J Appl Phycol (2009) 21:471–482
DOI 10.1007/s10811-008-9394-5

Harmonization is more important than experience—results of the first Nordic–Baltic diatom intercalibration exercise 2007 (stream monitoring)

Maria Kahlert · Raino-Lars Albert · Eeva-Leena Anttila · Roland Bengtsson · Christian Bigler · Tiina Eskola · Veronika Gälman · Steffi Gottschalk · Eva Herlitz · Amelie Jarlman · Jurate Kasperoviciene · Mikołaj Kokociński · Helen Luup · Juha Miettinen · Ieva Paunksnyte · Kai Piirsoo · Isabel Quintana · Janne Raunio · Bernt Sandell · Heikki Simola · Irene Sundberg · Sirje Vilbaste · Jan Weckström

Received: 24 September 2008 / Revised and accepted: 18 November 2008 / Published online: 13 December 2008

Abstract The goal of this study was a harmonization of diatom identification and counting among diatomists from the Scandinavian and Baltic countries to improve the comparison of diatom studies in this geographical area. An analysis of the results of 25 diatomists following the European standard EN 14407 during an intercalibration exercise showed that a high similarity was achieved by harmonization and not because of a long experience with diatoms. Sources of error were wrong calibration scales, overlooking of small taxa, especially small *Navicula* s.l., misidentifications (*Eunotia rhomboidea* was mistaken for *Eunotia incisa*) and unclear separation between certain taxa in the identification literature. The latter was discussed during a workshop with focus on the *Achnanthes minutis-*

M. Kahlert (✉) · S. Gottschalk · E. Herlitz · I. Quintana
Department of Aquatic Sciences and Assessment,
Swedish University of Agricultural Sciences,
Box 7050, S-750 07 Uppsala, Sweden
e-mail: maria.kahlert@ma.slu.se

R.-L. Albert · J. Miettinen
Ecomonitor,
Länsikatu 15,
FI-80110 Joensuu, Finland

E.-L. Anttila
Department of Geography,
University of Oulu,
P.O. Box 3000, FI-90014 Oulu, Finland

R. Bengtsson
Mikroalg,
Ölsåkra, Slottet,
S-340 37 Torpsbruk, Sweden

C. Bigler
Department of Ecology and Environmental Science,
Umeå University,
KBC plan 5, S-901 87 Umeå, Sweden

T. Eskola
Institution of Geosciences,
University of Oulu,
P.O. Box 3000, FI-90014 Oulu, Finland

V. Gälman
Environmental Change Assessment Group,
Department of Ecology and Environmental Science,
Umeå University,
S-901 87 Umeå, Sweden

A. Jarlman
JARLMAN HB,
Stora Tvärgatan 33,
223 52 Lund, Sweden

J. Kasperoviciene
Laboratory of Hydrobotany,
Institute of Botany,
Zaliuju Ezeru 49, LT-084 06 Vilnius, Lithuania

M. Kokociński
Adam Mickiewicz University,
Collegium Polonicum,
Kościuszki Str. 1, PL 69-100 Słubice, Poland

sima group, the separation of *Fragilaria capucina* var. *gracilis* from *F. capucina* var. *rumpens*, and *Nitzschia palea* var. *palea* from *N. palea* var. *debilis*. The exercise showed also that the Swedish standard diatom method tested here worked fine with acceptable error for the indices IPS (Indice de Polluo-sensibilité Spécifique) and ACID (ACidity Index for Diatoms) when diatomists with a low similarity (Bray–Curtis <60%) with the auditor in at least one of the samples are excluded.

Keywords Diatoms · Monitoring · Streams · Intercalibration exercise · Diatom index · Nordic–Baltic region

Introduction

One aim of this study was a test and quality control of the Swedish (SE) standard method using diatoms for biomonitoring of water quality in streams. The other aim was a harmonization of diatom identification and counting among diatomists from Sweden and its neighbouring Nordic and Baltic countries. This latter objective is relevant, as we are convinced that a comparison of diatom studies is only possible if similar identification criteria are applied.

The study was done as a first Nordic–Baltic diatom intercalibration exercise with 25 participants from six countries (Sweden, Finland, Estonia, Lithuania, Latvia and Poland) in 2007. Epilithic diatoms from six streams with different physico-chemical characteristics were counted and analysed following the European standard EN 14407 (CEN 2004) and the software OMNIDIA (Lecointe et al. 1993, http://perso.club-internet.fr/clci/tour_guide.htm) with some Swedish modifications (Naturvårdsverket 2007, Appendix). The counting results of the intercalibration participants were compared with the results from an auditor (Amelie Jarlman AJ, Jarlman HB Lund, in cooperation with Bart Van de Vijver BV, National Botanic Garden of Belgium, Meise). Two metrics, namely IPS (Indice de Polluo-sensibilité Spécifique, Cemagref 1982) and ACID (ACidity Index for Diatoms, Andrén and Jarlman 2008) were used in the comparison.

In 2004, Sweden started using benthic diatoms in biomonitoring of stream ecological quality. With the increasing widespread use of the SE method in regional and national monitoring programmes, it is now necessary to quantify the performance of the SE method and to harmonize interlaboratory approaches.

At an early planning stage of the Swedish intercalibration exercise, an interest in the neighbouring countries to join the harmonization process emerged. Therefore, we started collaboration in form of a network (NORBAF—Nordic–Baltic Network for Benthic Algae in Freshwater, http://www.norbaf.net) resulting in a diatom taxonomy course held in 2004 and a test intercalibration during a workshop in 2006. Harmonization of diatom analyses is required in the European Union (EU)'s Water Framework Directive (WFD), as stated in the reports of the Central-Baltic and Nordic geographical intercalibration groups (Kelly et al. 2007, 2008).

In general, there are few publications on diatom intercalibration exercises, despite the fact that they give basic knowledge on how diatom methods are performing in different regions and types of water. Many EU countries, as part of the implementation of the WFD, are now including benthic diatoms in bioassessment. However, little is known of the uncertainties associated with their use in ecological classification. In particular, the performance of the new SE acidity index ACID might be interesting for other countries lacking an acidity index in their standards.

The present intercalibration exercise focussed on the differences between diatomists as this difference has been shown to be the main factor of variance. According to Prygiel et al. (2002), 80% of the variance of the tested diatom index was due to the diatomist, 10% was due to sampling, 5% due to preparation of the sample and diatom

H. Luup · K. Piirsoo · S. Vilbaste
Institute of Agricultural and Environmental Sciences,
Estonian University of Life Sciences,
181 Riia Str., EE-510 14 Tartu, Estonia

I. Paunksnyte
Hydrobiology and Ecotoxicology Division,
Environmental Research Department,
Environmental Protection Agency,
A. Juozapaviciaus str. 9, LT-09311 Vilnius, Lithuania

J. Raunio
Water and Environment Association of the River Kymi,
Tapiontie 2 C, FI-451 60 Kouvola, Finland

B. Sandell
BS Sötvattenkonsult,
Roliasgatan 23B, S-553 39 Jönköping, Sweden

H. Simola
Ecological Research Institute,
Faculty of Biosciences,
University of Joensuu,
P.O. Box 111,
FI-801 01 Joensuu, Finland

I. Sundberg
Medins Biologi AB,
Företagsvägen 2,
S-435 33 Mölnlycke, Sweden

J. Weckström
Environmental Change Research Unit (ECRU),
Department of Biological and Environmental Sciences,
University of Helsinki,
P.O. Box 65 (Viikinkaari 1),
FI-00014 Helsinki, Finland

slides and 5% was due to the replicates on each slide. Moreover, according to a study by Lavoie et al. (2005), field sampling and laboratory methods are not important contributors to the variation of diatom community analyses across stream sites. Most of the observed variance in an index value is due to misidentification of certain diatom taxa. This has been demonstrated for the diatom indices IBD (Indice Biologique Diatomées) and IPS in France, an area where many intercalibration studies have been done. It is commonly noted that there are problematic groups of taxa, e.g. among the genera *Achnanthes* (*A. catenatum* (Bily & Marvan) Lange-Bertalot, *A. minutissimum* Kützing, *A. biasolettiana* Grunow), *Cocconeis* (*C. placentula* var. *placentula* Ehrenberg, *C. placentula* var. *lineata* (Ehrenberg) van Heurck, *C. placentula* var. *euglypta* (Ehrenberg) Grunow) and *Gomphonema* (*G. bourbonense* Reichardt & Lange-Bertalot, *G. pumilum* var. *rigidum* Reichardt & Lange-Bertalot, *G. pumilum* var. *pumilum* (Grunow) Reichardt & Lange-Bertalot, *G. minutum* Agardh; Luc Ector pers. comm., Prygiel et al. 2002). One study also pointed out the problem of overlooking small taxa, such as *Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot and *Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot (Luc Ector pers. comm.), which are classified as eutrophic and even pollution tolerant. Omitting these taxa will lead to a more oligotrophic classification of the studied stream.

Minimizing identification and counting problems also minimizes the error of the indices, giving a more reliable picture of the stream water quality (Prygiel et al. 2002). Therefore, intercomparison exercises are regularly performed, e.g. in France (Prygiel et al. 2002), and a study from UK showed that a high similarity between the diatom counts of a diatomist and an auditor gives a high probability that the difference of the index will be small (Kelly 2001).

Still, even if the differences between diatomists might be high, differences between samples from different streams are expected to be much greater (Luc Ector pers. comm.). The importance of among-site differences was also anticipated using the SE method and the Nordic–Baltic intercalibration exercise. Therefore, we tested the hypothesis that the Swedish method for assessing stream water quality with benthic diatoms using the indices IPS and ACID is reliable as long as differences between the analysing diatomists are minimized by intercalibration exercises and training.

Materials and methods

Organization of intercalibration exercise

The intercalibration exercise started with the distribution of readymade diatom slides to the 25 diatomists from the Nordic–Baltic countries during spring 2007. Each participant received a description of the method and a standardized taxa list. The lists were coded and collected by the Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, where the similarity with the auditor counts and the diatom indices were calculated. A final workshop was organized at the Norr Malma field station, Uppsala University, 12–15 November 2007, to discuss the results and harmonize our view on the identification of problematic diatom taxa.

Samples

Participating countries were invited to suggest national streams for the intercalibration exercise. The streams were selected to cover different ecological regions and water quality (Table 1). Epilithic samples were taken and permanent slides were prepared by the national diatomist following EU standards (CEN 2003, 2004). Because of time restrictions and somewhat suboptimal slide quality, samples 1 and 5 were made optional, whereas samples 2, 3, 4 and 6 were obligatory for participation.

Calculations

Similarity

Diatom counts were transformed as relative abundance of the total count. Comparisons between participants and auditor were made using Bray–Curtis (BC) similarity according to Kelly (2001). Bray–Curtis index can vary between 0 and 100%, where 100% would be the result of two samples with exactly the same number of valves counted for exactly the same taxa. Results >60% indicated replicate countings (Engelberg 1987; Kelly 2001) for all samples except for sample 5. Sample 5 had very low diversity (Hill's N2, Hill 1973; Kelly 2001), and the results were considered as replicates only if the Bray–Curtis similarity was >70%. Taxa lists were checked to determine which of the diatom taxa were contributing to the differences between the diatomists.

Auditor counts are also variable, but it has been shown that the variation between counts of the same sample are minor (1–5% of total variation, Prygiel et al. 2002; Lavoie et al. 2005). We are also aware that the auditor must be chosen with care, and that other diatom experts might have slightly deviating results than AJ. However, AJ was chosen as one of the diatomists with deepest insight into the taxonomy of Nordic diatom assemblages in streams. AJ was also involved in the development of the diatom method in Sweden and is currently preparing the new diatom flora of Swedish streams in cooperation with taxonomist BV. BV was consulted if questions occurred concerning diatom identification. Moreover, all participants knew about the intercalibration exercise conditions from the start and agreed on the use of AJ as the auditor (Table 2).

Table 1 Characteristics of the streams included in the intercalibration exercise

Sample Nr.	1		2		3		4		5		6	
Name	Bobr river		Martimojoki		Rökeån		Hammarbäcken		Lillån-Bosgård		Navesti	
x coordinate	152990		336026		136500		138290		133310		250254	
y coordinate	516190		734549		623325		688280		631840		583037	
Place	SW PL		N FI		S SE		central SE		S SE		EE	
	Min–max	Mean	Min–max	Mean	Min–max	Mean	Min–max	Mean	Min–max	Mean	Min–max	Mean
pH		6.9	5.6–7.8	6.6	5.5–6.9	6.5	5.3–7.3	6.5	4.4–5.8	5.1	7.6–8.4	8.1
Conductivity ($\mu s.cm^{-1}$)	250–270	257	10		70–140	100	12–46	24	40–60	50	290–580	480
Tot-P ($\mu g.L^{-1}$)	140–360	200	26		16–95	34	5–52	16	6–31	11	16–140	43
Tot-N ($\mu g.L^{-1}$)	2600–6500	4000	400		830–2100	1300	120–400	250	400–840	560	220–5000	2000
NH4-N ($\mu g.L^{-1}$)	50–750	430	2		13–260	95			21–240	64	2–170	31
Colour (mg $Pt.L^{-1}$)			140			ca. 200	25–250	130		ca. 150	30–200	90

The Achnanthes minutissima complex (AMIN)

One major problem came up during the calculation of the results: The modified SE method using diatoms as biomonitors simplifies the problem of the identification of the varieties of the *Achnanthes minutissima* complex (AMIN—main species including varieties following Tafel 32–34 in 'Süsswasserflora von Mitteleuropa Band 2/4, Krammer and Lange-Bertalot 1991), by using the mean width of 10–20 valves as substitute for the AMIN varieties. It has been shown that a mean width <2.2 μm is associated with highland oligotrophic streams (AMI1), 2.2–2.8 μm is associated with oligotrophic–mesotrophic streams (AMI2) and >2.8 μm is associated with eutrophic streams (AMI3; Amelie Jarlman pers. comm.). Both in sample 4 and 6, AMIN size was on the border between two groups (sample 4: AMI1/2, sample 6: AMI2/3), leading to different AMIN taxa groups for the diatomists even if the size of their measured AMIN did not necessarily differ very much. As AMIN was the dominating taxa in this study, this led to large differences in similarity. To ensure a fair comparison between the diatomists, we decided not to differentiate between AMI1 and AMI2 in sample 4 and AMI2 and AMI3 in sample 6. This was done by placing all counts of AMI2 into AMI1 in sample 4. AMI1 was chosen because both the auditor and most of the diatomists had encountered AMI1. For sample 6, all counts of AMI2 were transformed into AMI3 for the same reasons.

Calculations of the diatom indices IPS and ACID

The diatom indices IPS and the acidity groups used for ACID were calculated using OMNIDIA 4.2 (Lecointe et al. 1993, http://perso.club-internet.fr/clci/tour_guide.htm). Missing index values for some Nordic taxa were empirically derived, and in a few cases existing values were modified. Added and modified values are given in the appendix. To determine which diatom taxa were causing problems when calculating IPS and ACID, after removal of those diatomists with low similarity following Kelly (2001), the taxa lists were checked for those diatomists that deviated most from the auditor.

Statistics

To check for the reasons of the variability between the diatomists, a questionnaire was circulated, asking for factors that could be important for the outcome of a diatom analysis and count. The variables were the country in which the diatomist is active, experience (time) with diatom analyses, the teaching group a diatomist has been involved in (a group of diatomists who regularly exchange experience and harmonize their way of diatom identification), whether the SE standard literature was available to the diatomist while conducting the analysis, the availability of differential interference contrast (DIC) or phase contrast optics in the analysis, if the person participated in the test intercalibration 2006, the number of diatom courses done, the number of diatom samples counted per year, whether the samples are fossil or recent, and whether they mainly represent lakes or streams.

For the streams, the environmental variables nutrients (Tot-P, Tot-N), pH, conductivity and geographical region, divided into N–S and W–E, were used for characterization.

For statistical analysis, the raw data of the diatom counts, as percentage of the total count were arcsine squareroot transformed to ensure normal distribution, and a detrended

Table 2 Diatomist variables: country a diatomist was active, time of experience with diatom analyses, teaching group a diatomist was involved in, availability of the SE standard literature, availability of differential interference contrast (DIC) or phase contrast (PHA) optics, a person's participation in the test intercalibration 2006, number of diatom courses done, number of diatom samples counted per year, counted samples mainly recent (alternative: fossil), mainly streams (alternative: lakes)

Diatomist	Country	Years of experience	Teaching Group	SE literature	DIC	PHA	Intercal. 2006	Courses	Counted samples per year	Recent samples	Stream samples
Auditor	1	32	1	Yes	Yes	No	Yes	4	120	Yes	Yes
2	1	36	1	Yes	No	Yes	Yes	2	13	Yes	Yes
3	1	4	1	Yes	Yes	Yes	Yes	3	65	Yes	Yes
4	2	1	9	No	No	No	No	2	32	No	No
5	2	3	9	No	No	Yes	No	2	30	No	No
6	2	7	7	Yes	No	No	No	1	67	Yes	Yes
7	1	10	3	No	No	Yes	No	4	275	No	No
8	1	2	1	Yes	Yes	No	Yes	1	25	Yes	Yes
9	2	15	7	No	No	Yes	No	4	75	No	No
10	1	2	3	No	No	No	No	0	6	No	No
11	3	0	8	No	No	No	No	0	6	Yes	Yes
12	1	1	1	Yes	Yes	No	Yes	1	20	Yes	Yes
13	6	4	6	No	Yes	No	No	1	30	Yes	Yes
14	1	6	1	Yes	Yes	No	No	0	100	Yes	Yes
15	4	15	5	No	No	No	No	2	25	Yes	No
16	2	10	2	No	No	Yes	Yes	1	30	Yes	Yes
17	4	3,5	5	No	No	No	Yes	1	15	Yes	No
18	5	33	4	Yes	No	Yes	Yes	2	35	Yes	Yes
19	1	9	1	Yes	Yes	No	Yes	1	50	Yes	Yes
20	5	33	4	Yes	Yes	No	Yes	3	80	Yes	Yes
21	2	4	10	No	No	No	No	2	15	Yes	Yes
22	2	33	2	No	No	Yes	No	1	10	Yes	Yes
23	1	38	1	Yes	Yes	No	Yes	3	23	Yes	Yes
24	5	3	4	Yes	No	Yes	Yes	1	10	Yes	Yes
25	1	32	11	No	Yes	No	No	1	30	Yes	Yes

correspondence analysis (DCA) was performed to visually examine the distribution of the different individual streams and groups. The length of the first gradient was 6.15 SD indicating that a unimodal model should be used. One very inexperienced diatomist had overlooked the AMIN group completely, resulting in this diatomist's samples appearing as an outlier. The results from this diatomist were removed from further analyses. pCCA (partial canonical correspondence analysis) was used to determine the variance associated with diatomist-specific variables or the environmental variability associated with the streams. The shared variance was also calculated. A second pCCA was run to determine which variables could explain most of the variance between the diatomists. In this step, the environmental variables were run as covariables in a pCCA analysis, where all but one of the diatomist variables also were counted as covariables. As country and teaching group correlated very much as well as the use of SE standard literature and a DIC microscope and the counting of fossil samples coming from lakes, the variables country, DIC and lake were removed from the analysis. Monte Carlo permutation tests (999 unrestricted permutations) were used to assess the significance of the variables for determining the variance of the diatom counts.

A blocked multi-response permutation procedures (MRBP, variant of Multi-Response Permutation Procedures, MRPP) was used to test which one of the factors, stream versus operator, were related to differences in the data. Samples were a priori clustered into the six streams. The distance measure was the Euclidean distance. The method requires a balanced design, therefore the obligatory samples were used only, and operator 25 was deleted from the design. MRBP compares the variation within groups and among groups of samples. The group shows the samples from one site analysed by different diatomists. A-value ranges from −1 to +1. It is negative if the variation is larger within the group than among the groups. $A=0$ when the variation is equal within and among the groups and $A=1$ when the samples are identical within the groups.

Canoco for Windows 4.5 was used to calculate the DCA and pCCA and the Monte Carlo permutation tests. PC-ORD, 5.14 was used for MRBP.

Results

Results of the intercalibration exercise

According to the criteria proposed by Kelly (2001), ten out of the 24 participating diatomists have produced 'replicates' of the auditor's counts of all the obligatory samples 2, 3, 4 and 6 (Table 3).

IPS and ACID—impact of low similarity of diatom counts

The IPS and ACID index results of the auditor corresponded well to the water chemistry of the analysed streams when compared with the water chemistry typical for the index value derived from the SE validation of the SE standard (Naturvårdsverket 2007). IPS had, however, a wide error marginal when all operators were included (Fig. 1). The error did not decrease linearly with increasing similarity; even with Bray–Curtis similarities >60%, the threshold recommended by Kelly (2001) following Engelberg (1987), some IPS results deviated more than ±0.5 units in average—the level that is accepted in the SE standard method for samples with an IPS >13 (Naturvårdsverket 2007; Fig. 1). Obviously, a high similarity in only one sample does not necessarily guarantee similar diatom index results in other samples. On the other hand, a low BC similarity did not necessarily mean a high deviation of the IPS result from the auditor's, because BC does not give any credit for identifying 'almost' the right taxa with a similar ecology. However, the error decreased when only those diatomists who have a high similarity with the auditor according to Kelly (2001) in all samples are included (Fig. 1). In that case, the error for both IPS and ACID decreased to the level acceptable according to the SE standard (Naturvårdsverket 2007; Figs. 1 and 2), and 64% of all counts deviated less than the accepted threshold from the auditor.

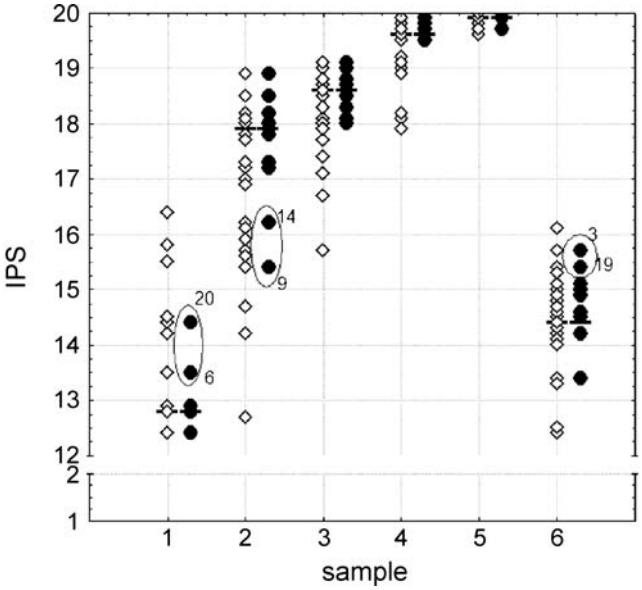

Fig. 1 IPS for samples 1–6 for all participants (*open diamond*), and for the participants who met the intercalibration criteria (Bray–Curtis similarity >60% for the obligatory samples 2, 3, 4 and 6) (*filled circle*); auditor (*dash*)

Table 3 Bray–Curtis similarity between intercalibration exercise participants and auditor (%). Hills N2 diversity of the samples is given in brackets. Optional samples (2, 3, 4, 6) in italics. [a]Participant near 60% BC

Diatomist	Sample (diversity Hills N2)						
	1 (10.5)	2 (4.3)	3 (5.7)	4 (5.1)	5 (2.9)	6 (6.3)	Similarity with auditor >60% BC in all obligatory samples?
2		68	75	72		77	Yes
3		73	77	86		81	Yes
4	*52*	14	61	64	*33*	60	No
5		19	31	81		52	No
6	*42*	71	62	70	*27*	66	Yes
7		32	26	69		69	No
8	*73*	72	76	85	*84*	79	Yes
9		69	68	68		72	Yes
10		29	26	78		68	No
11	*32*	11	21	12	*7*	31	No
12	*71*	74	72	82	*84*	70	Yes
13	*39*	11	56	51	*20*	69	No
14	*60*	72	74	83	*60*	70	Yes
15	*45*	62	66	56	*35*	62	No
16	*37*	73	60	65	*32*	66	No[a]
17	*46*	69	65	69	*63*	59	No[a]
18		74	62	81	*36*	68	Yes
19	*68*	76	76	87	*78*	84	Yes
20	*43*	68	60	66	*29*	69	No[a]
21	*30*	52	62	55		67	No
22		60	14	61		65	No
23		75	67	78		71	Yes
24		13	39	47		56	No
25		33		43		59	No

Multivariate analysis

In general, we found as expected that the variance depended more on the stream sampled than on the operator doing the analyses (after removal of the outlier, Fig. 3). The sum of the environmental variables from the streams explained significantly more (20%) of the total variance than the sum of the diatomist-specific variables (15%; pCCA, total inertia 10.1, sum of all canonical eigenvalues environment 2.02, Monte Carlo permutation test, $p<0.001$; sum of all canonical eigenvalues diatomist, 1.56, Monte Carlo permutation test, $p<0.001$). No variance was shared. Also, the MRBP showed that the variation between streams was larger than the variation within one stream among the operators (streams = one group, $A=0.26$, $p<0.0001$).

A certain group of experts had results very similar to those of the auditor; also, other diatomists were grouped together for the different samples. This indicated the

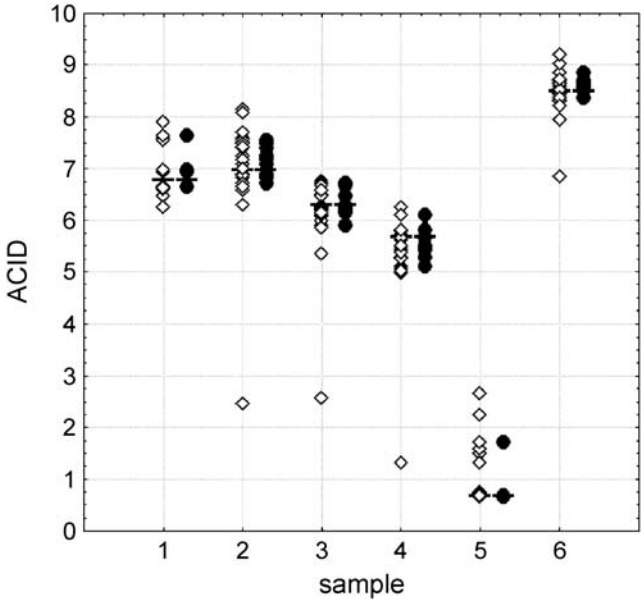

Fig. 2 ACID for samples 1–6 for all participants (*open diamond*), and for the participants who met the intercalibration criteria (Bray–Curtis similarity >60% for the obligatory samples 2, 3, 4 and 6) (*filled circle*); auditor (*dash*)

importance of some variables connected to those groups. The main factor steering the variance between the diatomists was the teaching group. This factor explained 10× more variance (9%) than any of the other factors, which all contributed about the same to the differences between diatomists (pCCA; Table 4). The fact that most diatomists were grouped near the ones trained by the same expert suggests that participation in harmonization exercises and discussions with colleagues is more important than the length of diatom expertise, the amount of diatom courses done or samples counted.

Identification problems

There were several reasons for the differences in similarity or index results between participants, including both identification problems but also incorrectly calibrated measurement scales.

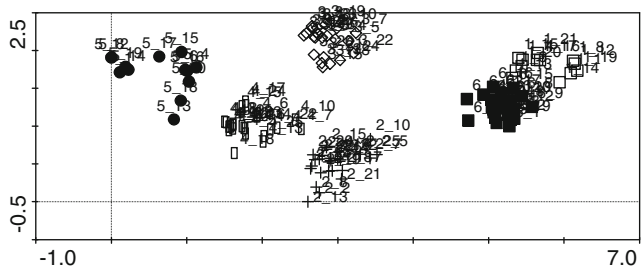

Fig. 3 DCA (data arcsine squareroot transformed, diatomist 11 removed as outlier)

Comparing diatom taxa lists and the grouping of the diatomists in the cluster analysis, it became clear that the largest source of variance was the taxa complex *Achnanthes minutissima* (AMIN). This taxa group was dominating in the samples 2, 3, 4 and 6 and differences in AMIN results led to high differences in similarity. The three AMIN groups have also somewhat differing IPS sensibility and indicator values in the SE method (AMI1 sensitivity value (S) 5, indicator value (I) 2, AMI2 5/1, AMI3 4/1), which can lead to differences of the IPS. For example, the fact that diatomists 7 and 10 counted AMI3 in sample 4 led to a grouping near sample 6, the same happened to diatomists 5, 7, 10 and 25 in sample 2. At least for diatomists 7 and 10 it was later shown that the measuring scale was incorrectly calibrated. The same may have been true for others. Incorrect calibration will also probably affect the identification of other species.

In sample 3, an additional problem was noted. The sample contained *Achnanthes abundans* Manguin (AABU; cited as *Psammothidium abundans* (Manguin) Bukhtiyarova et Round), a diatom that is not included in Krammer and Lange-Bertalot (1991a) and therefore probably often overlooked, even if it is quite common in the Nordic region

Table 4 Effect of diatomist variables on the variance of the intercalibration results (pCCA, Monte Carlo permutation test (999 unrestricted permutations)

Source of variance	Explained variance	(%)	Significance *p*
Total inertia	10.1		
Teaching group a diatomist was involved in	0.863	8.6	0.001
Time of experience with diatom analyses	0.082	0.8	0.006
Availability of differential interference contrast (DIC)	0.079	0.8	0.035
A person's participation in the test intercalibration 2006	0.081	0.8	0.022
Number of diatom samples counted per year	0.094	0.9	0.003
Counted samples mainly recent	0.116	1.1	0.002
Non-significant factors			
Availability of phase contrast (PHA) optics			>0.05
Number of diatom courses done			>0.05
Factors not considered in analyses because of high correlation with another factor	Correlated with		
Country a diatomist was active	Teaching group		
Availability of the se standard literature	Availability of differential interference contrast (DIC)		
Counted samples mainly streams	Counted samples mainly recent		

(Van de Vijver et al. 2008). If AABU is mistaken for AMIN, the larger valves of AABU will result in a too-high mean valve width for AMIN. This is a problem as a high AMIN mean width is associated with eutrophic waters, whereas AABU is present in oligotrophic to mesotrophic waters. As AABU was not very frequent in sample 3, the problem was of minor importance not affecting the AMIN groups. However, the trend towards a higher AMIN thickness was noted and significant (Fig. 4; r^2=0.3318, p=0.0246), and might be a problem when AABU is present in a sample in higher abundance.

The next major problem was the occurrence of a *Fragilaria* species subdominating (~20% of relative abundance) in the samples 2 and 4. Most of the participants and the auditor identified this species as *Fragilaria capucina* var. *gracilis* (Østrup) Hustedt (FCGR), but some of the participants identified this taxon as *F. capucina* var. *rumpens* Kützing (FCRU), as Krammer and Lange-Bertalot (1991b), a reference often used as standard identification literature, is ambiguous on how to separate these two taxa. Most of the valve forms and sizes resemble very much FCGR as shown in Krammer and Lange-Bertalot (1991b). However, Krammer and Lange-Bertalot (1991b) state that FCRU is usually broader than FCGR ('around 4 μm'), but that there are smaller forms that 'leiten so zu den *gracilis* Sippen über' (are leading to FCGR). No clear differentiation is given. According to Tuji & Williams (2006) and Tuji (2007), who examined the type material, FCGR is 2–3 μm wide and FCRU 3–4 μm wide. Further studies seem to be necessary to ensure the separation of these two taxa. This separation is important for biomonitoring as an identification of FCGR as FCRU gives lower IPS results because FCRU occurs in somewhat more nutrient rich waters (IPS S/I values 4/1) than FCGR (4.8/1).

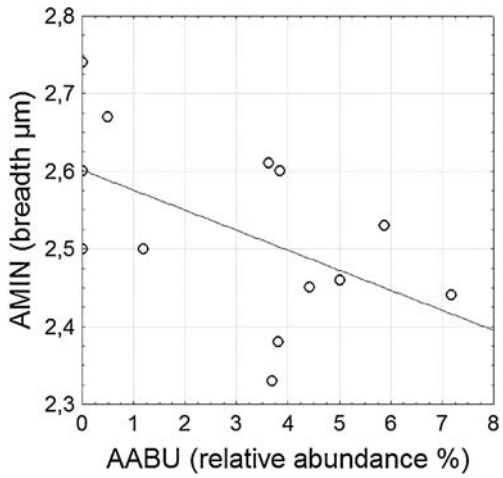

Fig. 4 Low counts (relative abundance %) of *Achnanthes abundans* Manguin (*AABU*) leads to erroneously high average breadth for the *Achanthes minutissima* group (*AMIN*; $AMIN_{width}=2.6013-0.0258\times AABU_{(\%)}$, r^2=0.3318, p=0.0246)

In sample 5, the major problem was that the dominating species *Eunotia rhomboidea* Hustedt (ERHO) was fully overlooked or underestimated in several counts because of a misidentification with *Eunotia incisa* Gregory (EINC), which was also present in the sample. As both species have the same IPS and ACID S/I values, this misidentification did not cause differences in the index results, but it separated diatomists in the multivariate analyses. The separation of these two species might also be important because ERHO seems to be less sensitive to anthropogenic acidification than EINC (Coring 1996).

A minor problem in samples 2 and 3, but certainly important in other samples of the Nordic–Baltic region, was the differentiation between *Gomphonema parvulum* Kützing (GPAR) and *G. exilissimum* (Grunow) Lange-Bertalot & Reichardt (GEXL). The literature often routinely used for identification (Krammer and Lange-Bertalot 1986, 1991a) is not quite clear about the separation, but the IPS S/I values are so different (GPAR 2/1, GEXL 5/1) that a differentiation is necessary. An analysis of the diatom lists showed that this problem was handled quite inconsistently by the different diatomists.

Finally, after removing the diatomists with low similarity with the auditor's results from the analysis, some discrepancy still remained, as indicated by the variance in the IPS results (Fig. 1).

The deviating IPS results for sample 1 for the diatomists 6 and 20 were due to the fact that they completely missed all small *Navicula* s.l. taxa, resulting in a too-high IPS result, because the small *Navicula* s.l. taxa present belonged almost all to taxa which are often occurring in streams impacted by eutrophication or organic pollution (IPS S/I values: *N. seminulum* Grunow NSEM: 1,5/2, *N. minima* Grunow NMIN: 3/1, *N. subminuscula* Manguin NSBM: 2/1, *N. atomus* var. *alcimonica* Reichardt NAAL: 4/1, *N. atomus* var. *permitis* (Hustedt) Lange-Bertalot NAPE: 2,3/1, *N. saprophila* Lange-Bertalot & Bonik NSAP: 2/1). Also other participants counted too few *Navicula* s.l. valves which had ~10% relative abundance in the obligatory sample 6 and ~20% in the optional sample 1. Overlooking small *Navicula* s.l. can be a severe problem when classifying the water quality of streams, as some of these taxa tend to be abundant in strongly impacted streams. Overlooking small *Navicula* s.l. may lead to erroneous classification.

The deviating results in sample 2 for the diatomists 9 and 14 resulted from the complicated distinction of *Nitzschia palea* var. *palea* (Kützing) W. Smith (NPAL) from *N. palea* var. *debilis* (Kützing) Grunow (NPAD). The SE standard has empirically changed the IPS S/I values for NPAD (from 1/3 to 3/1), as this taxa occurs quite frequently in less affected streams in SE. However, this can lead to too low index

results when NPAD is identified as NPAL. The somewhat deviating IPS results for sample 6 for diatomists 3 and 19 also partly depend on the NPAD/NPAL problem. The literature does not give a clear separation between NPAL and NPAD. NPAD is said to have narrower valves, but there is a consistent overlap in width of both taxa (Krammer and Lange-Bertalot 1997; Trobajo and Cox 2004).

Discussion

The intercalibration exercise gave credence to the use of the Swedish method for assessing stream water quality using benthic diatoms. The diatom indices IPS and ACID reflected the water quality well, despite differences between diatomists. These findings agree with an earlier study in France (Luc Ector pers. comm.). However, even if the taxonomic composition of the different diatomists were clearly separated between samples, still high differences between diatomists were found within each sample. Here, it should be kept in mind that the use of only one auditor in this Nordic–Baltic intercalibration exercise maybe led to a lower similarity of the auditor's and some of the participants' results. On the other hand, the participants were aware of the fact that the intercalibration slides had to be counted with care, thereby probably increasing the quality of the counts in comparison with a 'normal' slide. However, besides the fact that the calculation of the similarity done in this study might have had some drawbacks, we could show that the use of the threshold similarity ≤60% BC with the auditor was useful to minimize the index error for each stream to about the level accepted by the SE standard (±0.5 units for IPS, ±10% for ACID (Naturvårdsverket 2007)). We could also show that a high Bray–Curtis similarity (BC) between participant and the auditor in one sample did not always guarantee similar index results. The error could still be higher than accepted in the SE standard. The error was only within the SE standard when diatomists with BC values ≤60% in all of the obligatory samples were excluded from the analysis, as suggested by Kelly (2001), albeit with some exceptions. These exceptions depended mostly on overlooking small *Navicula* s.l. and the problem of distinguishing between NPAD and NPAL. Hence, care should be taken when dealing with these taxa.

Care should also be taken when comparing diatom taxa lists of people trained from different teaching groups. The intercalibration exercise verified the assumption that it is not enough to have a long experience with diatoms or to count many samples per year to get similar results as the auditor. The main point driving variable, ensuring low error was the discussion of taxonomy and methods. This was demonstrated by the fact that the main variable steering similarity between diatomists was their participation in different teaching groups.

Differences in diatom index values between participants were due to different reasons, and each diatomist can do a number of things to ensure the quality of his or her diatom counts. First, correct calibration of the measuring scales is crucial. The next step is to ensure that the standard methods are adhered to (in this case the SE standard), such as use of the proper literature and a high-quality microscope, preferably with DIC or at least with phase contrast. Frequent diatom analysis, i.e. counting many samples per year, will improve the quality of diatom counts as this will help to keep the taxa in memory. One lesson learned from this intercalibration exercise was that the mean size of AMIN should always be reported, not only the group (e.g. AMI1, 2, 3). In this way samples with an AMIN group near a size limit can easily be detected. However, a diatomist should also check a sample including AMIN very careful for other similar *Achnanthes* species to ensure that other species are not inferring with the size measurements. Finally meetings, training, agreement and harmonization can lead to more comparable diatom lists and indices, as the main problem still are misidentifications either due to missing knowledge, or due to the fact that the diatom literature sometimes is not clear on how to separate similar species or varieties.

The problem of small *Achnanthes* species resembling AMIN was already noted in France, and also problems when identifying certain *Gomphonema* taxa (Luc Ector pers. comm., Prygiel et al. 2002). However, the problematic taxa were different from those in the Nordic–Baltic region, for example AMIN was confused with *Achnanthes catenata* Bily & Marvan (cited as *Achnanthidium catenatum* (Bily & Marvan) Lange-Bertalot; Luc Ector pers. comm.) or *A. biasolettiana* Grunow (Prygiel et al. 2002). This confirms the importance of regional intercalibration exercises to ensure that diatomist's increase their knowledge of the most problematic taxa in their region. However, some problems seem to be common, and one is the problem with the taxa complex *Cocconeis placentula* var. *placentula, Cocconeis placentula* var. *euglypta and Cocconeis placentula* var. *lineata* (Luc Ector pers. comm., Prygiel et al. 2002). This was resolved in the SE standard by grouping all varieties together, as they are mostly found under the same ecological conditions. Another problem found for example in France (Luc Ector pers. comm.) is that some diatomists miss the occurrence of small *Navicula* s.l. taxa, a problem that is more likely to occur when a high-quality DIC microscope is not available.

To ensure the comparability and quality of diatom analyses in the Nordic–Baltic region, we discussed and suggested solutions for the main problems of diatom identification in the intercalibration exercise during a workshop at the Erken Laboratory, Uppsala University, Sweden. Results of this workshop are given at the NORBAF homepage http://www.norbaf.net.

Conclusions

In conclusion, we could show that the results of different experts counting diatoms from the Nordic–Baltic region are similar when diatomists are harmonized, i.e. are using the same method, the same literature and most of all, are exchanging information about diatom identification problems. This harmonization was shown to be more important than a long experience with diatoms. With some exceptions, a high similarity also means a high comparability of calculated diatom indices, shown by the successful test of the Swedish method using diatoms as bioindicators in streams with the indices IPS and ACID. As in this case, an experienced diatomist must be chosen as auditor to achieve reliable results. Sources of error were erroneous calibration of measuring scales, overlooking small taxa (especially small *Navicula* s.l.), misidentifications (*Eunotia rhomboidea* was mistaken for *E. incisa*) and unclear separation between some similar species/varieties in the identification literature. Regarding the last point, we aim to try to use the suggested test criteria presented at http://www.norbaf.net with focus on the *Achnanthes minutissima* group, the separation of *Fragilaria capucina* var. *gracile* from *F. capucina* var. *rumpens*, and *Nitzschia palea* var. *palea* from *N. palea* var. *debilis* to improve the comparability of counting results in the Nordic–Baltic region.

Acknowledgements The course was partly funded by Nordplus Neighbour, a program of the Nordic Council of Ministers. We thank the Swedish Environmental Protection Agency for funding of part of the auditor's work. Microscopes were kindly provided by Olympus (Olympus Sverige AB) and Nikon (Tekno Optik AB Skärholmen). Some literature was kindly provided by Koeltz Scientific Books and E. Schweizerbart'sche Verlagsbuchhandlung. The Estonian river sampling and the participation of the Estonian diatomists was funded by Estonian target funding project SF 0170011508, and by grant 6580 from Estonian Science Foundation. We thank also Martyn Kelly for the constructive comments on the manuscript.

Appendix

Added* and changed** indicator values in OMNIDIA 4.2 according to the SE standard (valid September 2008. List is regularly updated)

		IPS sensibility value	IPS indicator value	acidity value
	code	S	I	
Achnanthes abundans	AABU	5*	1*	3*
Achnanthes altaica	AALT	5*	2*	
Achnanthes curtissima	ACUR			3*
Achnanthes didyma	ADID	5*	1*	3*
Achnanthes kriegeri	AKRG			3*
Achnanthes lanceolata ssp. dubia	ALDU			4*
Achnanthes lanceolata ssp. frequentissima var. rostratiformis	ALFF			4*
Achnanthes microscopica	AMCP			3*
Achnanthes minutissima group I (<2,2 μm)	AMI1	5*	2*	3*
Achnanthes minutissima group II (2,2-2,8 μm)	AMI2	5	1	3
Achnanthes minutissima group III (>2,8 μm)	AMI3	4*	1*	3*
Achnanthes nodosa	ANOD	5*	2*	3*
Achnanthes saccula	ASCL			3*
Achnanthes scotica	ASCT	5*	1*	2*
Amphipleura kriegeriana	AKRI	5*	3*	
Amphipleura pellucida	APEL	4**	1**	
Aulacoseira muzzanensis	AMUZ			4*
Aulacoseira "pseudodistans"	AUPD			3*
Aulacoseira valida	AUVA			2*
Brachysira procera	BPRO			2*
Brachysira zellensis	BZEL			3*
Cyclotella rossii	CROS			3*
Cymbella excisiformis	CEXF	5*	1*	4*
Cymbella laevis	CLAE			3*
Cymbella lange-bertalotii	CLBE			4*
Cymbella perparva	CPPV			4*
Cymbella subhelvetica	CSBH			4*
Encyonema lange-bertalotii	ENLB	4**	1**	3*
Encyonema minutiforme	ENMF	5*	1*	
Encyonema pergracile	EPRG			2*
Encyonema reichardtii	ENRE			3*
Encyonema simile	ENSI	5*	2*	3*
Encyonema ventricosum	ENVE			3*
Encyonopsis cesatii var. geitleri	ECGE	5*	2*	3*
Encyonopsis krammeri	ECKR	5*	2*	
Encyonopsis minuta	ECPM	4**	2**	4*
Encyonopsis perborealis	ECPB	5*	3*	
Eucocconeis alpestris	EUAL	5*	3*	
Eunotia bilunaris	EBIL			2**
Eunotia boreotenuis	EBOR			2*
Eunotia chelonia	ECHE			2*
Eunotia circumborealis	ECIR			2*
Eunotia curtagrunowii	ECTG			2*
Eunotia eurycephaloides	EECP			2*
Eunotia exsecta	EEXS			2*
Eunotia formica	EFOR		1**	
Eunotia genuflexa	EGEN			2*
Eunotia hexaglyphis	EHEX			2*
Eunotia iatriensis	EIAT			2*
Eunotia inflata	EINF			2*
Eunotia muscicola	EMUS			2*
Eunotia muscicola var. perminuta	EMPE			2*
Eunotia muscicola var. tridentula	EMTR			2*
Eunotia pectinalis var. ventralis	EPVE			2*

Eunotia pseudoparallelloides	EPDP	5*	1*	2*
Eunotia rhomboidea	ERHO	5**	1**	
Eunotia seminulum	ESEM	5*	1*	2*
Eunotia steineckii	ESTK			2*
Eunotia sp.	EUNS			2*
Fragilaria nanoides	FNNO			3*
Fragilaria oldenburgioides	FODD			3*
Fragilaria opacolineata	FOPA			3*
Fragilaria pinnata var. intercedens	FPII	4*	1*	4*
Fragilaria pseudoconstruens	FPCO			3*
Frustulia erifuga	FERI	5**	2**	
Frustulia krammeri	FKRA	5*	2*	2*
Frustulia quadrisinuata	FQDS	5*	2*	2*
Gomphonema angustatum	GANG			3*
Gomphonema coronatum	GCOR	5**		3*
Gomphonema cymbelliclinum	GCBC			4*
Gomphonema pseudobohemicum	GPBO	5*	1*	2*
Gomphonema pumilum group	GPUM	4.5**		4*
Hippodonta coxiae	HCOX			4*
Melosira distans var. tenella	MDTE			2*
Navicula arctotenelloides	NATT	5*	1*	
Navicula germainii	NGER			4*
Navicula maceria	NMCE	5*	1*	2**
Navicula minima	NMIN	2.2**		
Navicula schmassmanni	NSMM			3*
Navicula subalpina	NSBN	4.5*	1*	4*
Navicula subhamulata	NSBH	4**	1**	
Navicula suchlandtii	NSUC		1**	3*
Navicula upsaliensis	NUSA	4*		4*
Naviculadicta sp. (Icon 2. 27:17-18, 28:6-9, 28:21-23)	NVDI**	5*	1*	
Naviculadicta litos	NLTO**	5*	1*	
Nitzschia agnita	NAGN			4*
Nitzschia alpina	NZAL			3*
Nitzschia bavarica	NBAV			3*
Nitzschia palea var. debilis	NPAD	3**	1**	
Nitzschia parvula	NPAR			4*
Peronia fibula	PFIB			2*
Pinnularia ivaloensis	PIVA	5*	2*	2*
Pinnularia perirrorata	PPRI	5*	2*	2*
Pinnularia silvatica	PSIL			2*
Pinnularia sinistra	PSIN			2*

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