizes and taxonomic resolution, leading to cost-effective environmental monitoring.

Material and methods

Sampling and analysis of northern Swedish streams

A number of 73 streams in northern Sweden (Västerbotten County) were selected in close cooperation with the local water authorities, including sites that have been monitored for water chemistry. Epilithic diatoms were sampled using a specifically designed sampler, the so-called Stone Brusher (Persson et al. 2006). The sampling was carried out from August 16 to September 4 in 2007, at all except three streams that were sampled in October.

Diatom analyses were performed according to the Swedish standard method SS-EN 14407 (SIS Swedish Standard Institute 2005) and guidelines from the Swedish Environmental Protection Agency (Naturvårdsverket 2005). In each sample, ca. 400 diatom valves were identified and enumerated at the highest possible taxonomical level. IPS and ACID indices were then calculated according to Kahlert et al. (2007) and evaluated following the guidelines from the Swedish Environmental Protection Agency (Naturvårdsverket 2007). The threshold IPS index values for high, good, moderate, and poor water quality are 17.5, 14.5, 11.0, and 8.0, respectively, and values <8.0 indicate bad water quality. Similarly, the ACID index classifies streams into five categories: alkaline, near-neutral, moderately acidic, acidic, and very acidic, with ACID indices of ≥7.5 (mean annual pH ≥7.3), 5.8 to 7.5 (pH 6.5 to 7.3), 4.2 to 5.8 (pH 5.9 to 6.5), 2.2 to 4.2 (pH 5.5 to 5.9), and ≤2.2 (pH <5.5), respectively.

Numerical methods

To generate reduced counting sum sizes for diatoms from each stream sample, subsets at 10% intervals between 10% and 100% were selected from the entire counting sum size (ca. 400 diatom valves). We randomly selected single diatom valves and generated artificial counting sum sizes of ca. 40, 80, 120, 160, 200, 240, 280, 320, 360, and 400 valves, respectively. For each sample and each counting sum size, this procedure was repeated 20 times. IPS and ACID indices were then calculated using the derived subsets, resulting in 200 simulations for each stream. The variation of the IPS and ACID indices was characterized by calculating mean values and 95% confidence intervals (2 standard deviation units).

The influence of rare taxa on the IPS and ACID indices was investigated by excluding from each sample, in a stepwise process, diatom taxa that occurred with relative abundances of <1.0%, <2.5%, and <5.0%. For exclusion, the rare taxa were simply transferred to the group of undetermined taxa. However, as the first part of the ACID index is based simply on the frequency ratio between *Achnanthes minutissima* complex and *Eunotia* taxa, we kept the relative abundances of these two groups unchanged for the ACID index and assessed the impact of rare taxa only on the second part of the index. Subsequently, IPS and ACID indices were calculated according to the procedure described above.

The effect of taxonomic resolution on the IPS index (not applicable to the ACID index) was evaluated by combining one taxonomic grouping as an example. The *A. minutissima* complex had a relative abundance of 22% in the 73 stream samples. According to the IPS index guidelines, the *A. minutissima* complex is divided into three subgroups, based on valve width (mean valve width <2.2, 2.2–2.8, and >2.8 µm for subgroups I, II, and III, respectively). We merged all *A. minutissima* subgroups into a single group and averaged sensitivity (I=4.66) and indicator (V=1.66) values, prior to calculating IPS classifications.

Results

Effects of counting sum size reductions on IPS and ACID indices

The IPS index of many of the sampled streams in northern Sweden was not affected by the reductions of the counting sum size. To illustrate the effects of the reductions, we have grouped the streams into six categories (Fig. 1). The first

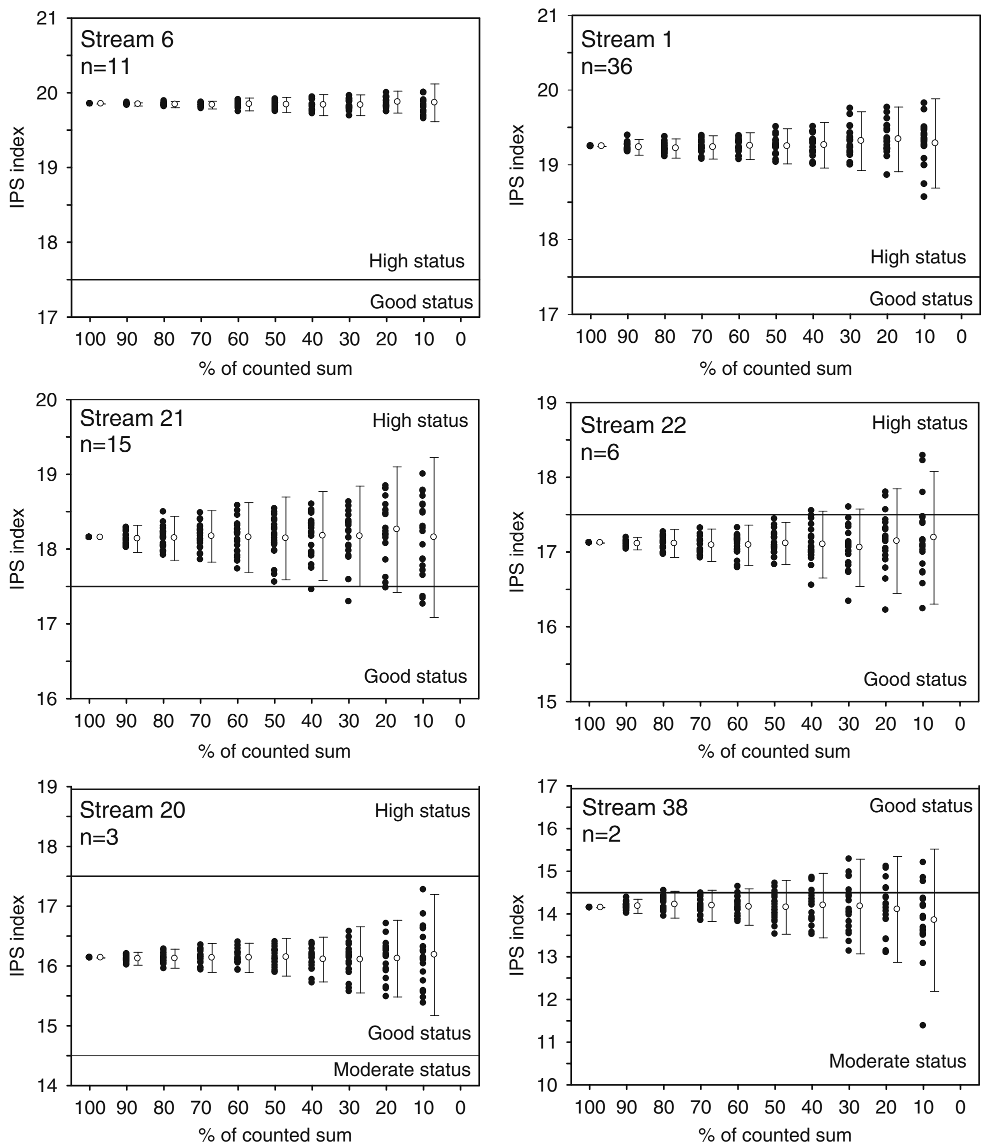


Fig. 1 IPS indices of six selected streams in relation to simulated counting sum size reductions (relative to the stipulated 400), including mean values and standard deviations. The six streams have been selected to represent typical patterns encountered within the entire data set (n=73) while reducing the counting sum size. *Stream 6* represents the high status streams (n=11) for which the counting sum size has very little effect on the IPS index (<0.5 standard deviation at 10% counting sum size). *Stream 1* represents the high status streams (n=36) for which the counting sum size has some effect on the IPS index (>0.5 standard deviation at 10% counting sum size), but it would not lead to any misclassifications. *Stream 21* is an example of high status streams (n=15) for which the reduction of the counting sum size would lead to some misclassifications. Similarly, *stream 22* that represents the good status streams (n=6) for which the reduction of the counting sum size would lead to some misclassifications. *Stream 20* represents the good status streams (n=3) for which the reduction of the counting sum size has no effect on the classification, whereas *stream 38* represents the moderate status streams (n=2) for which the reduction of the counting sum size leads to misclassifications

category includes 11 streams (e.g., *stream 6*) that originally had a high IPS status and retained this status after reducing the counting sum size by up to 90% (ca. 40 diatom valves per count). Moreover, the standard deviation of the IPS values of these streams, based on the 10% count, was below 0.5, highlighting the robustness of their IPS values. The second category includes 36 streams (e.g., *stream 1*) that were also originally classified as high status streams, differing from the first group only in having a higher standard deviation (>0.5, based on 10% of the original counting sum size). Together, these two categories include 47 high status streams (64% of the entire data set) that would have been given identically high IPS indices, based on 10% of the original counting sum size.

The third category includes 15 streams (e.g., *stream 21*) with originally high status for which the IPS index was affected by the counting sum size reductions, resulting in some incorrect classifications (good rather than high). The opposite pattern was recorded for the fourth category, which includes six streams (e.g., *stream 22*). These were originally classified as streams with good quality water, but the reduction of the counting sum size improved the classification to high in some cases. The fifth category includes three streams (e.g., *stream 20*) that originally had a "good" classification and retained this status when the counting sum size was reduced by up to 90%. Finally, the last category includes two streams (e.g., *stream 38*) that originally had a moderate IPS classification, which was raised to good in some simulations (Fig. 1).

Overall, the results of the stepwise reduction of the counting sum size used to calculate IPS indices for our 73 streams in Västerbotten show that there is considerable

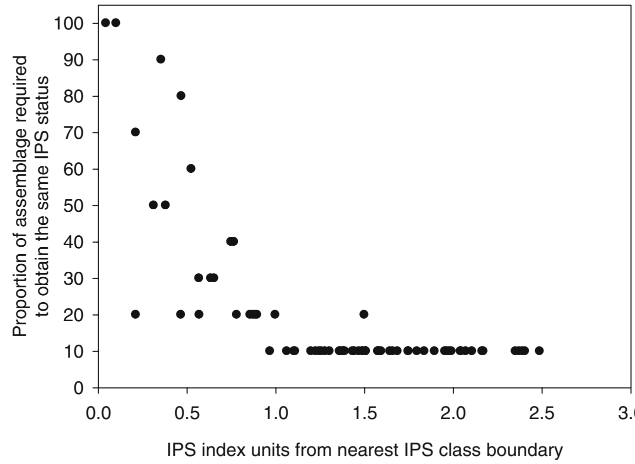


Fig. 3 The relationship between sufficient counting sum size and distance of the IPS index from the closest classification boundary. Stream samples that have an IPS index close to a class boundary require considerably higher counting sum sizes, compared with samples yielding an IPS index distant from a class boundary

scope for such reduction, without severely compromising the reliability of the classification. For 50 of the 73 streams, it would have been sufficient to count only ca. 40 diatom valves (10% of the stipulated 400 diatom valves) to obtain an identical IPS classification (Fig. 2). For an additional ten streams, 20% of the counting sum size would have been sufficient. A counting sum size of at least 200 diatom valves (≥50% of the stipulated number) would have been required for just six streams. Furthermore, the initial IPS status of all six of these streams was very close (<0.5) to a class boundary, indicating that a higher counting sum size is

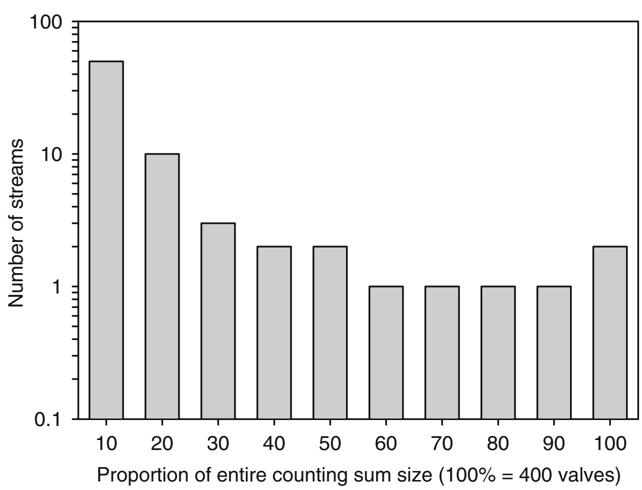
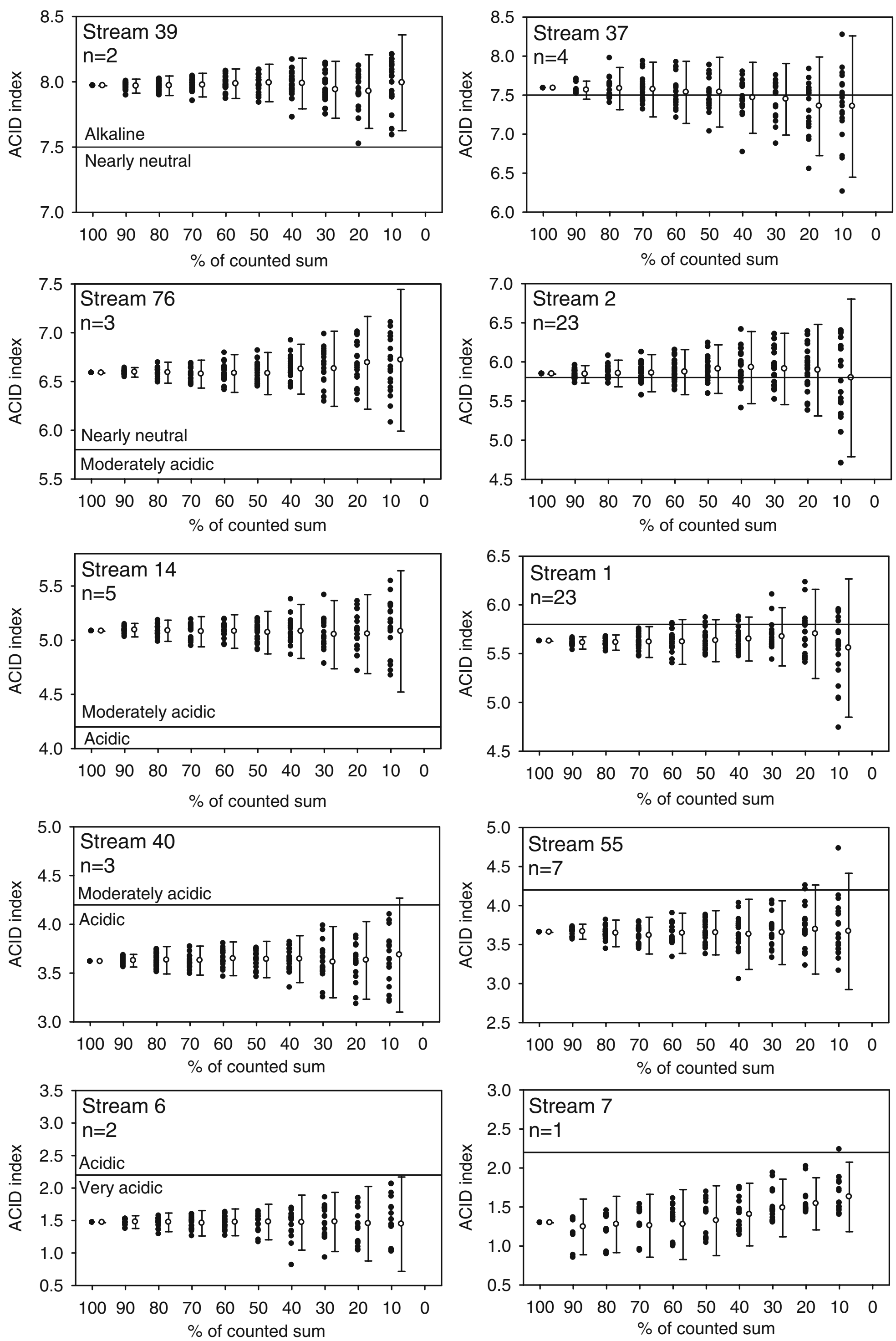


Fig. 2 Number of streams versus the proportion of the full counting sum size (100%=400 valves) required to obtain the same IPS index (with 95% confidence). For example, it would have been sufficient to count only 40 diatom valves to obtain the same IPS index as that obtained using 400 valves for 50 out of 73 streams

Fig. 4 ACID indices of ten selected streams in relation to simulated ▶ counting sum size reductions, including mean values and standard deviations. The ten streams have been selected to represent typical patterns encountered within the entire data set (n=73) while reducing the counting sum size. *Stream 39* represents the alkaline streams (n=2) for which the counting sum size reduction would not lead to any misclassifications, while *stream 37* represents the alkaline streams (n=4) for which the reduction of the counting sum size would lead to some misclassifications. *Stream 76* represents the near-neutral streams (n=3) for which the counting sum size reduction would not lead to any misclassifications, while *stream 2* represents the near-neutral streams (n=23) for which the reduction of the counting sum size would lead to some misclassifications. *Stream 14* represents the moderately acidic streams (n=5) for which the counting sum size reduction would not lead to any misclassifications, while *stream 1* represents the moderately acidic streams (n=23) for which the reduction of the counting sum size would lead to some misclassifications. *Stream 40* represents the acidic streams (n=3) for which the counting sum size reduction would not lead to any misclassifications, while *stream 55* represents the acidic streams (n=7) for which the reduction of the counting sum size would lead to some misclassifications. Finally, *stream 6* represents the very acidic streams (n=2) for which the counting sum size reduction would not lead to any misclassifications, and *stream 7* is the only very acidic stream (n=1) for which the reduction of the counting sum size would lead to some misclassifications needed for sites that have an ambiguous status. The calculated counting sum sizes required to obtain identical IPS status classifications to the initial classifications (displayed in relation to the nearest class boundaries in Fig. 3) show that only 10% of the stipulated counting sum size would be needed for almost all samples with IPS values >1.0 from the nearest class boundary.

We also evaluated the effect of stepwise reductions of the counting sum size on the ACID index and grouped streams according to their ACID index and the effect of the counting sum size reduction on their acidity classification (Fig. 4). Six streams were classified as alkaline. The reduction of the counting sum size by up to 90% did not affect the classification of two of these streams (e.g., *stream*



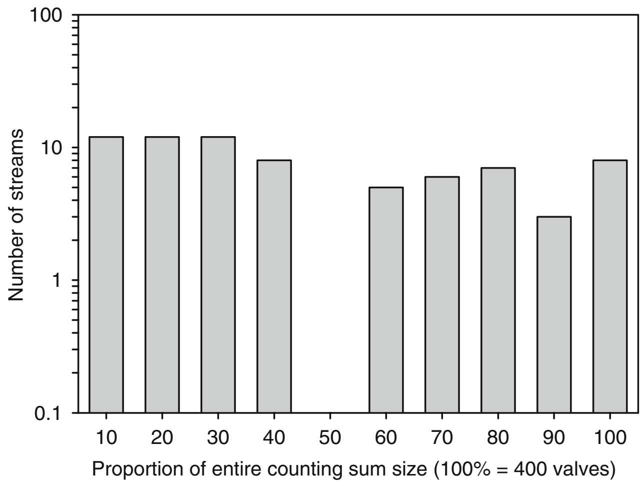


Fig. 5 Number of streams versus the proportion of the full counting sum size (100%=400 valves) required to obtain the same ACID index (with 95% confidence). For example, it would have been sufficient to count only 40 diatom valves to obtain the same ACID index as that obtained using 400 valves for 12 out of 73 streams

39) but led to some misclassifications for the other four (e.g., *stream 37*). Twenty-six streams were classified as nearly neutral, only three of which (e.g., *stream 76*) were unaffected by the 90% counting sum size reduction, while the other 23 streams (e.g., *stream 2*), mostly with ACID indices close to a class boundary, were affected to some extent by the counting sum size reduction. Similarly, the status of only five of the 28 streams (e.g., *stream 14*) classified as moderately acidic were unchanged by a 90% decrease in the counting sum size, whereas the classification of the other 23 was affected. Of the streams classified as acidic, three (e.g., *stream 40*) out of ten were not affected by the counting sum size reduction, but seven were misclassified in some simulations when the counting sum size was reduced. Finally, three streams are classified as very acidic, for two of which the classification was not sensitive to the counting sum size reductions (e.g., *stream 6*), but one (*stream 7*) was misclassified when the counting sum size was reduced by 90% (Fig. 4).

In comparison with the IPS index count simulations, the results of the ACID index were more sensitive to a reduction in counting sum size. Only 12 streams retained their original ACID indices when they were calculated using 10% of the stipulated counting sum size with a 95% confidence limit (Fig. 5). An additional 12 streams retained their initial indices with 20% of the counting sum size, and 30% of the stipulated 400 diatom valves were required to obtain identical to initial values for a further 12 streams. Furthermore, a counting sum size of at least 200 diatom valves (≥50% of the stipulated 400) would have been required for 29 streams, far more than the corresponding number (six) obtained from the IPS index simulations. However, simulated misclassifications affected streams with index values close to class boundaries, and considerably more streams in our dataset had ACID index values that were close to class boundaries (Fig. 6). Only nine streams (12%) had IPS indices closer than 0.5 units to a class boundary, while 45 had ACID indices closer than 0.5 units to a class boundary, considerably increasing the probability of simulated misclassifications when reducing the counting sum size.

Effects of removing rare taxa and reducing taxonomic resolution

In a first simulation test to evaluate the effect of rare taxa, we excluded all diatom taxa that occurred at less than 1.0% frequency (four valves per 400 counted valves). This affected the IPS status classification of only one stream (*stream 51*), which had an IPS value (17.40) close to the class boundary between good and high (17.5). The IPS classifications of the remaining 72 streams were not affected by removing diatom taxa with a relative abundance of <1.0%. In a second simulation test, all taxa occurring with a relative abundance of less than 2.5% (ten valves per 400 counted valves) were excluded, which again affected the classification of only one stream (*stream 79*), which also had an IPS value (17.29) close to a class boundary. Interestingly, the third simulation test, in which all taxa with relative abundances of <5.0% (20 valves per 400 counted valves) were excluded, classified all 73 streams with the same IPS status as initially calculated using the full taxonomic resolution. This highlights the minute effects of rare taxa on the IPS status classification.

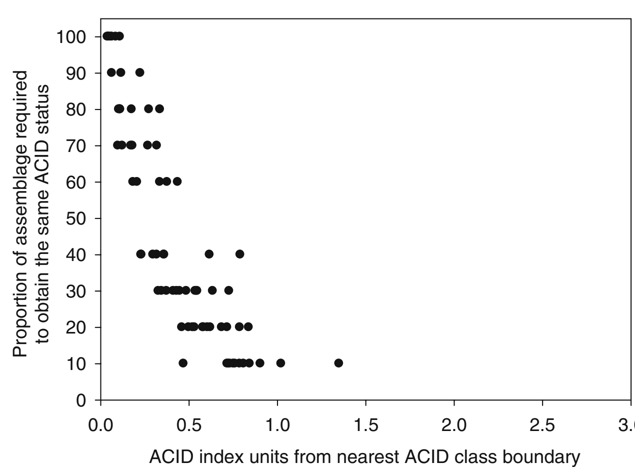


Fig. 6 The relationship between sufficient counting sum size and distance of the ACID index from the closest classification boundary. Stream samples that have an ACID index close to a class boundary require considerably higher counting sum sizes, compared with samples yielding an ACID index distant from a class boundary

Similarly, rare taxa were removed at 1.0%, 2.5%, and 5.0% levels prior to calculating ACID index values. Since the first part of the ACID index is based simply on the relative abundances of *A. minutissima* complex and *Eunotia*, the impact of rare taxa was assessed only on the second part of the ACID index, based on the acidification sensitivity classes for all diatom taxa recorded in a sample. Removal of taxa occurring with less than 1.0% relative abundance did not affect the ACID index of any stream. In comparison, five streams were given incorrect ACID status classifications when taxa occurring at <2.5% frequencies were removed, and those of 12 streams were incorrect when taxa with <5.0% frequencies were removed. As for the IPS index, streams with indices close to class boundaries were more likely to be wrongly classified.

Species from the *A. minutissima* complex are common floristic elements in northern Swedish streams, occurring in our samples with an average relative abundance of 22%. *A. minutissima* group 2 was present in 61 streams and the most common *A. minutissima* group in our northern Swedish streams, whereas group 1 and group 3 were found in one and seven streams, respectively. To explore the effect on the IPS index of the three subgroups (based on valve width) within the *A. minutissima* complex, we simulated the IPS index for all streams after combining the three subgroups into a single group. This affected the IPS classification of four streams (*streams 36, 38, 41, and 51*) that originally had an IPS index close (0.0 to 0.6) to a class boundary. The IPS classification of the remaining 69 streams was not affected.

Discussion

Diatom counts in ecological and paleoecological contexts are often expressed as percentages. Consequently, the count size is an inherent factor constraining their precision. The accuracy of percentage data depends on the frequency of each taxon, which is in turn related to the size of the entire counting sum. Several pioneering studies have discussed these relationships with regard to pollen analysis, with the aim of developing confidence limits for stratigraphical data (Mosimann 1965; Maher 1972). According to Battarbee (1986), the findings based on pollen analysis can also be applied to diatom analysis. Obviously, determining an adequate count size has been the subject of discussion for a long time, not least for optimizing the cost–benefits of the time-consuming analyses.

The optimal counting sum size is, clearly, dependent on the purpose of the analysis. While a high counting sum size is essential for capturing the entire biodiversity or floristic composition of a sample, a lower counting sum size is sufficient for determining, for example, biostratigraphic boundaries (Battarbee 1986). In a study based on a sample from Växsjösjön, percentage data and number of taxa were recorded at counts ranging between 100 and 600 diatom valves. These results indicated that the largest shifts in relative abundances and increases in the number of detected taxa occur between 100 and 200 counted valves (Battarbee 1986; Battarbee et al. 2001). Based on shifts in relative abundances and number of taxa recorded, a counting sum size of 300 to 600 valves was recommended for routine analyses (Battarbee 1986).

The IPS index, which is now used in many countries to describe the environmental status of streams, is not intended to capture the entire floristic composition in a given stream but to provide a robust indication of its overall water quality. Therefore, knowledge about the presence or absence of rare taxa is of secondary importance, as long as the classification is not affected. Our simulation results clearly indicate that the IPS index (and to some extent the ACID index) is very robust, providing highly reproducible classification of streams, even when the counting sum size is reduced by up to 90%, and ignoring rare taxa has a minute influence on the stream classification. The results also show that the IPS index is robust, that the stipulated counting sum size is larger than necessary, and that disproportionate attention is paid to rare taxa. However, our dataset is geographically restricted to northern Sweden with largely unpolluted, high status streams, and analyses including streams from a larger geographical range, and more polluted streams are necessary to fully evaluate the geographical validity of our findings. A diatom taxonomy harmonization study among 24 analysts from Scandinavia also indicates that an evaluation over larger geographical range is required, as the variation of the obtained IPS index seems to increase in polluted streams (Kahlert et al. 2009).

Diatoms are usually very abundant in streams, and in epilithic samples, the number of diatoms recovered is often sufficiently high to be able to count the stipulated sum of 400 diatom valves. Methodological developments and thorough statistical analyses of the effects on counting sum sizes therefore originate mainly from fields where the biological organisms are so rare that they limit the counting sum size. One example is chironomid head capsules, indicators recovered from lacustrine sediment cores used to infer, inter alia, past climatic conditions (Walker et al. 1991). Due to their low abundances, statistical analyses on the counting sum size have been carried out to assess the reliability of quantified chironomid-inferred temperature estimates. In a simulation study using methods comparable with our approach, Heiri and Lotter (2001) showed that counting sum sizes of ca. 50 head capsules generally resulted in similar temperature inferences to those obtained using larger counting sum sizes (100–200), but use of <50 counting sums led to higher variation and less reliable temperature estimates. These results, obtained from simulations based on samples collected from a few Swiss lakes, are corroborated by studies reaching similar conclusions from Sweden (Larocque 2001) and Canada (Quinlan and Smol 2001). It is also worth noting that the cited simulation results for testing the reliability of temperature inferences were obtained using chironomids and the weighted averaging partial least squares approach, whereas we obtained comparable results using epilithic diatoms and the IPS (and ACID) indices.

Overall, our results clearly indicate that considerable methodological simplifications are possible in relatively unpolluted regions such as northern Sweden, without compromising the outcome of index-based stream quality assessments. A future sustainable monitoring strategy that fully implements the outcome of this study would adopt the following approach. First, only ca. 50 diatom valves per sample would be identified and enumerated and used for a preliminary IPS or ACID status classification. In cases where the preliminary status is clearly separated (>1.0 index units) from a class boundary, counting 50 diatom valves would be sufficient. In cases where the preliminary status is close (0.5–1.0 index units) or very close (<0.5 index units) to a class boundary, the counting sum sizes would need to be expanded to 200 diatom valves and 400, respectively. Using this approach, the same status classification would be obtained, with 95% confidence, to that obtained when 400 diatom valves are enumerated in each sample. Moreover, as rare diatom taxa hardly affect the IPS and ACID status, according to our simulations, we suggest that rare diatom taxa, occurring at very low abundances (<1.0%), can be neglected. However, the suggested introduction of methodological simplifications could also have some negative consequences, for instance when epilithic diatom data are used for alternative purposes than IPS or ACID status evaluation, such as, for example, biodiversity assessments.

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References

Andrén C, Jarlman A (2008) Benthic diatoms as indicators of acidity in streams. Fundam Appl Limnol 173:237–253

Battarbee RW (1986) Diatom analysis. In: Berglund BE (ed) Handbook of holocene palaeoecology and palaeohydrology. Wiley, Chichester, pp 527–570

Battarbee RW, Jones VJ, Flower RJ, Cameron NG, Bennion H, Carvalho L, Juggins S (2001) Diatoms. In: Last WM, Smol JP (eds) Tracking environmental change using lake sediments. Volume 3: Terrestrial, algal and siliceous indicators. Kluwer Academic, Dordrecht, pp 155–202

Cemagref (1982) Etude des méthodes biologiques quantitatives d'appréciation de la qualité des eaux. Pierre-Bénite, Rapport Division Qualité des Eaux Lyon—Agence financière de Bassin Rhône—Méditerranée—Corse: 28 pp

Chessman BC, Bate N, Gell PA, Newall P (2007) A diatom species index for bioassessment of Australian rivers. Mar Freshw Res 58:542–557

European Council (2000) EU Water Framework Directive. Official Journal of the European Communities 327, 72 pp

Feio MJ, Almeida SFP, Craveiro SC, Calado AJ (2009) A comparison between biotic indices and predictive models in stream water quality assessment based on benthic diatom communities. Ecol Ind 9:497–507

Gälman V, Bigler C, Renberg I (2009) Kiselalger som miljöindikatorer i små vattendrag. Meddelande 3 2009. Länsstyrelsen i Västerbotten, Umeå

Heiri O, Lotter AF (2001) Effect of low count sums on quantitative environmental reconstructions: an example using subfossil chironomids. J Paleolimnol 26:343–350

Jüttner I, Sharma S, Dahal BM, Ormerod SJ, Chimonides PJ, Cox EJ (2003) Diatoms as indicators of stream quality in the Kathmandu Valley and Middle Hills of Nepal and India. Freshw Biol 48:2065–2084

Kahlert M, Andrén C, Jarlman A (2007) Bakgrundsrapport för revideringen 2007 av bedömningsgrunder för Påväxt—kiselalger i vattendrag. Uppsala, SLU, Inst. för miljöanalys: Rapport 2007: 23

Kahlert M, Anttila EM, Bengtsson R, Bigler C, Gälman V, Gottschalk S, Miettinen J, Piirsoo K, Raunio J, Sandell B, Simola H, Weckström J, Vilbaste S (2009) Harmonization more important than experience: results of the first Nordic–Baltic diatom intercalibration exercise 2007 (stream monitoring). J Appl Phycol 21:471–482

Larocque I (2001) How many chironomid head capsules are enough? A statistical approach to determine sample size for palaeoclimatic reconstructions. Palaeogeogr Palaeoclimatol Palaeoecol 172:133–142

Maher LJ (1972) Nomograms for computing 0.95 confidence limits of pollen data. Rev Palaeobot Palynol 13:85–93

Mosimann JE (1965) Statistical methods for the pollen analyst: multinomial and negative multinomial techniques. In: Kummel B, Raup D (eds) Handbook of palaeontological techniques. Freeman, San Francisco

Naturvårdsverket (2005) Påväxt i rinnande vatten—kiselalgsanalys, Version 2:2, 2005-07-19

Naturvårdsverket (2007) Status, potential och kvalitetskrav för sjöar, vattendrag, kustvatten och vatten i övergångszon, 415 pp

Persson J, Hansson H, Renberg I (2006) The Stone Brusher, a new sampler for submerged epilithic material in shallow streams and lakes. Hydrobiologia 560:385–392

Quinlan R, Smol JP (2001) Setting minimum head capsule abundance and taxa deletion criteria in chironomid-based inference models. J Paleolimnol 26:327–342

SIS Swedish Standard Institute (2005) Svensk Standard, SS-EN 14407:2005, water quality—guidance identification, enumeration and interpretation of benthic diatom samples from running waters

Walker IR, Smol JP, Engstrom DR, Birks HJB (1991) An assessment of Chironomidae as quantitative indicators of past climatic change. Can J Fish Aquat Sci 48:975–987