FIRST NORDIC-BALTIC DIATOM INTERCALIBRATION EXERCISE 2007 (STREAM MONITORING)

Results of workshop at the Erken Laboratory, Uppsala University, Sweden, 11.-16.11.2007.

Names of authors:

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

Under 2006, the first Nordic-Baltic diatom intercalibration exercise with 25 participants from six countries (Sweden, Finland, Estonia, Lithuania, Latvia and Poland) took place. The results from this intercalibration will be published in Journal of Applied Phycology soon. Epilithic diatoms from six streams with different physico-chemical characteristics were counted and analysed following the European standard EN 14407 and the software OMNIDIA (Lecointe et al. 1993, http://perso.club-internet.fr/clci/tour_guide.htm) with some Swedish modifications (Naturvårdsverket 2007). 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. The goal of the intercalibration 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 showed that a high similarity between auditor and participant 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 *E. incisa*) and unclear separation between certain taxa in the identification literature. The latter was discussed during a workshop with focus on the *Achnanthes minutissima* 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.

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, 11.-16.11.2006. Results of this workshop are given here.

Suggestions to solve identification problems

Difference between Fragilaria capucina var. gracilis (Oestrup) Hustedt and F. capucina var. rumpens (Kützing) Lange-Bertalot

About 20 % of the diatoms in samples 2 and 4 consisted of a *Fragilaria* species which was identified as *Fragilaria capucina* var. *gracilis* (Oestrup) Hustedt (FCGR) by the auditor and most of the participants. However, some of the participants identified this taxon as *F. capucina* var. *rumpens* (Kützing) Lange-Bertalot (FCRU) and the question arose as to how to separate these two taxa, especially as the auditor also noted some few FCRU in sample 4.

In sample 2, most of these *Fragilaria* valves were around 23-29 µm long, and 1.7-2.3 µm broad, with about 20-23 striae/10 µm (Fig. 1), although single individuals were even shorter. All of the valves had more or less parallel sides, most of them had heads, and the striae were nearly perfectly parallel. These forms and sizes resemble very much FCGR, with a tendency to heads as in (Krammer and Lange-Bertalot, 1991b), Fig. 110:10, 111:1, 113:22, 24, 25, and Lange-Bertalot 1996, Fig. 7:9-12. The fact that the breadth often is under 2 µm does not disagree with lower limit given in Krammer and Lange-Bertalot (1991b), as these authors only state that the valves should be 'about' 2-3µm broad. In the oligotrophic Nordic water systems diatom valves might become somewhat thinner than in Central Europe.

Other species do not fit the taxa of sample 2, they are either broader (*F. capucina* var. *capucina* and *F. capucina* var. *vaucheriae)* or longer (*F. nanana, F. nanoides, F. tenera,* and *F. delicatissima)* (see table 1). Furthermore, the valve form and the amount and characteristics of the striae are often different in these taxa.

The closest diatom taxa would be *F. capucina* var. *rumpens* (FCRU)*,* but this variety should have valves broader than FCGR. According to (Krammer and Lange-Bertalot, 1991b) the valve breadth is 'about' 4 µm. According to Tuji and Williams (2006), the type material of FCRU ('Synedra rumpens') had valve breadths of 3-4 µm. That means that narrower forms 'leiten so zu den *gracilis* Sippen über' (are leading to FCGR, (Krammer and Lange-Bertalot, 1991b), which makes differentiation difficult. As FCGR is more typical for clean water streams than FCRU, which is also reflected in their IPS S/I values (4.8/1 resp. 4/1), it is important to try a separation. We agreed to try to use a valve breadth of 3 µm as a limit: ≤ 3 µm was classified as FCGR, > 3 was classified as *F. capucina* var. *rumpens.*

Using this definition, it was easier to find an acceptable name for the *Fragilaria* taxa in sample 4, which was not as homogenous as the one in sample 2 (Fig. 2). Here, we found typical FCGR mixed with a similar form as in sample 2, additionally there was a larger amount of short valves. All of the valves had about 20-23 striae/10 µm. The long valves and the short valves narrower than 3 µm were identified as FCGR, whereas some single short valves broader than 3 µm were identified as FCRU, resembling Krammer and Lange-Bertalot (1991b)) Fig. 108:21, and 110: 4, 5.

Table 1. Valve size and features from Krammer and Lange-Bertalot (1991b) and Lange-Bertalot and Metzeltin (1996). [+] from Tuji and Williams (2006). Additionally features suggested on the Nordic-Baltic intercalibration workshop marked with *, suggested later for the SE standard method marked with **.

FRAGILARIA	Length [µm]	Breadth [µm]	Striae/10µm	characteristics
F.capucina	< 10 - > 100	2-6.5	9-22	
F.capucina var.*capucina*		3.5-4.5	12-17	
F.capucina var.*gracilis*		ca. 2-3, in SE down to 1.7*	ca. 20	striae ± parallel sides ± parallel
F.capucina var. *rumpens*		> 3* (ca. 4 and less) (3-4) [+]	18-20	striae ± alternate
F.capucina var.*vaucheriae*		(3.5) 4-5	9-14	
*F.capucina 'group 1'**		3-3.5*	9-15*	thinner than *vaucheriae* similar to var. *austriaca/amphicephala*
*F.capucina 'group 2'**		3-3.5*	15-18*	thinner than *vaucheriae* between 'group 1' and 'var. *rumpens'*
*F.capucina 'group 3'**		ca. 2-3**	9-15**	thinner than 'group 1'
F.nanana	40-90	1.5-2	22-25	needle shaped, L/B extremely high, no heads, striae very delicate
F.nanoides	40-90	1.8-2.4	22.5-23	spindle shaped, L/B extremely high, heads, striae more gross than in *F.nanana*
F.delicatissima	30-100	2.5-3	14-16	spindle shaped, heads
F.tenera	30-100 typical: 70-100	2-3	17-20	needle shaped, heads, striae alternate

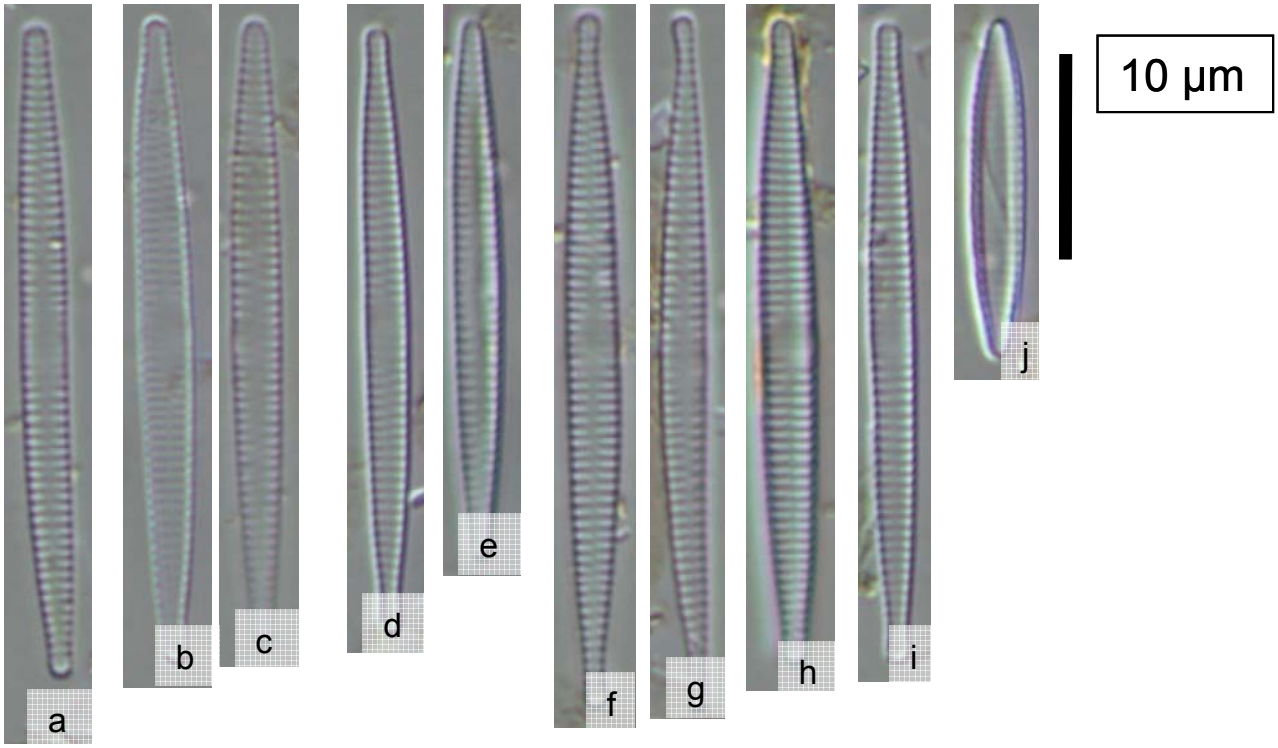

Fig. 1. *Fragilaria capucina* var. *gracilis* (Oestrup) Hustedt (a-j) (sample 2, Martimojoki, FI)

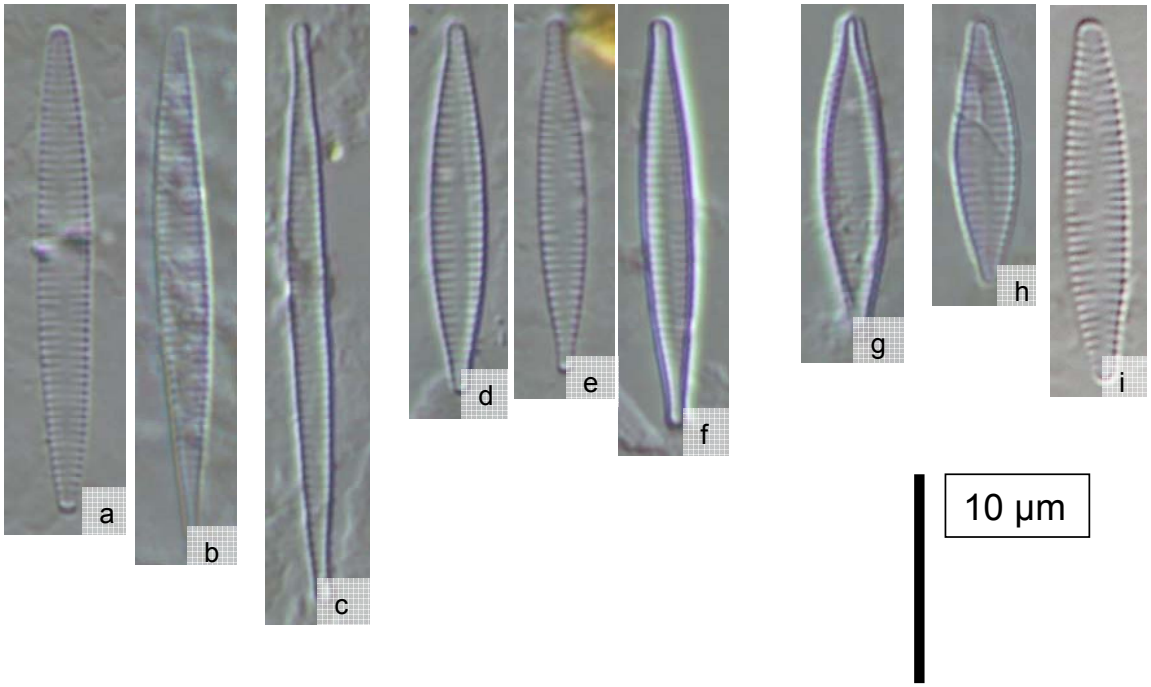

Fig. 2. *Fragilaria capucina* var. *gracilis* (Oestrup) Hustedt (a-f), *F. capucina* cf. var. *rumpens* (g-i) (sample 4, Hammarbäcken, SE)

Difference between Nitzschia palea var. *palea (Kützing) W.Smith, N. palea* var. *debilis (Kütz.) Grunow (NPAD) and N. gracilis Hantzsch*

About 2-5 % of the diatoms in sample 2 belonged to *Nitzschia palea*. The auditor and six of the participants identified it as *N. palea* var. *debilis* (Kütz.) Grunow (NPAD), whereas several of the other participants identified it as *N. palea* (Kützing) W.Smith var. *palea* (NPAL) or *N. gracilis* Hantzsch (NIGR), and one participant classified the taxon as *N. palea* (Kützing) W.Smith var. *tenuirostris* (NPAT). Three participants used the name *N. paleacea* Grunow (NPAE). The separation of NPAE from the others is however quite simple, as there is a wide space between the two middle fibulae of NPAE, whereas this is not the case for *N. palea* complex and NIGR.

Most of the valves of the *N. palea* variety in question were 20-33 µm long, 2.7-3 µm broad, had about 13-17 striae/10 µm, a length/breadth ratio ranging from 7.5-11.4 and the two middle fibulae were equally spaced. However, the issue became more complicated as the auditor also found NIGR in the sample in small amounts.

Following the text in Krammer and Lange-Bertalot (1997), *N. palea* length should be 15-70 µm, breadth 2.5-5 µm, and 9-17 striae/10 µm. NPAD is described as more narrow with denser striae as the nominate form, no exact limits are given. The characteristics of NPAT are described as a mixture of NPAD and *N. gracilis.* Seen from an ecological point of view, it would be necessary to separate NPAL and NPAD, as the former occurs in α-mesosaprobic to polysaprobic water, tolerating even raw sewage, whereas the NPAD variety usually occurs in electrolyte poor, oligotrophic waters (Krammer and Lange-Bertalot, 1997). The SE method therefore uses different IPS S/I values for the forms NPAL (1/3) and NPAD (3/1), which makes a clear separation of the two varieties even more important.

The figures in Krammer and Lange-Bertalot (1997) show an overlap of the breadth of the two *N. palea* forms: NPAL starts at 3 µm, whereas NPAD is smaller than 3.5 µm. For practical purposes, we suggested the limit of 3.5 µm as separation between the two varieties.
To separate NPAD from NIGR, which can also be difficult as both have similar microstructure (Krammer and Lange-Bertalot, 1997), we agreed to try to use a limit of 15 for the length to breadth ratio, with the long valves belonging to NIGR (Fig. 3). Valves with a length > 70 µm are always assigned to NIGR (Table 2).

For practical reasons, we propose not to use NPAT as this variety can morphologically not be distinguished from NPAD and NIGR according to Krammer and Lange-Bertalot (1997).

Table 2. Valve size and features from Krammer and Lange-Bertalot (1997) (taken from text[t] & figures[f]). Additionally features suggested on the Nordic-Baltic intercalibration workshop marked with *.

NITZSCHIA	Length [µm]	Breadth [µm]	Length/ breadth ratio	Fibulae/ 10µm	Striae/ 10µm	Ecology
N. palea	15-70[t]	2.5-5[t]		9-17[t]	28-40[t]	
N. palea var. *palea NPAL*		≥ 3.5* (≥ 3[f])	≤ 10[f]			α-mesosaprobic to polysaprobic
N. palea var. *debilis NPAD*		< 3.5[f]	≤ 15* (7-14[f])	14-17[f]	≥ 37[f]	oligotrophic
N. gracilis NIGR	30-110[t] if > 70*: = NIGR ≠ *N. palea*	2.5-4	> 15f*	12-18[t]	38-42[t]	oligotrophic
N. palea var. *tenuirostris*	*Habitus overlap of NPAD and NIGR*					*unclear*

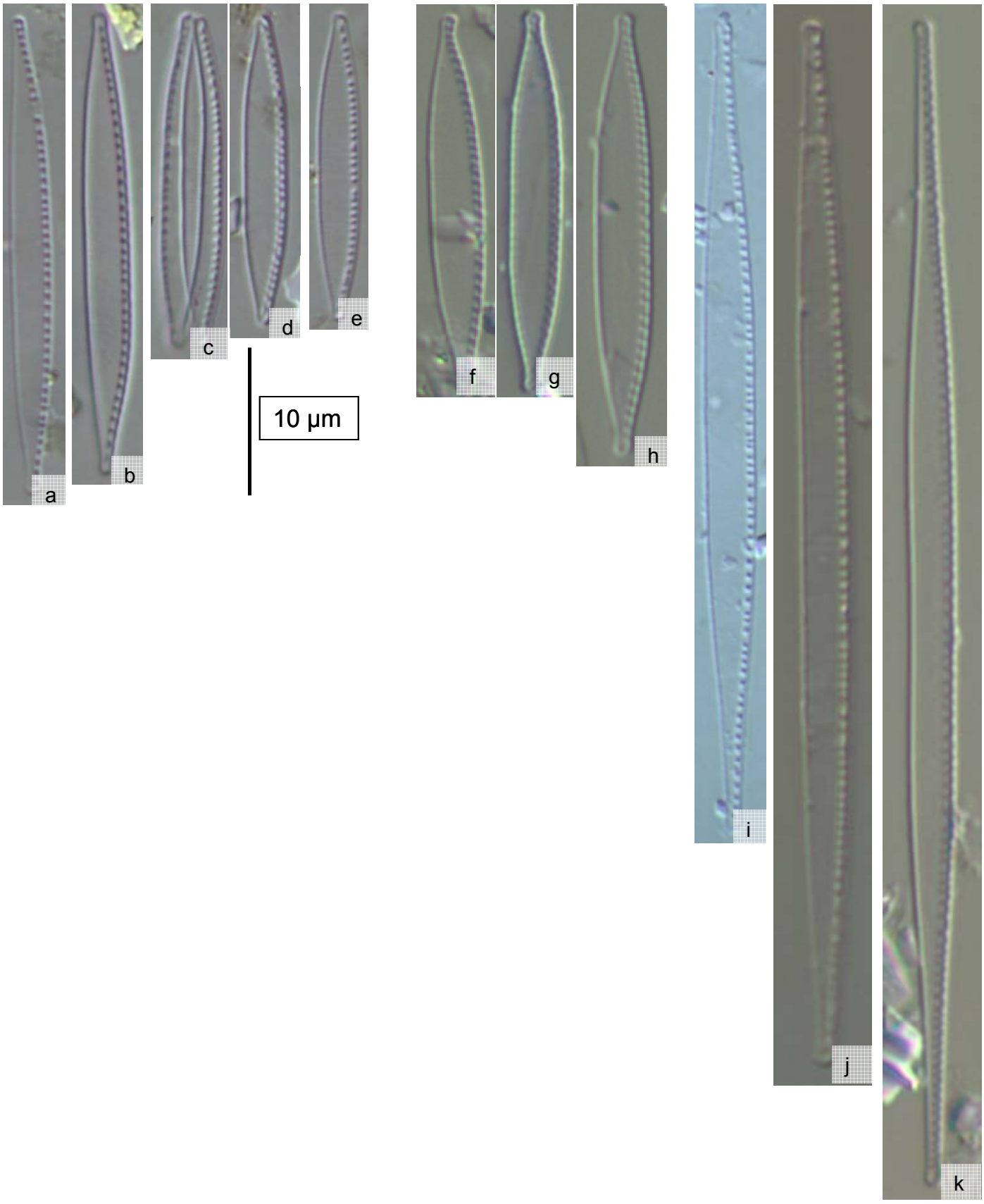

Fig. 3. *Nitzschia palea* var. *debilis* (Kütz.) Grunow (a-e) (sample 2, Martimojoki, FI). For comparison: *Nitzschia palea* (Kützing) W.Smith var. *palea* (f-h) (f,g: Getåbäcken, SE, h: Stångån Nykvarn, SE), *Nitzschia gracilis* Hantzsch (i-k) (i: Morabäcken, SE, j,k: Ringsån, SE)

Small Navicula s.l. taxa

Small species of *Navicula* s.l. were not counted correctly by all participants, which in turn partly explained the differences of the intercalibration. Obviously, these small species were overlooked by some of the participants. This is a severe problem when classifying the water quality of streams, as these small *Navicula* s.l. tend to be abundant in strongly impacted streams, and therefore overlooking them may lead to erroneous classification.

Both samples 1 and 6 contained such small *Navicula* s.l. taxa, the obligatory sample 6 at least 10 % of total valves and sample 1 more than 20 %. Some of the typical taxa for an impacted stream are shown in figure 4 (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. The problem of overlooking small taxa is enhanced when a sample slide is prepared with a high density of diatom valves, as had been done for sample 1, which is why we excluded this sample from the obligatory list. Regarding the obligatory sample 6, eight participants out of 24 counted no NMIN at all, whereas the auditor noted 10 % NMIN in this sample. The participants without NMIN had usually only large *Navicula* taxa in their species lists. Despite the relative dense diatom slide of sample 1, four participants out of 13 counted about the same amount of small *Navicula* s.l. taxa as the auditor, but again, the other participants had very low numbers, and three participants missed small *Navicula* s.l. taxa completely.

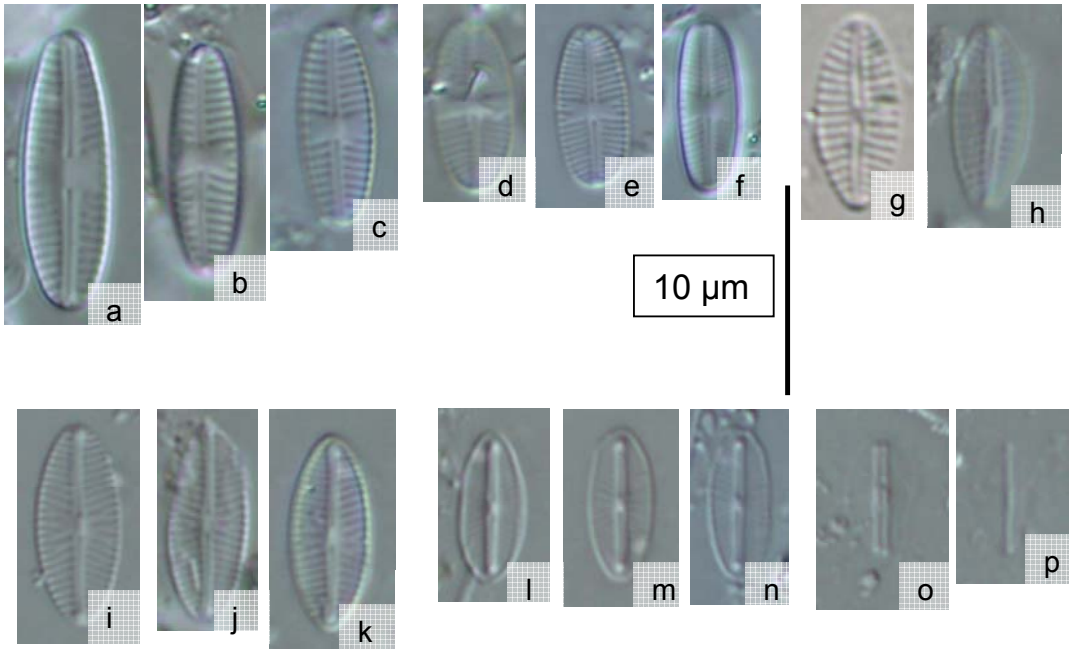

Fig. 4. Small taxa of *Navicula* s.l..: *N. seminulum* Grunow (a-c), *N. minima* Grunow (d-f), *N. subminuscula* Manguin (g,h), *N. atomus* var.*alcimonica* Reichardt (i-k), *N. atomus* var.*permitis* (Hustedt) Lange-Bertalot (l-n), *N. saprophila* Lange-Bertalot & Bonik (o, p) (sample 1, Bobr River, PL).

Difference between Gomphonema parvulum Kützing and G. exilissimum (Grunow) Lange-Bertalot & Reichardt

About 5 % of the diatoms in samples 2 and 3 consisted of two species in the *Gomphonema parvulum* group: *Gomphonema parvulum* Kützing (GPAR) and *G. exilissimum* (Grunow) Lange-Bertalot & Reichardt (GEXL). It is important to separate these two species, as they indicate very different ecological conditions, which is also expressed in their IPS S/I values (GPAR: 2/1, GEXL: 5/1). GPAR is typical for mesotrophic to polytrophic, usually mesosaprobic water whereas GEXL is typical for oligotrophic and oligosaprobic water (Krammer and Lange-Bertalot, 1991a). The intercalibration exercise performed in 2006 included two samples with very distinct populations of these two varieties (Fig. 11). However, the distinction is unfortunately not always as simple and has resulted in identification problems in the intercalibration exercise of 2007 (Fig. 5, 6). Furthermore, both species can occur in the same sample, which was the case in sample 2 and 3.
It should be noted that GEXL in the 2006 samples was mostly represented by a form with long, slender valves. According to Lange-Bertalot and Metzeltin (1996), Fig. 62:23-27, also these forms most probably represent GEXL.
In the intercalibration workshop, we suggested that valves with a length to breadth ratio > 4 and a breadth < 6 µm should be counted as GEXL (or *G.* cf. *exilissimum*), while valves with a length to breadth ratio ≤ 4 or a breadth ≥ 6 µm should be counted as GPAR. We are aware that this separation does not solve all questions (see especially Fig. 6), but it should help when analysing diatoms for biomonitoring.

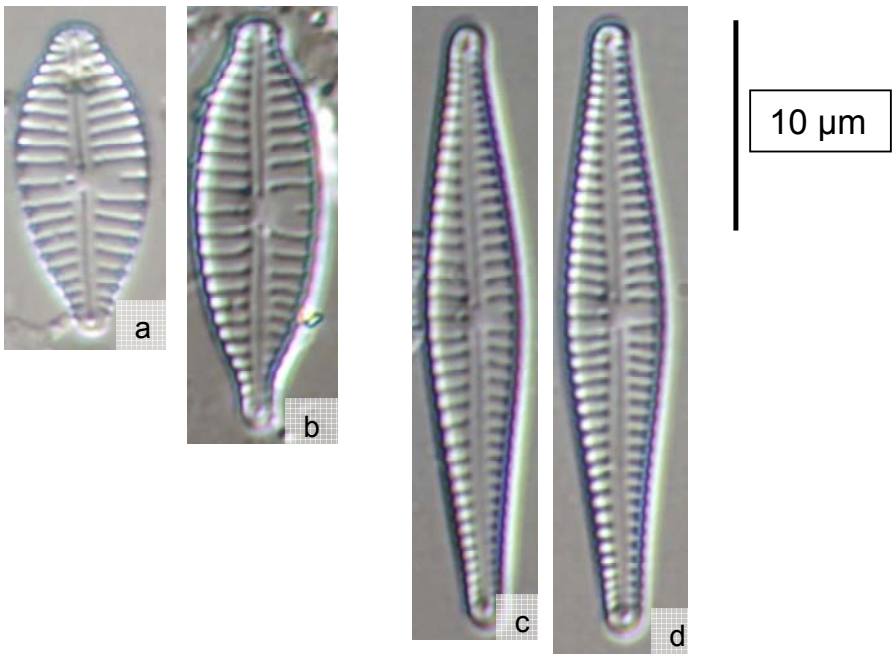

Fig 5. *Gomphonema parvulum* Kützing (a, b) (Örupsån, SE) vs. *G. exilissimum* (Grunow) Lange-Bertalot & Reichardt (c, d). (Mälskarbäcken, SE).

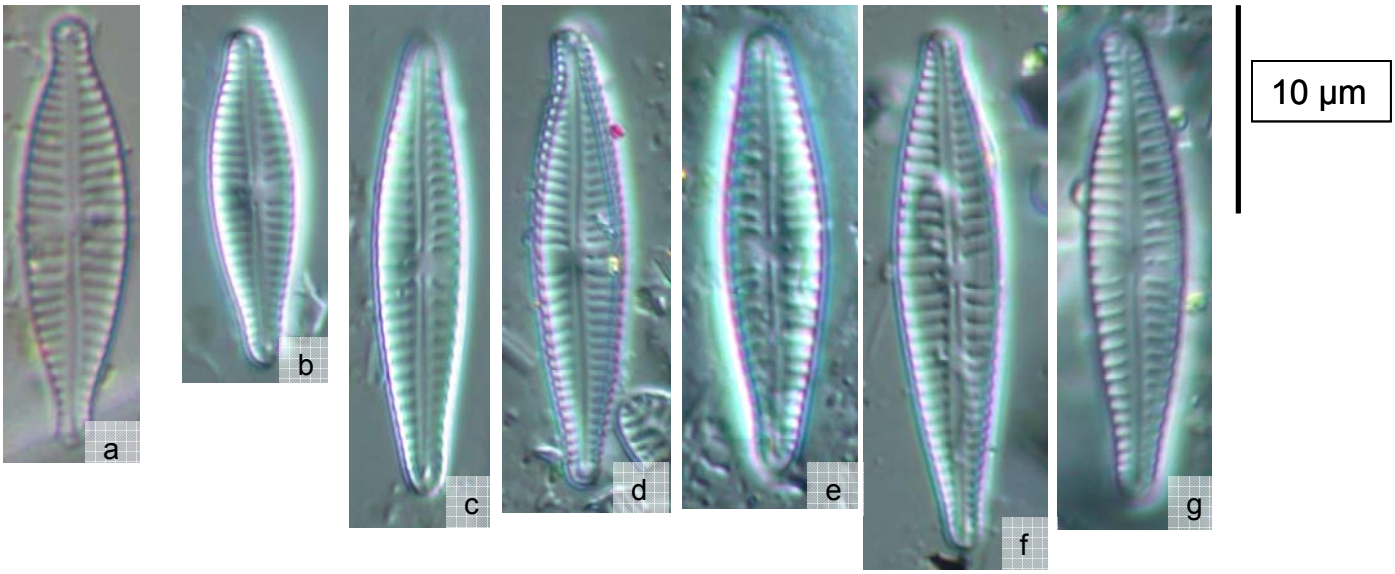

Fig 5. *Gomphonema parvulum* Kützing (a) vs. *G. exilissimum/G.* cf. *exilissimum* (Grunow) Lange-Bertalot & Reichardt (b-g) (sample 3 Rökeån, SE).

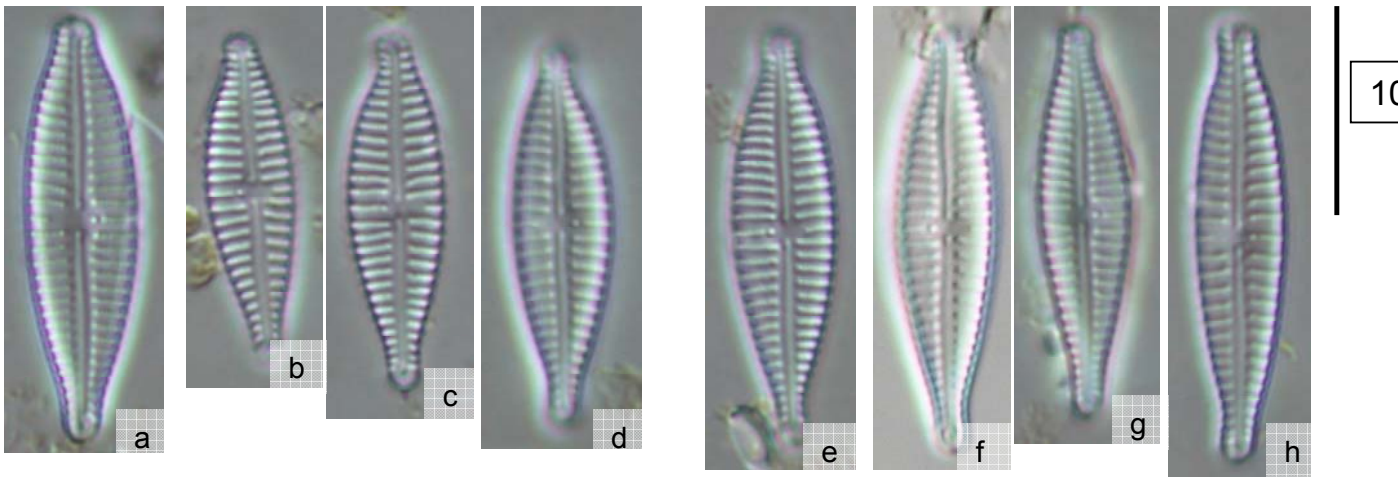

Fig. 6. *Gomphonema parvulum* Kützing (a), *G.* aff. *parvulum* (b-d) and *G.* aff. *exilissimum* (e) according to separation suggestion of intercalibration exercise, *Gomphonema exilissimum* (Grunow) Lange-Bertalot & Reichardt (f-h) (sample 2, Martimojoki, FI).

Distinction between Eunotia incisa Gregory and E. rhomboidea Hustedt

The main differences regarding the sample 5 emanated from the identification of *Eunotia rhomboidea* Hustedt (ERHO). The sample was actually dominated by ERHO, but only few participants actually counted so many ERHO valves. Instead, the valves were counted, at least in part, as *Eunotia incisa* Gregory (EINC). Most of the individual diatoms were laying in girdle view and therefore often counted as *Eunotia* spec. However, it is possible to identify these girdles by carefully checking the ends: The nodules of EINC are far from the ends of the girdle, leaving a long 'nose', whereas the nodules of ERHO lay near the ends, resulting in a short 'nose' (see Fig. 7). The separation of these two species is probably important because ERHO might be less sensitive to anthropogenic acidification than EINC (Coring, 1996).

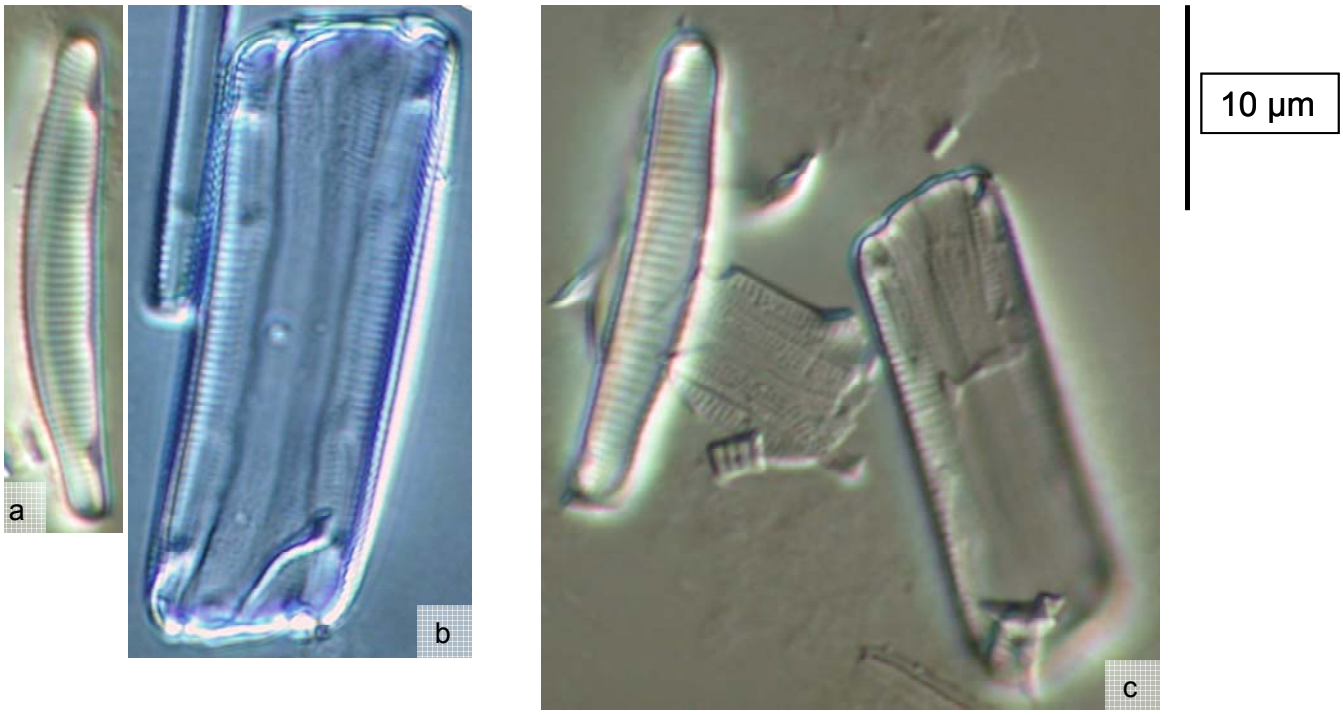

Fig. 7. Valve and girdle view of *Eunotia incisa* Gregory (a, b) vs. *Eunotia rhomboidea* Hustedt (c), (sample 5, Lillån-Bosgård, SE).

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