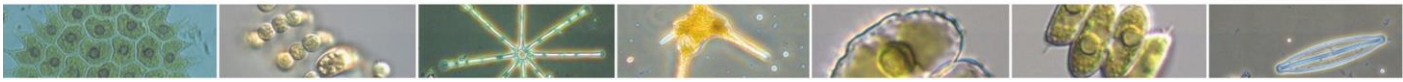
External Quality Assessment Trials Phytoplankton



Second German Benthic Diatom Intercalibration Exercise

2014/2015

Report / November 2019

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1. Introduction

Beginning in 2000, the European Water Framework Directive (WFD; European Union, 2000) has regulated water politics within the entire European Community. Accordingly, the countries developed protocols for identifying the ecological status of surface waters that is specified for each lake- and river-type. The species composition and abundance of several organism groups are used to identify their ecological status.

In Germany, the PHYLIB-method was developed and is continuously adjusted for assessing the water quality with macrophytes and phytobenthos (Schaumburg et al. 2006b, 2007b, 2011a, b, c, 2012, 2014). For diatoms the PHYLIB-instruction protocol provides details for site selection, sampling, sample processing and preparation, microscopic analysis and water quality assessment (Schaumburg et al. 2011c, 2012). For microscopic analysis, the protocol requires the identification of diatoms to at least species level, and sometimes up to variety-level. Accordingly, the analyst should have a comprehensive knowledge of diatom taxonomy and ideally long-term experience with diatom identification.

To ensure a good quality of diatom taxonomy for water quality assessments, it is essential to participate in intercalibration exercises, taxonomic workshops as well as, exchange and communicate ideas with other diatomists (Kelly & Lewis 1997, Prygiel et al. 2002, Kahlert et al. 2009, Dreßler et al. 2014, Kahlert et al. 2016). Intercalibration exercises are a valuable part of quality assurance, as they examine the quality of the results of participating laboratories and validate the applied method (DIN 38402-42, DIN 38402-45).

The first German intercalibration exercise for benthic diatoms took place in 2011 and 2012 (Dreßler et al. 2014). The analysis of the counting results and the discussions at the associated workshop identified taxonomic problems and generated recommendations on taxonomic resolution. Therefore, the previous intercalibration exercise helped to improve the quality of diatom counting results (Dreßler et al. 2014, 2015). Also, the results demonstrated that the quality of counting results affected the water quality assessment when using the PHYLIB-method and consequently demonstrated the importance of intercalibration exercises as they help reduce the variability of counting results (Werner et al. 2016). Recommendations for counting diatoms were generated to improve the PHYLIB-method that assesses the water quality in German surface waters (Dreßler et al. 2014).

This report summarises the second German intercalibration exercise for benthic diatom that was conducted in 2014 and 2015. Forty participants counted and identified diatoms from two diatom slides according to the German instruction protocols (Schaumburg et al. 2011c & 2012), one from a lake and one from a stream site from the North German Lowlands. The auditors were three internationally renowned diatom specialists (Krisztina Buczko, Hungarian Natural History Museum, Hungary; Bart Van De Vijver, Botanic Garden Meise, Belgium; Luc Ector, Luxembourg Institute of Science and Technology (LIST), Luxemburg). In July 2015, two auditors participated in the two-day long workshop at the Technische Universität Braunschweig (Germany) that was associated with the intercalibration exercise. At the workshop the results of the intercalibration exercise were presented. Together we discussed taxonomically difficult diatom-groups and the related characteristics that facilitate their identifications and formulated suggestions on how to improve the PHYLIB-method.

Based on this intercalibration exercise and associated workshop, this report presents the counting results of the second German intercalibration exercise, recommends how to consistently deal with the difficult to differentiate diatom groups identified in this report and suggests potential improvements to the German PHYLIB-method.

2. Declaration

Staff from the Technische Universität Braunschweig, Germany, and Universität Rostock, Germany, selected the sampling sites, conducted the sampling and sample preparation, sent the slides to the participants, assessed the counting results of the participants, and did not participate in the intercalibration exercise itself. This ensured no competition or bias with any of the participating consultants. The statistical expert of this study does not know which laboratory-code refers to which participant. Overall, it was understood that the counting of different slides of the same sample would lead to some variation among counting results.

3. Materials and methods

The "Second German intercalibration exercise benthic diatoms 2014/2015" is based on one stream-sample and one lake-sample from the North German Lowlands (Table 1). The stream-sample (Diat. FG F) is from the Stream Saaler Bach near the village of Wiepkenhagen (Mecklenburg-Western Pomerania, Germany) and was sampled in September of 2008. The lake-sample (Diat. S F) is from Lake Lychensee (Northern Brandenburg, Germany) and was sampled in late July of 2010.

Table 1. Sample site characteristics. WFD-type = European Water Framework Directive (WFD) - water body-type according to Schaumburg et al. (2011c, 2012), VQ = volume ratio = size of catchment area / lake volume in km^2 / $10^6 m^3$

	Diat. FG F, Stream Saaler Bach	**Diat. S F, Lake Lychensee**
Ecoregion	North German Lowlands	North German Lowlands
WFD-type	14 (sandy stream)	10 (i.e. VQ > 1.5, dimictic)
Diatom-water body-type	12.1	10.1
Catchment area geology	carbonate-rich	carbonate-rich
Catchment area size	27.2 km^2	175.7 km^2

Samples were taken according to the German instructions (Schaumburg et al. 2011c, 2012) for implementing the European Water Framework Directive, i.e. the PHYLIB-method for lotic systems and lakes. The periphyton sample of each site was collected in a 500 ml plastic bottle and preserved with ethanol. Diatom samples were subsequently oxidised and prepared with hydrochloric acid (HCl), water peroxide (H_2O_2), sulphuric acid (H_2SO_4), potassium permanganate ($KMnO_4$) and oxalic acid ($C2H_2O_4$) following modified methods from Kalbe & Werner (1974). The resulting slurry of each sample was used to create diatom slides for all participants and auditors. The slurry was shaken repeatedly during the procedure. Diatom slurries were dried on cover slips in two densities and then mounted with Naphrax® (refraction index 1.71) onto slides. Slides were prepared by one person (Technische Universität Braunschweig, Germany) for consistency. Early in October of 2014, the slides were sent to participants (Table 2). Participants were allowed another slide, if their slides were insufficient in quality or diatom density (no one made use of this option).

Table 2. Time schedule of intercalibration exercise

Task	**Date**
Dispatch of slides to participants	01.10.2014
Deadline to submit counting results	28.02.2015
Workshop at Technische Universität Braunschweig, Germany	18.-19.07.2015
Dispatch of certificates	23.09.2015

Auditor participants ('auditors' in the following) for this intercalibration exercise were: Dr. Krisztina Buczko (Hungarian Natural History Museum, Budapest), Prof. Dr. Bart Van De Vijver (Botanic Garden Meise, Belgium) and Dr. Luc Ector (Luxembourg Institute of Science and Technology (LIST), Luxemburg). They are three internationally renowned diatom-specialists with more than 20 years of experience of analysing diatom slides. All three auditors analysed both one stream-sample (Diat. FG F) and one lake-sample (Diat. S F).

For the **stream sample**, participants and auditors were instructed to base their counts on the most current German instructions for lotic systems (Schaumburg et al. 2012 (in German)) and on the instructions in the letter accompanying the samples. The letter detailed any new and relevant

changes of instructions given in Schaumburg et al. 2006a (in English) compared to the instructions given in Schaumburg et al. (2012). Additionally, the letter drew attention to the following particularly important mandatory instructions:

- Counting of at least 400 objects along transects; not field of views.
- Including girdle band valve views in the count and broken valves (> 50 % of an intact valve).
- Each frustule within a chain of girdle bands is one object (Hofmann, personal communication). Valves at the end of the chains are also one object.
- Excluding pennate taxa that are assumed to exclusively inhabit the pelagic zone, listed in Table 2, p. 30 in Schaumburg (2012).

For the **lake sample**, counts had to be based on the most current German instructions for lakes (Schaumburg et al. 2011c (in German)) and letter details. The slide-accompanying letter contained any new and relevant changes of instructions given in Schaumburg et al. 2007a (in English) compared to the instructions given in Schaumburg et al. (2011c). Additionally, the letter stated that:

- At least 500 diatom-objects need to be counted.
- For identifying rare taxa, the slide had to be scanned subsequent to counting 500 objects for another 30 minutes. These taxa had to be entered with the value "0" in the entry mask for the counting results.
- Also, above notes about girdle band views, broken valves and planktonic pennates exclusion (Table 4, S. 26, Schaumburg et al., 2011c - the list was included in the letter) applied.

Mandatory identification literature for the intercalibration exercise was also provided in the letter. The standard identification literature was Hofmann et al. 2011 or 2013 (2nd edition). Additionally, identification had to be based on the supplementary books Krammer & Lange-Bertalot (1986-2004), Lange-Bertalot (1993, 2001), Lange-Bertalot & Moser (1994), Lange-Bertalot & Metzeltin (1996), Krammer (1997a, 1997b, 2000, 2002, 2003), Reichardt (1999), Witkowski et al. (2000) and Levkov (2009).

Participants and auditors entered their data via the EQAT-webpage www.planktonforum.eu and the EQAT entry mask using their unique laboratory code, which they received with the accompanying letter. Laboratory codes ran from 1 to 41 for 40 participants. Number 23 was used for a test run. Auditor codes ran from 42 to 44. Analysis, evaluation and presentation of counting results were based solely on the laboratory codes. Participants and auditors had to enter the number of identified objects of each taxon together with the associated most recent German data processing number of each taxon (dv-numbers) (Mauch et al., 2003, version 2011). In addition to the counting results, mandatory microscope information was entered, i.e. magnification used, lens (type, aperture) and optical illumination technique.

For data analysis and evaluation of counting results the same method was used as that for the first German intercalibration exercise of benthic diatoms (Dreßler et al., 2014). First, the relative abundances were calculated from the number of counted diatom objects. Then, the number of diatom-objects (sum) per sample was identified, as well as the number of identified taxa, the number of taxa determined with uncertainty (sp., cf., aff., *Pennales*), the number of taxa that occurred in greater than 1 % abundance and the number of taxa that were found during the scan for rare taxa. The similarity of counting results per sample were determined using both Bray-Curtis dissimilarity distance and a multivariate graph (Detrended Correspondence Analysis = DCA). These two

independent methods were used to confirm and ensure the assessment of the counting results of each participant.

Based on the relative abundance data of the three auditors, the average, standard deviation and corresponding 95 %-confidence interval of the Bray-Curtis dissimilarity distances of each sample were calculated and represented the similarity of the results of the auditors. If the Bray-Curtis distance of a participant was inside of this confidence interval around all three auditors, then the result of the participant was considered to be similar to the auditor results. Results that differed statistically significantly from all three auditors, were deemed to be counted unsuccessfully. These participants were marked with a red circle in the DCA (see Chapter 4.1.). Subsequently, the Bray-Curtis distances (coloured circles) were visually compared to the results of the DCA figure (if appropriate also in the third dimension) for further confirmation of findings.

Prior to calculating the Bray-Curtis-distances and creating the DCA, the problematic taxa were pooled into groups (listed in Chapter 4.1). These were:

1. Taxa that were both, difficult to identify (see no. 4) and named with and without uncertainty, i.e. taxa that were sometimes labelled with 'cf.' or 'aff.' by the auditors or participants.
2. Identical taxa with varying names (synonyms) were grouped, if they were based on identical taxonomical concepts. Examples: *Planothidium lanceolatum* and *Achnanthes lanceolata* ssp. *lanceolata* or *Fragilaria vaucheriae* and *Fragilaria capucina* var. *vaucheriae*.
3. Species and their nominate variety, form or morphotype: Species and nominate variety were grouped, if no other varieties exist, e.g. *Fragilaria brevistriata, Fragilaria famelica*, *Navicula cryptocephala*, *Nitzschia capitellata* or *Nitzschia recta*. Also, species were grouped, if their variety, form or morphotype were both difficult to identify (Hofmann et al. 2011 and 2014, Dreßler et al. 2014) and other varieties/forms/morphotypes were not found.
4. Taxa that were taxonomically difficult to differentiate using light microscopy: This applied to taxa that could not be separated unambiguously with the taxa description in the current identification literature.

These groups were created to ensure a realistic evaluation of the participants, i.e. to avoid unfavourable evaluations due to these taxonomic problems. Therefore, only serious taxa confusion, misidentifications or misnomers affected the evaluation of the counting results. The pooled groups are listed in Chapters 4.1.1 and 4.1.2. With the exception of grouping no. 3, taxa were not pooled to these groups for the detailed discussion of taxonomic problems (Chapters 4.3 and 4.4).

Next to above taxonomic evaluation, participants were also evaluated based on deviations from the given official German instruction protocols (Schaumburg et al. 2011c and 2012, respectively). The following criteria were used to assess consistency with the protocol and were noted on the certificates of the participants:

- Number of counted objects were distinctly too low, i.e. <380 objects in the stream sample and <475 objects in the lake sample.
- Slides were not scanned for rare taxa in the lake sample.
- Obligatory planktonic diatoms were listed.
- Taxa names did not correspond to the names provided in the requested literature, i.e. the names were either outdated or wrong due to a new taxon concept.

Based on the above, participants received either no (0), minor (1) or substantial deviations (2) from the instruction protocol on their certificates. Minor deviations were noted, if one of the above parameters were neglected. Substantial deviations were noted, if at least two or more of above criteria pertained.

The ecological status class of each sample was assessed based on the diatom assemblages using the PHYLIB-software version 5.3.0 from 11. December 2015 (Chapter 4.5; Schaumburg et al. 2011c and 2012, respectively). The effects of counting result variances on the ecological assessment with PHYLIB was assessed using DI_{Seen} (Diatom $Index_{lakes}$) and $DI_{Fließgewässer}$ (Diatom $Index_{running\ waters}$).

Finally, all diatom pictures in this report were taken with the camera ProgRes®SpeedXTcore3 (Jenoptik) attached to an Axioplan light microscope (Zeiss) with differential interference contrast (DIC), 100x oil-immersion objective Plan-Apochromat (aperture 1.4) at an overall magnification of 1000x. Valves were measured using the software analySIS® (Soft Imaging System GmbH).

4. Results and discussion

4.1 Diatom assemblages, counting results and evaluation of participants

4.1.1 Stream Saaler Bach (Diat. FG F)

The sample **Diat. FG F** was taken from the Stream Saaler Bach near the village Wiepkenhagen in Mecklenburg-Western Pommerania, Germany, and will be referred to as ‘Stream’ sample in the following discussion. The following diatom taxa dominated the assemblage according to the three auditors: *Navicula gregaria* Donkin, *Melosira varians* C. Agardh and *Navicula cryptocephala* Kützing (Table 3). Also abundant (present at least once with >3.5 % relative abundance) were *Cocconeis placentula* var. *lineata* (Ehrenberg) van Heurck, *C. placentula* var. *placentula* Ehrenberg, *Nitzschia paleacea* Grunow, *Gomphonema parvulum* (Kützing) Kützing var. *parvulum* f. *parvulum, Gomphonema parvulum* (Kützing) Kützing var. *parvulum, Achnanthidium* cf. *minutissimum* (Kützing) Czarnecki, *Achnanthidium* cf. *minutissimum* (Kützing) Czarnecki var. *minutissimum, Nitzschia palea* (Kützing) W. Smith var. *palea, Nitzschia palea* var. *debilis* (Kützing) Grunow and *Nitzschia archibaldii* Lange-Bertalot (Table 3).

Table 3. Relative abundances (%), average (%) and standard deviation (SD) (%) of the dominant diatom taxa in the ‘Stream’ sample based on the results of the three auditors. L42-L44 = laboratory codes 42 to 44.

	Auditor 1	**Auditor 2**	**Auditor 3**		
Taxon	**L 42**	**L 43**	**L 44**	**Average**	**SD**
Navicula gregaria	8.8	16.8	15.8	13.6	4.3
Melosira varians	8.5	10.8	12.2	10.5	1.8
Navicula cryptocephala	8.8	8.5	6.8	8.0	1.1
Cocconeis placentula var. *lineata*	0.0	4.3	7.1	3.8	3.6
Cocconeis placentula var. *placentula*	4.8	0.0	0.0	1.6	2.7
Nitzschia paleacea	6.0	4.3	3.2	4.5	1.4
Gomphonema parvulum var. *parvulum* f. *parvulum*	5.0	0.0	2.7	2.6	2.5
Gomphonema parvulum var. *parvulum*	0.0	4.0	0.0	1.3	2.3
Achnanthidium cf. *minutissimum*	0.0	5.0	0.0	1.7	2.9
Achnanthidium cf. *minutissimum* var. *minutissimum*	3.8	0.0	0.0	1.3	2.2
Nitzschia palea var. *palea*	3.3	0.0	3.6	2.3	2.0
Nitzschia palea var. *debilis*	3.5	1.5	0.0	1.7	1.8
Nitzschia archibaldii	3.5	3.5	3.2	3.4	0.2

The abundance of each taxon occurs with a certain statistical dispersion on the slides of all participants due to a natural variability of the samples and the slide preparation (Chapter 3). This variability is reflected in the results and the extent of this "natural variability" is particularly visible in the standard deviation of the dominant taxa identified by the three auditors (Table 3). The standard deviations of the taxa abundance were 1.1 % for *Navicula cryptocephala,* with an average relative abundance of 8.0 % (n=3), 1.8 % for *Melosira varians* (average: 10.5 %) and 4.3 % for *Navicula gregaria* (average 13.6 %). It should be noted that the entire extent of "natural variability" cannot be represented by the results of the auditors, as the sample size (three) is too low.

Further deviations among the results of the auditors were due to different taxonomic allocations, e.g. to *Cocconeis placentula* var. *lineata* or *C. placentula* var. *placentula*, *Gomphonema parvulum* var. *parvulum* f. *parvulum* or *Gomphonema parvulum* var. *parvulum* and *Achnanthidium* cf. *minutissimum* or *Achnanthidium* cf. *minutissimum* var. *minutissimum*. These taxa have either ambiguous taxonomic concepts (e.g. *Cocconeis placentula*-aggregate, see Chapter 4.3.1), or the auditors determined the taxa to different taxonomic levels (e.g. *Gomphonema parvulum*-aggregate), or the taxa were determined with uncertainty, as they were labelled with "cf." (*Achnanthidium minutissimum*-aggregate).

The participants of the intercalibration exercise, and to a very small extent, also the auditors, had difficulties identifying certain diatoms from the ‘Stream’ sample. Particularly difficult were the allocation of taxa from the *Cocconeis placentula*-aggregate, the *Gomphonema parvulum*-aggregate and the *Nitzschia palea*-aggregate. Additionally, severe taxonomic problems occurred when identifying *Navicula cryptocephala*, *Nitzschia paleacea*, *Planothidium lanceolatum* and *P. frequentissimum*. The counting results of these taxa and groups are presented and discussed in detail in Chapter 4.3.

Next to the taxonomic evaluation of the participant results, participants were also evaluated based on deviations from the given official German instruction protocol (Schaumburg et al. 2012) (see Chapter 3). As the ‘Stream’ sample is from a lotic system, at least 400 diatom-objects had to be counted according to the instruction protocol (Schaumburg et al. 2012). Two of the 40 participants deviated distinctly from the target (counting < 380 objects) (Table 4). Also, obligatory planktonic living diatoms had to be excluded from the count. In contrast, six of the 40 participants listed planktonic diatoms in their results from the ‘Stream’ sample, as they listed *Nitzschia acicularis* or *N. acicularis* var. *acicularis* (laboratory codes 2, 12, 14, 29 and 37) or *Cyclotella meneghiniana* and *C. cyclopuncta* (Laboratory Code 31). The results (taxa names) of two participants (laboratory codes 28 and 39) suggest the use of outdated identification literature. Therefore, it was understood that 31 participants complied with the official German instruction protocol (Schaumburg et al. 2012).

The auditors identified a total of 57 to 58 diatom taxa in the ‘Stream’ sample. The participants found 12 to 72 taxa, on average 50 taxa (n=40; Table 4). The number of taxa that occurred with more than 1.0 % relative abundance ranged from 25 to 28 for the auditors, and for the participants from 8 to 29 taxa (Table 4). The sum of the relative abundance of all taxa that were identified with uncertainty ("sp.", "cf.", "aff.", *Pennales*) were 0 %, 5.5 % and 6.5 %, respectively, for the auditors and up to 52.7 % for the participants. Six participants (and one auditor) determined all identified taxa for the river sample with certainty, i.e. they did not use "sp.", "cf.", "aff." or "*Pennales*". Nine participants (and two auditors) labelled more than 5 % of the identified taxa with uncertainty (Table 4). A detailed discussion about how to deal with taxonomic uncertainties and recommendations when using PHYLIB and how to advance PHYLIB is presented in the report of the first German intercalibration exercise for benthic diatoms (Dreßler et al. 2014).

Table 4. Basic counting parameters and Bray-Curtis-Distances of the participants (laboratory codes 1-41) and auditors (laboratory codes 42-44, shaded in green) for the 'Stream' sample: **Lab Code** =laboratory-code. Number of counted diatom-objects (**Objects**), objects minus planktonic objects (**Obj-B**), number of identified taxa during the count (**NTC**), during the search for rare taxa after the count (**NTS**, not required as this is a lotic system sample) and with a relative abundance >1 % (**NT>1 %**) and sum of the relative abundance of all ambiguously determined diatom objects, i.e. taxa labelled with "sp.", "cf.", "aff.", *Pennales* (**cf (%)**). Also given are the Bray-Curtis-Distances of the participants compared to auditor 42 (**Diff 1**), 43 (**Diff 2**) and 44 (**Diff 3**), respectively. Red and bold Bray-Curtis-Distances were outside the 95 %-confidence interval.

Lab Code	Basic counting parameters						Bray-Curtis-Distances		
	Objects	Obj-B	NTC	NTS	NT>1 %	cf (%)	Diff 1	Diff 2	Diff 3
1	438	438	51	0	18	1.1	**0.4653**	0.3559	0.3374
2	547	522	69	3	29	1.9	0.2819	0.3162	0.3314
3	400	400	51	0	24	3.5	0.2512	0.2140	0.2634
4	400	400	52	1	21	0.8	0.3066	0.2190	0.2275
5	401	401	43	0	19	0.0	0.2263	0.1701	0.2156
6	457	457	50	0	22	13.3	0.3402	0.2743	0.3535
7	400	400	58	0	24	8.3	0.3615	0.2966	0.2977
8	451	451	57	0	22	0.7	0.2765	0.2244	0.2815
9	400	400	60	16	25	1.0	0.2713	0.2491	0.2698
10	407	407	47	0	23	1.0	0.2916	0.1677	0.2275
11	417	417	47	17	22	0.0	**0.4479**	0.4201	0.4303
12	477	474	55	0	20	1.5	0.3928	0.3711	0.4020
13	408	408	43	7	19	0.2	0.3282	0.2639	0.3045
14	520	498	52	4	22	8.6	0.3107	0.2956	0.3229
15	435	435	44	0	21	0.0	0.4346	0.3913	0.4139
16	401	401	68	10	23	2.5	0.3048	0.2553	0.2811
17	473	473	67	0	21	0.0	0.3225	0.2792	0.2664
18	239	239	29	0	19	1.7	**0.8111**	**0.8537**	**0.8488**
19	400	400	44	0	21	0.5	0.3640	0.2661	0.2563
20	423	423	42	10	18	0.9	0.3468	0.2604	0.3496
21	412	412	61	0	18	1.0	0.2709	0.1884	0.2105
22	407	407	44	0	21	0.5	0.3497	0.2867	0.2966
24	400	400	51	0	23	1.0	0.2869	0.2068	0.2816
25	400	400	48	0	18	5.3	0.3293	0.2140	0.2732
26	406	406	62	0	22	2.7	0.2444	0.2141	0.3047
27	400	400	66	0	25	2.5	0.2741	0.2464	0.2319
28	540	540	20	0	13	14.3	**0.9499**	**0.9098**	**0.9197**
29	469	382	28	0	18	24.6	**0.7841**	**0.7938**	**0.8017**
30	400	400	54	0	20	1.3	0.2961	0.2314	0.2723
31	425	387	12	0	8	52.7	**0.8946**	**0.8897**	**0.8856**
32	400	400	49	0	22	5.5	0.3441	0.2694	0.2752
33	439	439	58	0	26	0.2	0.3252	0.2509	0.3004
34	513	511	36	0	17	0.0	0.3831	0.3635	0.3877
35	400	400	72	0	22	1.5	0.3092	0.2466	0.2480
36	415	415	57	0	24	3.9	0.2603	0.1971	0.2543
37	374	359	61	0	27	12.3	0.3483	0.2895	0.3215
38	400	400	49	0	19	3.8	0.3165	0.2969	0.3231
39	397	397	41	19	23	2.3	0.3628	0.3140	0.3228
40	443	443	47	0	19	0.0	**0.4543**	0.3399	0.3263
41	408	408	54	0	20	0.0	0.3063	0.2351	0.3023
42	399	399	57	16	28	6.5	**0.0000**	0.2406	0.2948
43	400	399	57	0	26	5.5	0.2406	**0.0000**	0.2186
44	411	411	58	21	25	0.0	0.2948	0.2186	**0.0000**

As described in Chapter 3, the Bray-Curtis-Distances were calculated and the counting results were depicted in a DCA to evaluate the participants. For both methods, the following taxa were pooled into groups prior to analysis for the ‘Stream’ sample:

1. Taxa were pooled that were both difficult to identify and named with and without uncertainty, i.e. taxa that were sometimes labelled with 'cf.' or 'aff.' by the auditors or participants:

   Group 1 (*Achnanthidium saprophilum*, *Achnanthidium* cf. *saprophilum*), **Group 2** (*Amphora inariensis*, *Amphora* cf. *inariensis*), **Group 3** (*Encyonema ventricosum*, *Encyonema* cf. *ventricosum*), **Group 4** (*Eolimna minima*, *Eolimna* cf. *minima*), **Group 5** (*Fragilaria rumpens*, *Fragilaria* cf. *rumpens*), **Group 6** (*Mayamaea atomus* var. *permitis*, *Mayamaea atomus* cf. var. *permitis*), **Group 7** (*Nitzschia paleacea*, *Nitzschia* cf. *paleacea*)

2. Identical taxa with varying names (synonyms) were pooled, in combination with previous criterion:

   Group 8 (*Planothidium lanceolatum*, *Achnanthes lanceolata* ssp. *lanceolata*), **Group 9** (*Fragilaria capucina* var. *vaucheriae*, *Fragilaria vaucheriae*, *Fragilaria capucina* cf./aff. var. *vaucheriae*, *Fragilaria* cf./aff. *vaucheriae*), **Group 10** (*Fragilaria gracilis*, *Fragilaria capucina* var. *gracilis*, *Fragilaria* cf./aff. *gracilis*, *Fragilaria capucina* cf./aff. var. *gracilis*)

3. Species were pooled with their nominate variety or form; where applicable in combination with above criteria:

   Group 11 (*Encyonema silesiacum*, *Encyonema* cf. *silesiacum*, *Encyonema silesiacum* var. *silesiacum*), **Group 12** (*Eunotia bilunaris*, *Eunotia bilunaris* var. *bilunaris*), **Group 13** (*Fragilaria brevistriata*, *Fragilaria brevistriata* var. *brevistriata*), **Group 14** (*Fragilaria famelica*, *Fragilaria famelica* var. *famelica*), **Group 15** (*Fragilaria ulna*, *Fragilaria ulna* var. *ulna*), **Group 16** (*Gomphonema olivaceum*, *Gomphonema olivaceum* var. *olivaceum*), **Group 17** (*Gomphonema parvulum* var. *parvulum*, *Gomphonema parvulum* var. *parvulum* f. *parvulum*), **Group 18** (*Mayamaea fossalis*, *Mayamaea fossalis* var. *fossalis*), **Group 19** (*Meridion circulare*, *Meridion circulare* var. *circulare*), **Group 20** (*Navicula cryptocephala*, *Navicula* cf. *cryptocephala*, *Navicula cryptocephala* var. *cryptocephala*, *Navicula cryptocephala* cf. var. *cryptocephala*), **Group 21** (*Nitzschia capitellata*, *Nitzschia capitellata* var. *capitellata*), **Group 22** (*Nitzschia recta*, *Nitzschia recta* var. *recta*), **Group 23** (*Sellaphora pupula*, *Sellaphora pupula* var. *pupula*)

   Gomphonema parvulum var. *parvulum* and *Gomphonema parvulum* var. *parvulum* f. *parvulum* were grouped (**Group 17)**, because *Gomphonema parvulum* var. *parvulum* f. *saprophilum* is usually distinctly different from f. *parvulum* and is currently considered to be a separate species (Abarca et al. 2014). Therefore, the form *parvulum* would be void. For a detailed discussion see Chapter 4.3.2.

4. Taxa were pooled into groups that are taxonomically difficult to differentiate using light microscopy; where applicable in combination with above criteria:

   Group 24 (*Planothidium frequentissimum*, *Planothidium frequentissimum* var. *frequentissimum*, *Planothidium frequentissimum* var. *magnum*, *Planothidium frequentissimum* var. *minus*), **Group 25** (*Achnanthidium minutissimum*, *Achnanthidium minutissimum* var. *minutissimum*, *Achnanthidium minutissimum* var. *jackii*, *Achnanthidium microcephalum*, *Achnanthidium lineare* W. Smith, *Achnanthidium* cf. *minutissimum*, *Achnanthidium minutissimum* cf. var. *minutissimum*, *Achnanthidium minutissimum* cf. var. *jackii*), **Group 26** (*Cocconeis placentula*, *Cocconeis placentula* var. *placentula*, *Cocconeis placentula* var. *lineata*, *Cocconeis placentula* var. *euglypta*), **Group 27** (*Navicula reichardtiana*, *Navicula reichardtiana*

var. *reichardtiana*, *Navicula* cf. *reichardtiana*, *Navicula caterva*), **Group 28** (*Nitzschia fonticola*, *Nitzschia fonticola* var. *fonticola*, *Nitzschia* cf. *fonticola*, *Nitzschia costei*), **Group 29** (*Nitzschia palea*, *Nitzschia palea* var. *debilis*, *Nitzschia palea* var. *palea*, *Nitzschia palea* var. *tenuirostris*, *Nitzschia* cf. *palea*, *Nitzschia palea* cf. var. *debilis*, *Nitzschia palea* cf. var. *palea*, *Nitzschia palea* cf. var. *tenuirostris*, *Nitzschia archibaldii*)

The similarity among counting results was assessed using the Bray-Curtis-Distance. The average Bray-Curtis-Distance of the counting results among auditors was 0.2513 with a standard deviation of 0.0392 and therefore a 95 %-confidence interval of permitted range of **0.0564 to 0.4463** for the ‘Stream’ sample. If a Bray-Curtis-Distance of a participant was outside this confidence interval of an auditor, the distance is marked red and bold in Table 4. If the Bray-Curtis-Distance of a participant was outside the confidence interval of all three auditors, then the results were too dissimilar to the results of the auditors, i.e. the sample was counted "unsuccessfully". This dissimilarity pertained to four participants (10 %, Table 4).

The Detrended Correspondence Analysis (DCA) confirms the results of the Bray-Curtis-Distances (Fig. 1, displaying the first and second axis), when also reviewing the third dimension (not shown). Four participants with high Bray-Curtis-Distances (Table 4, red outline in Fig. 1) are distinctly apart from the counting results of the auditors (L42-L44). The counting results of the auditors were similar and therefore are located very close to each other and to most participants (90 %, Fig. 1).

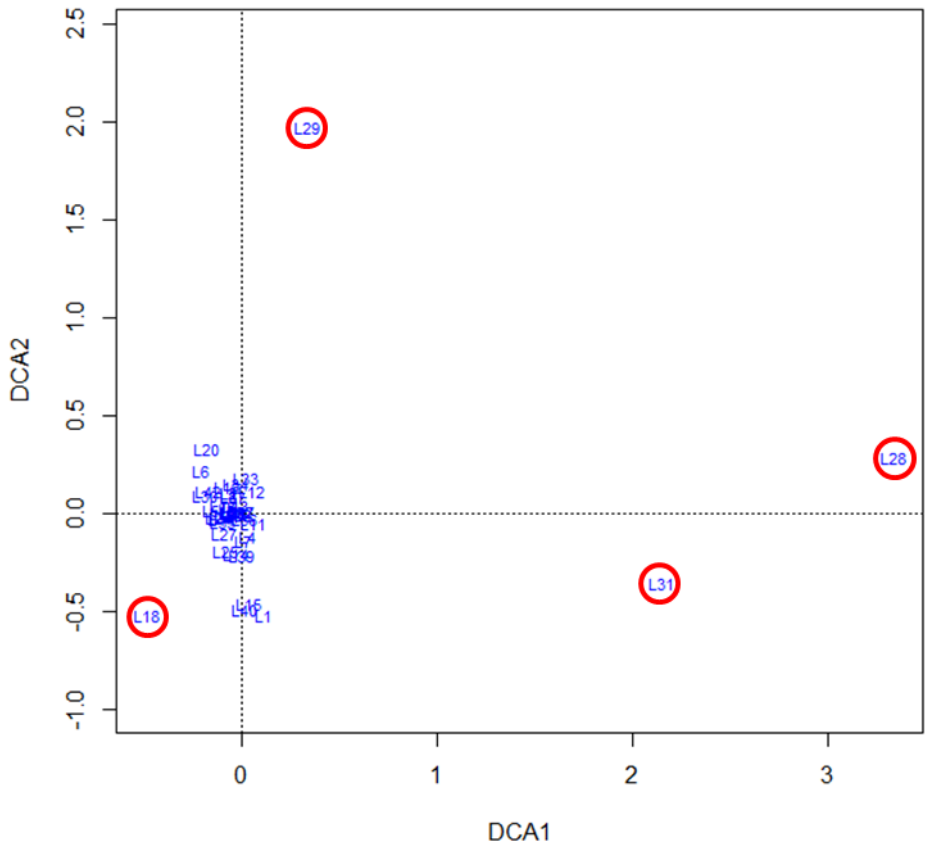


Figure 1: First and second axis of the DCA based on the diatom results of all participants and auditors of the ‘Stream’ sample. Numbers correspond to laboratory codes (L1-L44). Results from participants of the red-rimmed laboratory codes were outside the confidence-intervals of all three auditors based on the Bray-Curtis-Distances, i.e. these participants counted this sample unsuccessfully.

4.1.2 Lake Lychensee (Diat. S F)

The sample **Diat. S F** was taken from Lake Lychensee in Northern Brandenburg, Germany (Chapter 3, Table 1) and will be called ‘Lake’ sample in the following. Overall, the results of the auditors agree well with each other. The following diatom taxa dominated the assemblage according to the auditors: *Achnanthidium minutissimum* (Kützing) Czarnecki var. *minutissimum*, *Cymbella vulgata* Krammer and *Encyonopsis minuta* Krammer & Reichardt. Also abundant (present at least once with >3.2 % relative abundance) were: *Epithemia adnata* (Kützing) Brébisson, *Fragilaria brevistriata* (Grunow) Grunow var. *brevistriata*, *Navicula cryptotenelloides* Lange-Bertalot, *Encyonopsis subminuta* Krammer & Reichardt, *Encyonopsis thumensis* Krammer, *Gomphonema exilissimum* (Grunow) Lange-Bertalot & Reichardt, *Achnanthidium lineare* W. Smith (entered as *Pennales*), *Epithemia sorex* Kützing and *Encyonopsis microcephala* (Grunow) Krammer (Table 5).

Some differences occurred in auditor results due to "natural variability" among slides, as reflected in the standard deviation (SD) among auditors of *Cymbella vulgata* (average relative abundance of 10.6 %, SD 2.2 %, n=3), *Epithemia adnata* (average 8.1 %, SD 1.2 %), *Navicula cryptotenelloides* (average 7.2 %, SD 0.9 %) and *Epithemia sorex* (average 2.9 %, SD 0.4 %). Additionally, some differences occurred due to different taxonomic allocations. For example, only one of the three auditor identified *Encyonopsis thumensis* (with 4.8 %) or E. *microcephala* (with 3.2 %)(Table 5).

Table 5. Relative abundances (%), average (%) and standard deviation (SD) (%) of dominant taxa in the ‘Lake’ sample based on the results of the three auditors. L42-L44 = laboratory codes 42 to 44.

	Auditor 1	**Auditor 2**	**Auditor 3**		
Taxon	**L 42**	**L 43**	**L 44**	**Average**	**SD**
Achnanthidium minutissimum var. *minutissimum*	15.4	8.8	11.1	11.8	3.3
Cymbella vulgata	13.0	10.2	8.7	10.6	2.2
Encyonopsis minuta	8.2	11.4	8.3	9.3	1.8
Encyonopsis subminuta	6.8	4.0	3.6	4.8	1.7
Encyonopsis thumensis	0.0	4.8	0.0	1.6	2.8
Encyonopsis microcephala	0.0	0.0	3.2	1.1	1.9
Epithemia adnata	7.4	7.4	9.5	8.1	1.2
Fragilaria brevistriata var. *brevistriata*	6.6	7.0	8.3	7.3	0.9
Navicula cryptotenelloides	6.2	8.0	7.5	7.2	0.9
Gomphonema exilissimum	3.6	0.0	0.4	1.3	2.0
Pennales/Achanthidium lineare	2.4	3.4	1.8	2.5	0.8
Epithemia sorex	2.4	3.0	3.2	2.9	0.4

For the ‘Lake’ sample, the analysis of the counting results and the discussions during the workshop identified taxa that are difficult to identify, particularly *Cymbella vulgata* and other *Cymbella*-species, *Encyonopsis minuta* and *E. subminuta,* taxa of the *Achnanthidium minutissimum*-aggregate, *Fragilaria brevistriata*, *Navicula cryptotenella* and *N. cryptotenelloides*. The counting results of these taxa or groups are presented and discussed in detail in Chapter 4.4.
In addition to the taxonomic evaluation of the participant results, participants were also evaluated based on deviations from the given official German instruction protocol (Schaumburg et al. 2011c) (see Chapter 3) for the ‘Lake’ sample. The German protocol (Schaumburg et al. 2011c) stipulates to identify at least 500 diatom-objects. One participant counted only 82 diatom-objects, i.e. distinctly

too few (<475) (Table 6). Also, obligatory planktonic diatoms had to be excluded from the count. However, two participants (laboratory codes 31 and 41) each listed one planktonic object (*Cyclotella meneghiniana* and *Asterionella formosa*, respectively).

One participant (Laboratory Code 20) and one auditor identified one and two objects, respectively, as *Fragilaria saxoplanctonica* (Lange-Bertalot & S. Ulrich). They listed the taxon correctly as „*Pennales*“ according to Schaumburg et al. (2011c), as *F*. *saxoplanctonica* was only recently described (in: Lange-Bertalot & Ulrich 2014) and therefore, did not have a German data processing number (dv-number) yet (Mauch et al. 2003, version of 2011). However, for the analysis of this intercalibration exercise, these objects were subtracted from the total number of counted objects, because *F. saxoplanctonica* is definitely planktonic.

The three auditors identified 59 to 69 diatom taxa in the ‘Lake’ sample. The participants found 13 to 71 taxa, on average 52 taxa (n=40; Table 6). The auditors identified 10 to 12 additional taxa during the required search for rare taxa after the count (mandatory for lake-samples in Germany; Schaumburg et al. 2011c). Four participants did not search for rare taxa. The remaining participants identified one to 21 additional taxa during the search for rare taxa (Table 6). The number of taxa that occurred with more than 1.0 % relative abundance ranged from 14 taxa to 24 taxa for the auditors, and from 10 to 24 taxa for the participants (Table 6). The results (taxa names) of three participants (laboratory codes 14, 28 and 39) suggest the use of outdated identification literature. Therefore, overall 33 of the 40 participants complied with the accountable facts of the official German instruction protocol (Schaumburg et al. 2011c).

The sum of the relative abundance of all taxa that were identified with uncertainty ("sp.", "cf.", "aff.", *Pennales*) were 0 %, 1.4 % and 5.0 %, respectively, for the auditors and up to 21.1 % for the participants. Six participants (and one auditor) determined all identified taxa for the ‘Lake’ sample with certainty, i.e. they did not use "sp.", "cf.", "aff." or "*Pennales*". Fourteen participants and one auditor labelled more than 5 % of the identified taxa with uncertainty (Table 6). A detailed discussion about how to deal with taxonomic uncertainties and recommendations when using PHYLIB and how to advance PHYLIB is presented in the report of the first German intercalibration exercise for benthic diatoms (Dreßler et al. 2014).

Table 6. Basic counting parameters and Bray-Curtis-Distances of the participants (laboratory codes 1-41) and auditors (laboratory codes 42-44, shaded in green) for the 'Lake' sample: **Lab Code** =laboratory-code. Number of counted diatom-objects (**Objects**), objects minus planktonic objects (**Obj-B**), i.e. number of benthic objects; number of identified taxa during the count (**NTC**), during the search for rare taxa after the count (**NTS**) and with a relative abundance >1 % (**NT>1 %**) and sum of the relative abundance of all ambiguously determined diatom objects, i.e. taxa labelled with "sp.", "cf.", "aff." or *Pennales* (**cf (%)**). Also given are the Bray-Curtis-Distances of the participants compared to auditor 42 (**Diff 1**), 43 (**Diff 2**) and 44 (**Diff 3**), respectively. Marked red and bold are Bray-Curtis-Distances that are outside the 95 %-confidence interval.

Lab Code	Basic counting parameters						Bray-Curtis-Distances		
	Objects	Obj-B	NTC	NTS	NT>1 %	cf (%)	Diff 1	Diff 2	Diff 3
1	565	565	39	3	18	0.2	**0.4959**	**0.4360**	**0.4547**
2	542	542	71	7	22	14.2	0.3116	0.3132	0.3465
3	499	499	56	18	17	2.2	0.2711	0.2465	0.2983
4	500	500	68	1	22	16.0	0.3840	0.3735	0.3774
5	513	513	52	18	17	21.1	**0.4096**	0.3419	**0.4017**
6	500	500	45	4	13	6.0	0.3120	0.3234	0.3200
7	500	500	61	6	18	6.4	0.3200	0.2816	0.2945
8	512	512	59	4	15	1.4	0.2685	0.1944	0.2961
9	500	500	61	19	17	4.2	0.3720	0.3354	0.3083
10	516	516	55	10	22	1.9	0.2739	0.2325	0.3118
11	495	495	57	6	24	0.0	**0.4684**	**0.4087**	**0.4747**
12	623	623	51	4	20	0.6	**0.5639**	**0.5902**	**0.5716**
13	516	516	49	3	17	0.8	**0.4106**	**0.4315**	0.3739
14	544	544	50	4	11	3.7	**0.6091**	**0.6452**	**0.6405**
15	510	510	41	11	17	0.0	**0.4634**	**0.5134**	**0.4933**
16	500	500	70	12	18	6.8	0.2840	0.3194	0.3323
17	546	546	58	21	21	0.0	0.3679	0.3130	0.2955
18	82	82	14	0	14	19.5	**0.9596**	**0.9556**	**0.9433**
19	503	503	51	11	14	0.2	**0.3938**	**0.4027**	**0.4086**
20	521	519	49	8	16	2.3	0.2889	0.2840	0.3723
21	549	549	64	5	17	4.0	0.3029	0.2458	0.2542
22	502	502	46	5	13	1.6	0.3015	0.2979	0.2567
24	500	500	50	6	17	0.2	0.2920	0.2573	0.3047
25	500	500	51	9	21	18.2	**0.5520**	**0.5276**	**0.5260**
26	504	504	67	12	17	7.1	0.2953	0.2275	0.2649
27	500	500	66	20	15	0.6	0.2840	0.2552	0.2840
28	605	605	25	0	12	6.8	**0.8022**	**0.7957**	**0.7752**
29	531	531	26	0	22	4.7	**0.5944**	**0.5799**	**0.6298**
30	504	504	55	18	18	7.1	0.3319	0.3530	0.3473
31	512	511	13	0	10	0.0	**0.8120**	**0.8397**	**0.8508**
32	503	503	52	2	17	5.4	0.3202	0.3416	0.3085
33	502	502	61	9	18	1.2	0.2917	0.2624	0.3193
34	739	739	51	2	19	0.0	0.3154	0.2461	0.2888
35	500	500	63	21	16	0.4	0.2860	0.2491	0.2938
36	506	506	71	8	21	16.0	0.2899	0.2858	0.3064
37	502	502	65	10	20	11.6	0.3163	0.3009	0.3080
38	500	500	46	11	16	3.2	**0.5160**	**0.5034**	**0.4468**
39	510	510	44	5	16	0.0	**0.5423**	**0.5460**	**0.5295**
40	520	520	42	4	13	1.3	**0.5681**	**0.5003**	**0.5068**
41	500	499	52	3	26	1.6	0.3573	0.2906	0.3282
42	500	500	61	10	14	1.4	**0.0000**	0.2613	0.2949
43	500	499	69	12	18	5.0	0.2613	**0.0000**	0.2496
44	495	495	59	10	24	0.0	0.2949	0.2496	**0.0000**

The following taxa groups were created prior to calculating the Bray-Curtis-Distances for the ‘Lake’ sample:

1. Taxa were pooled that were both difficult to identify and named with and without uncertainty, i.e. taxa that were sometimes labelled with 'cf.' or 'aff.' by the auditors or participants:

   Group 1 (*Achnanthidium caledonicum*, *Achnanthidium* cf. *caledonicum*), **Group 2** (*Achnanthidium eutrophilum*, *Achnanthidium* cf. *eutrophilum*), **Group 3** (*Amphora indistincta*, *Amphora* cf. *indistincta*), **Group 4** (*Amphora pediculus*, *Amphora* cf. *pediculus*), **Group 5** (*Cymbella lange-bertalotii*, *Cymbella* cf. *lange-bertalotii*), **Group 6** (*Encyonopsis krammeri*, *Encyonopsis* cf. *krammeri*), **Group 7** (*Encyonopsis microcephala*, *Encyonopsis* cf. *microcephala*), **Group 8** (*Encyonopsis minuta*, *Encyonopsis* cf. *minuta*), **Group 9** (*Encyonopsis subminuta*, *Encyonopsis* cf. *subminuta*), **Group 10** (*Encyonopsis thumensis*, *Encyonopsis* cf. *thumensis*), **Group 11** (*Fragilaria construens* f. *venter*, *Fragilaria construens* cf. f. *venter*), **Group 12** (*Gomphonema exilissimum*, *Gomphonema* cf. *exilissimum*), **Group 13** (*Gomphonema minusculum*, *Gomphonema* cf. *minusculum*), **Group 14** (*Navicula cryptotenella*, *Navicula* cf. *cryptotenella*), **Group 15** (*Navicula cryptotenelloides*, *Navicula* cf. *cryptotenelloides*)

2. Identical taxa with varying names (synonyms) were pooled, in combination with previous criterion:

   Group 16 (*Fragilaria capucina* var. *vaucheriae*, *Fragilaria vaucheriae*, *Fragilaria capucina* cf. var. *vaucheriae*, *Fragilaria* cf. *vaucheriae*)

3. Species were pooled with their nominate variety, morphotype or form, where applicable in combination with above criteria:

   Group 17 (*Cymbella cymbiformis*, *Cymbella cymbiformis* var. *cymbiformis*), **Group 18** (*Cymbella helvetica*, *Cymbella helvetica* var. *helvetica*), **Group 19** (*Encyonema cespitosum*, *Encyonema cespitosum* var. *cespitosum*), **Group 20** (*Encyonema ventricosum*, *Encyonema ventricosum* morphotype 2), **Group 21** (*Fragilaria brevistriata*, *Staurosira brevistriata*, *Staurosira* cf. *brevistriata*, *Fragilaria brevistriata* var. *brevistriata*), **Group 22** (*Fragilaria ulna*, *Fragilaria ulna* var. *ulna*), **Group 23** (*Gomphonema parvulum* var. *parvulum*, *Gomphonema parvulum* var. *parvulum* f. *parvulum*), **Group 24** (*Gomphonema pumilum*, *Gomphonema pumilum* var. *pumilum*), **Group 25** (*Navicula radiosa*, *Navicula radiosa* var. *radiosa*), **Group 26** (*Rhopalodia gibba*, *Rhopalodia gibba* var. *gibba*)

4. Taxa were pooled into groups that are taxonomically difficult to differentiate using light microscopy; where applicable in combination with above criteria:

   Group 27 (*Planothidium frequentissimum*, *Planothidium frequentissimum* var. *frequentissimum*, *Planothidium frequentissimum* var. *magnum*), **Group 28** (*Achnanthidium minutissimum*, *Achnanthidium minutissimum* var. *minutissimum*, *Achnanthidium minutissimum* cf. var. *minutissimum*, *Achnanthidium minutissimum* var. *jackii*, *Achnanthidium minutissimum* cf. var. *jackii*, *Achnanthidium lineare*, *Achnanthidium neomicrocephalum*, *Achnanthidium pseudolineare*), **Group 29** (*Cocconeis placentula*, *Cocconeis placentula* var. *placentula*, *Cocconeis placentula* var. *lineata*, *Cocconeis placentula* var. *euglypta*), **Group 30** (*Cymbella neoleptoceros*, *Cymbella neoleptoceros* var. *neoleptoceros*, *Cymbella neoleptoceros* var. *tenuistriata*), **Group 31** (*Fragilaria pinnata*, *Fragilaria pinnata* var. *pinnata*, *Fragilaria pinnata* var. *lancettula*), **Group 32** (*Karayevia clevei*, *Karayevia clevei* var. *clevei*, *Karayevia clevei* var. *rostrata*), **Group 33** (*Nitzschia fonticola*, *Nitzschia fonticola* var. *fonticola*, *Nitzschia fonticola* cf. var. *fonticola*, *Nitzschia costei*), **Group 34** (*Nitzschia palea*, *Nitzschia palea* var. *debilis*, *Nitzschia palea* var. *palea*, *Nitzschia palea* var. *tenuirostris*), **Group 35** (*Cymbella excisa*,

Cymbella excisa var. *excisa*, *Cymbella excisiformis*, *Cymbella* cf. *excisiformis*, *Cymbella parva*, *Cymbella* cf. *parva*, *Cymbella perparva*, *Cymbella* cf. *perparva*, *Cymbella kappii*, *Cymbella vulgata*, *Cymbella* cf. *vulgata*, *Cymbella vulgata* var. *vulgata*, *Cymbella vulgata* cf. var. *vulgata*)

About half of the participants had large difficulties with identifying *Cymbella vulgata* in the ‘Lake’ sample (see Chapter 4.4.2). Therefore, *Cymbella* (cf.) (var.) *vulgata* was pooled into one group with species that are similar to *Cymbella vulgata* according to Krammer (2002) and that were found by the participants (**Group 35**). Additionally, *Cymbella kappii* was added to Group 35, as it is very similar to *C. perparva* (see discussion in Chapter 4.4.2).

The similarity among counting results (relative abundances of the diatom objects) of participants and auditors was assessed using the Bray-Curtis-Distance. The average Bray-Curtis-Distance of the counting results among auditors was 0.2686 with a standard deviation of 0.0235 and thus a 95 %-confidence interval of permitted range of **0.1517 to 0.3855** for the **‘Lake’ sample**. If a Bray-Curtis-Distance of a participant was outside this confidence interval of an auditor, the distance is marked in red and bold in Table 6. If the Bray-Curtis-Distance of a participant was outside the confidence interval of all three auditors, then the results were too dissimilar to the results of the auditors, i.e. the sample was counted "unsuccessfully". For the ‘Lake’ sample this dissimilarity pertained to 14 participants (35 %, Table 6).

For the ‘Lake’ sample the Detrended Correspondence Analysis (DCA) confirms the results of the Bray-Curtis-Distances (Fig. 2, displaying the first and second axis), when also reviewing the third dimension (not shown). The counting results of 14 participants with high Bray-Curtis-Distances (Table 6, red outline in Fig. 2) are distinctly apart from the counting results of the auditors (L42-L44). The counting results of the auditors were similar and therefore are located very close to each other and to most participants (65 %, Fig. 2).

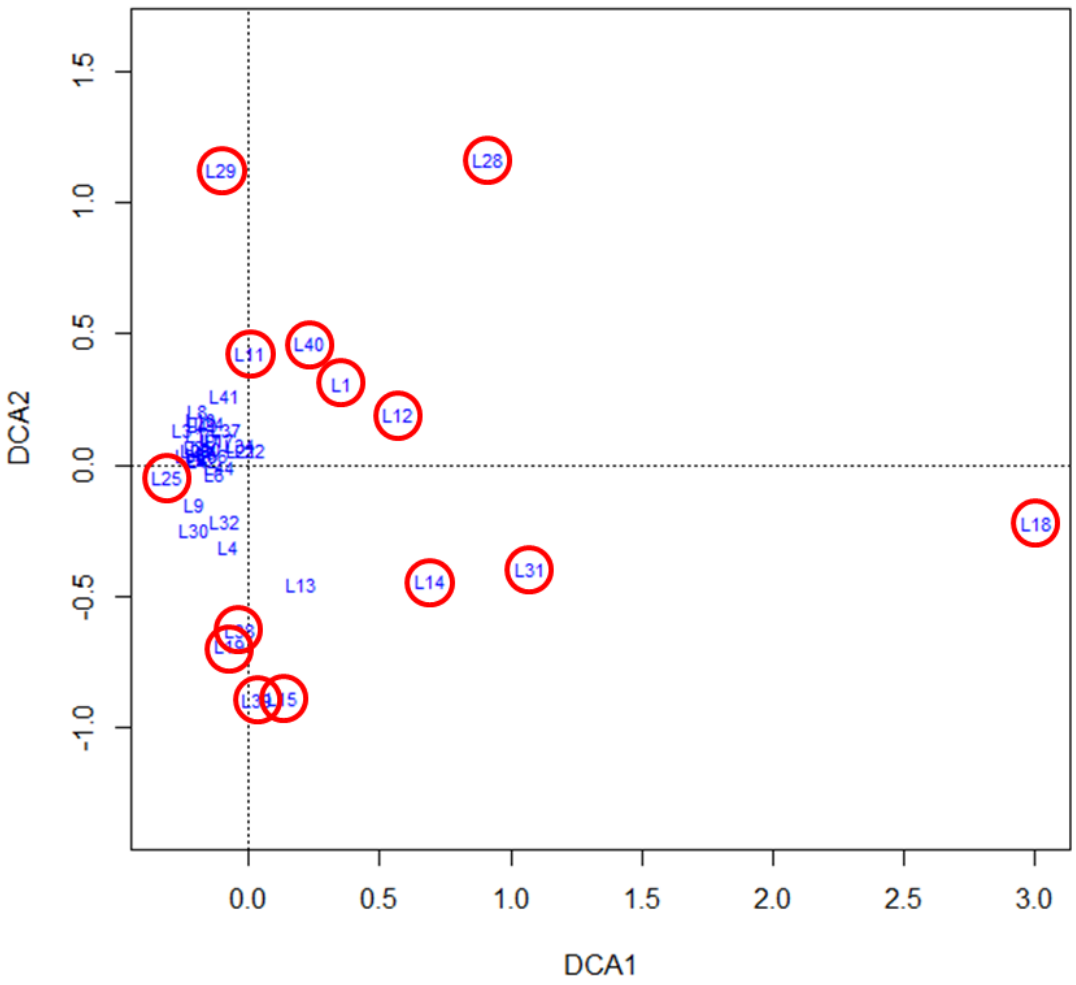


Figure 2: First and second axis of the DCA based on the diatom results of all participants and auditors of the ‘Lake’ sample. Numbers correspond to the laboratory codes. Results from participants of the red-rimmed laboratory codes were outside the confidence-intervals of all three auditors based on the Bray-Curtis-Distances, i.e. these participants counted this sample unsuccessfully.

4.2 Microscope Specifications

In addition to the counting results, all participants and auditors had to provide information about the equipment used for counting (Fig. 3).

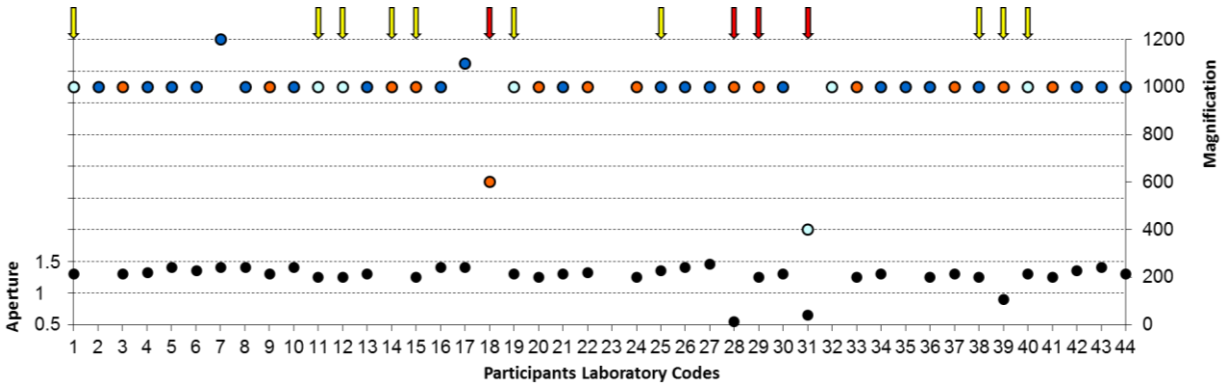


Figure 3. Microscope specifications used by participants and auditors. Legend: Coloured circles indicate used magnification and used optical illumination technique (light blue: bright-field, dark blue: differential interference contrast (DIC), orange: phase-contrast). Solid circles: aperture of lens. Arrows mark the participants that counted one (yellow) or both (red) samples unsuccessfully.

Overall, most participants and all auditors conducted the diatom analyses using differential interference contrast (DIC; 51.2 %, n=22/43), an aperture of ≥ 1.3 (58.1 %) and a magnification of ≥ 1000 X (95.3 %)(Fig. 3). Two participants that used a lower magnification than 1000 X (400 X, 600 X) counted the samples unsuccessfully (Fig. 3). Also, 35 % of the participants used phase-contrast and 17.5 % of the participants used bright-field. Most of these participants counted at least one sample unsuccessfully, while the 26 participants that counted both samples successfully used DIC or phase-contrast and only in one case bright-field (Fig. 3).

The auditors used lens apertures of 1.35, 1.4 and 1.3, respectively. Five participants did not specify the lens aperture used, while ten participants used an aperture of 1.25. Participants that counted the samples unsuccessful mainly used a low aperture, including distinctly lower apertures of 0.55, 0.65 and 0.9. Only five of the 26 successful participants used a lens aperture of 1.25, the remaining 21 participants used a higher aperture (>1.25, Fig. 3).

4.3 Details of taxonomic problems in the ‘Stream’ sample

4.3.1 *Cocconeis placentula*-aggregate

The participants of the intercalibration exercise had no problem identifying the species *Cocconeis placentula*. However, they had large difficulties to differentiate the varieties of the *Cocconeis placentula*-aggregate. Similarly, the auditors differed in their allocation of the varieties (Fig. 4-8).

In the ‘Stream’ sample the auditors identified the *C. placentula*-aggregate (*C. placentula* with and without differentiation of varieties) with a relative abundance of 5.0 %, 6.5 % and 7.1 % (Fig. 4). The participants identified this aggregate with 0.8 % to 16.3 %, with an average of 7.6 % relative abundance (n=39). Therefore, the counting results of three participants differed distinctly from both, the results of the auditors and the average of the participants, with 0 %, 0.8 % and 16.3 % relative abundances of the *C. placentula*-aggregate, respectively (Fig. 4).

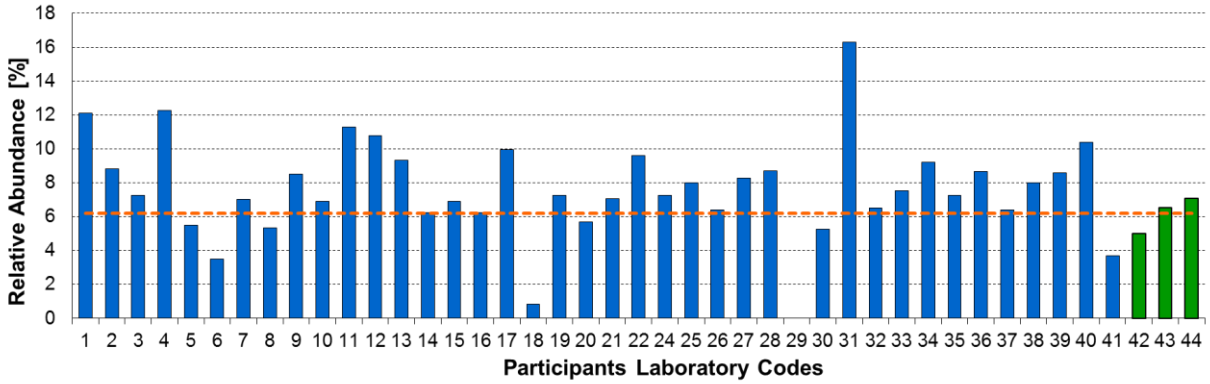


Figure 4. The relative abundance of ***Cocconeis placentula*-aggregate** (*Cocconeis placentula*, *C. placentula* var. *lineata*, *C. placentula* var. *euglypta* and *C. placentula* var. *placentula*) determined by each participant in the ‘Stream’ sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

Cocconeis placentula var. *lineata* was detected by 24 participants with a relative abundance of 0.2 % to 12.1 % (average: 3.6 %, n=24) in the ‘Stream’ sample (Fig. 5). Also, 21 participants found *C. placentula* var. *euglypta* with 0.5 % to 7.5 % (average: 3.4 %) (Fig. 6). *C. placentula* var. *placentula* was detected by 13 participants with 0.2 % to 5.3 % (average: 2.1 %) (Fig. 7) and *C. placentula* without differentiation of the varieties by 16 participants with 0.8 % to 16.3 % (average: 7.5 %)(Fig. 8). Of these 16 participants, only four additionally identified one or more varieties of *C. placentula*.

Two of the three auditors detected *C. placentula* var. *lineata* with a relative abundance of 4.3 % and 7.1 %. *C. placentula* var. *euglypta* was identified by two auditors with 0.3 % and 2.0 %. One auditor identified *C. placentula* var. *placentula* with 4.8 % (Fig. 5-7). Therefore, the three varieties (*lineata*, *euglypta* and *placentula*) are hardly distinguishable following the current taxonomic concept (Plate 1).

Despite the difficulties of most participants in identifying the varieties *placentula*, *lineata* and *euglyta* of *C. placentula*, no participants (and also no auditor) listed a taxon as determined with uncertainty (designation of ”cf.“ or ”aff.“).

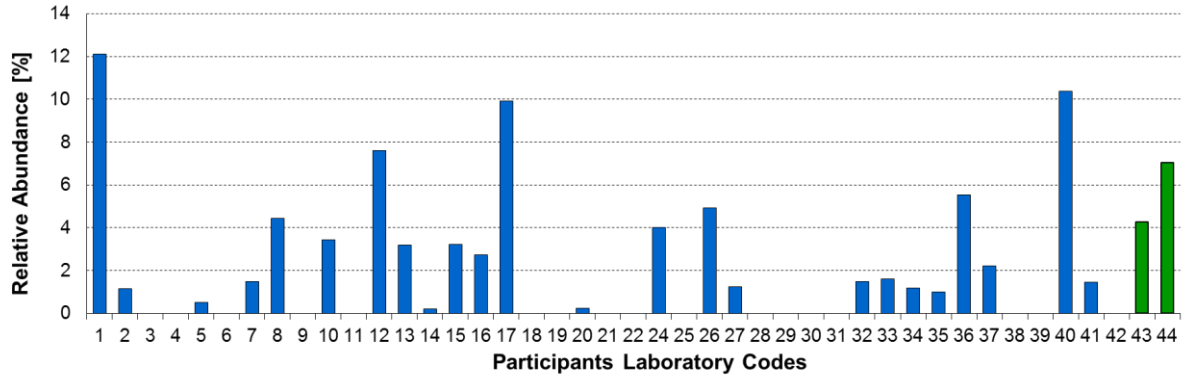


Figure 5. The relative abundance of ***C. placentula* var. *lineata*** determined by each participant in the ‘Stream’ sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44).

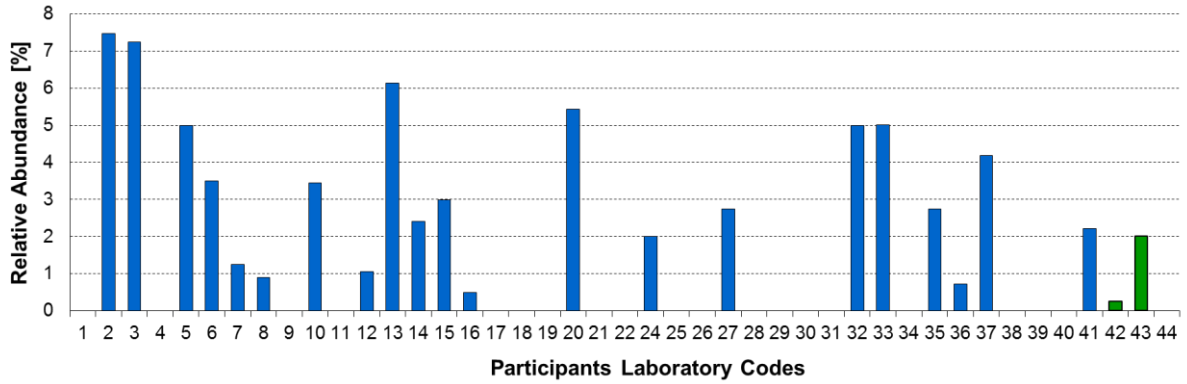


Figure 6. The relative abundance of ***C. placentula* var. *euglypta*** determined by each participant in the ‘Stream’ sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44).

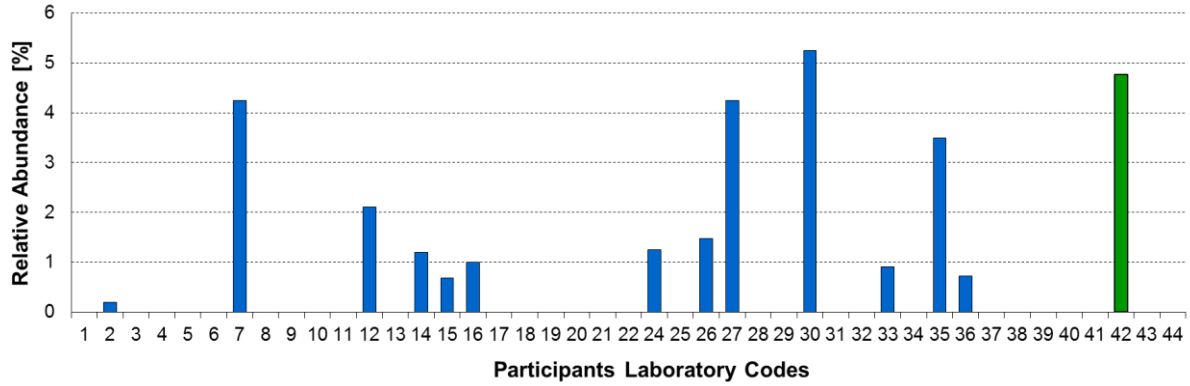


Figure 7. The relative abundance of ***C. placentula* var. *placentula*** determined by each participant in the ‘Stream’ sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44).

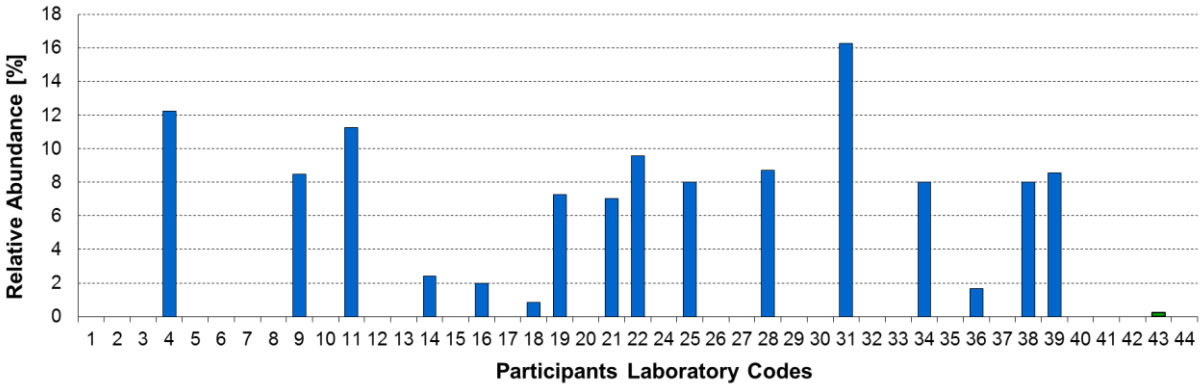


Figure 8. The relative abundance of ***C. placentula*** without differentiation of varieties determined by each participant in the ‘Stream’ sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44).

A detailed discussion about the taxonomic differentiation of the varieties of *Cocconeis placentula* and how to deal with the *C. placentula*-aggregate when inferring the water quality using the German PHYLIB-method is provided in the report of the first German intercalibration exercise (Dreßler et al. 2014). The importance of a harmonized differentiation of the varieties also becomes apparent, when assessing the water quality using the German PHYLIB-method. A different allocation of the very common varieties may lead to different assessments. For example, if a single reference species occurs with more than 40 %, the sum of the reference species abundances is reduced by 20 %, which may easily reduce the water quality assessment by one class (e.g. from good to moderate). For most lotic system water-types, the varieties *lineata* and *euglypta* are type-specific reference species. Thus, if the *C. placentula* abundances are allocated only to one variety, the 40 % limit is more likely reached than allocating the abundances to two varieties.

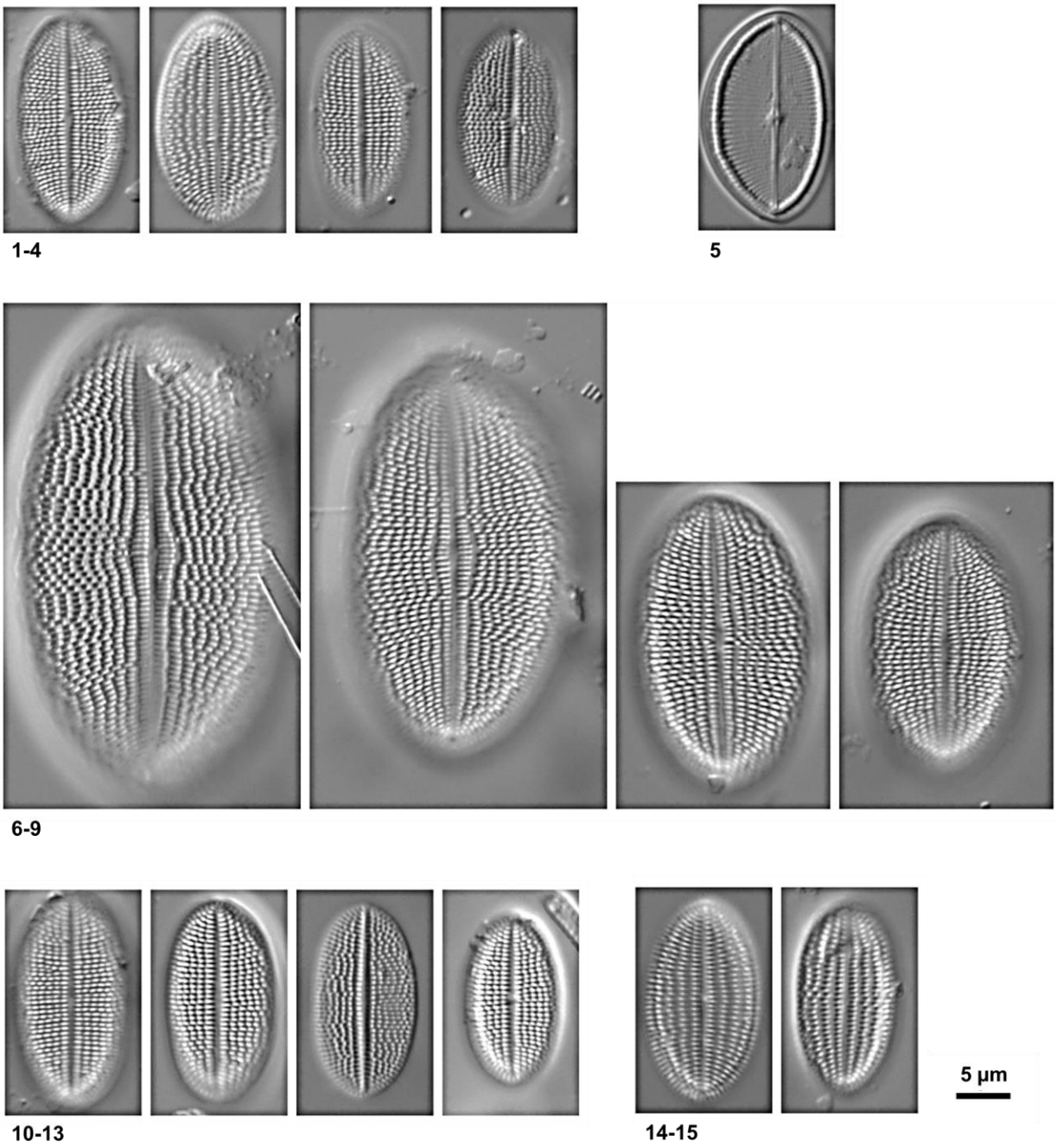


Plate 1. Valves of *Cocconeis placentula*-varieties according to the taxonomic concept of Krammer & Lange-Bertalot (1986-2004) from the ‘Stream’ sample. **1-4:** *C. placentula* var. *placentula* (as striae density is 24-26 in 10 µm), appearance more like: *C. placentula* var. *lineata*; **5:** raphe valve; **6-13:** *C. placentula* var. *lineata* (23 or less striae in 10 µm and more than five areolae / stria); **14-15:** *C. placentula* var. *euglypta* (21 striae in 10 µm and 4 areolae / stria; rows of striae almost straight, i.e. not distinctly zigzagging; however, both valves could also be identified as *C. placentula* var. *lineata*, as the characteristics also match).

4.3.2 *Gomphonema parvulum*-aggregate

In this intercalibration exercise the identification of *Gomphonema parvulum* and its differentiation from other *Gomphonema*-species and its other forms (*parvulum* and *saprophilum*) posed problems.

The auditors determined the *G. parvulum*-aggregate (including: *G. parvulum* var. *parvulum*, *G. parvulum* var. *parvulum* f. *parvulum* and *G. parvulum* var. *parvulum* f. *saprophilum*) with relative abundances ranging from 2.7 - 5.0 % (average: 3.9 %) in the ‘Stream’ sample (Fig. 9). Most participants (37) found the aggregate with 0.7 % to 6.0 % (average: 2.8 %). Three participants did not detect any valves from the *G. parvulum*-aggregate (Fig. 9). According to Hofmann et al. (2013), *Gomphonema parvulum* var. *parvulum* may be mistaken for *G. exilissimum* and *G. innocens*. The latter was not identified by any participants in this intercalibration exercise. However, *G. exilissimum* was identified at low abundances by four participants, of which only one participant indicated some ambiguity (0.2 % of *G.* cf. *exilissimum* by Laboratory Code 33) (Fig. 10). The auditors did not determine any *G. exilissimum* in the ‘Stream’ sample.

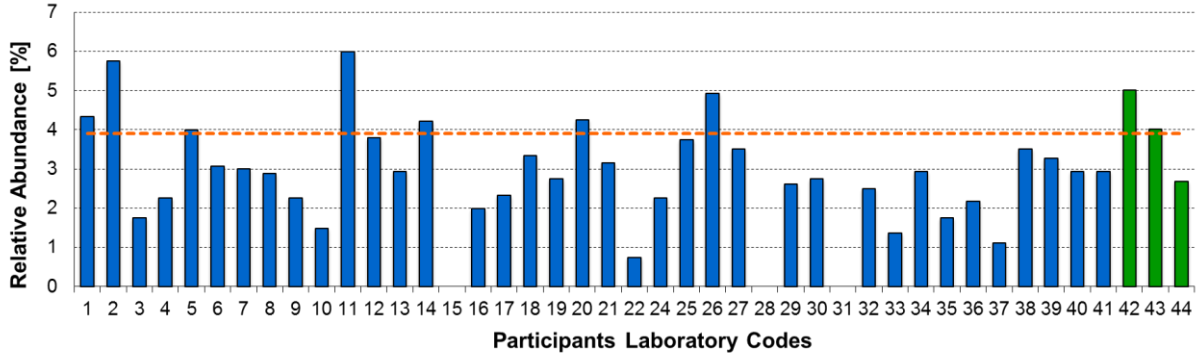


Figure 9. The relative abundance of ***Gomphonema parvulum*-aggregate** determined by each participant in the ‘Stream’ sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

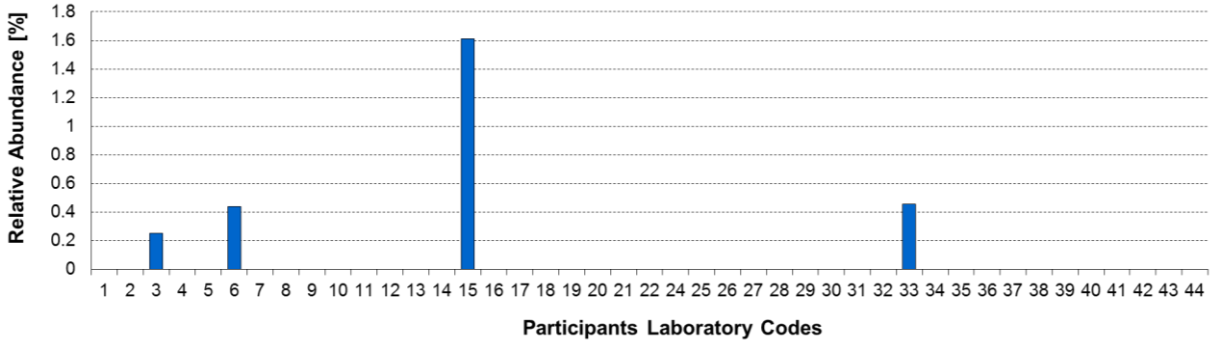


Figure 10. The relative abundance of ***Gomphonema exilissimum*** determined by each participant in the ‘Stream’ sample. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

According to the German instruction protocols (Schaumburg et al. 2011c & 2012) each diatom taxon needs to be identified to the highest possible taxonomic level. One auditor and 11 of the 38 participants that identified *G. parvulum* var. parvulum, did not differentiate the forms (Fig. 11). *G. parvulum* var. *parvulum* f. *parvulum* was determined by two auditors and 25 participants (Fig. 12). No

auditor identified the form *saprophilum*. One participant identified only the form *saprophilum* of *G. parvulum* var. *parvulum* with a relative abundance of 6.0 %. Three participants also identified *G. parvulum* var. *parvulum* f. *saprophilum* in abundances <1.0 % in addition to the form *parvulum* (Fig. 13).

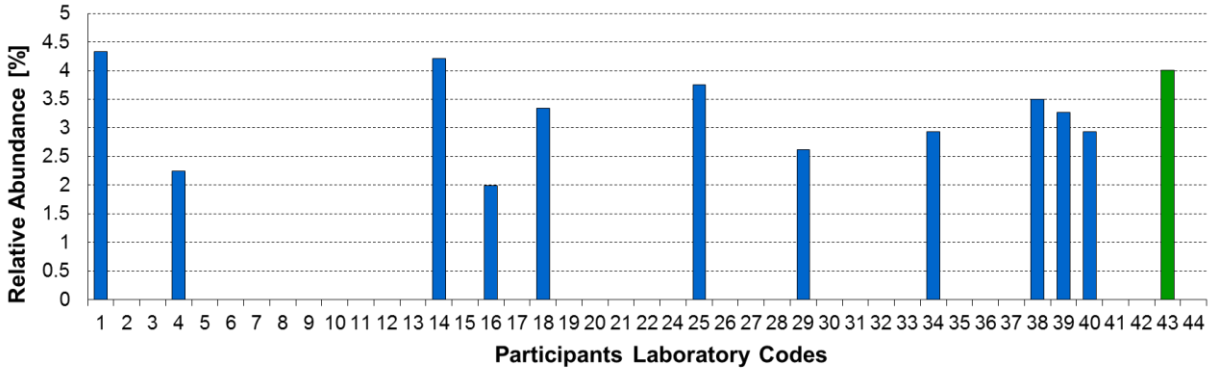


Figure 11. The relative abundance of ***Gomphonema parvulum* var. *parvulum*** determined by each participant in the ‘Stream’ sample. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

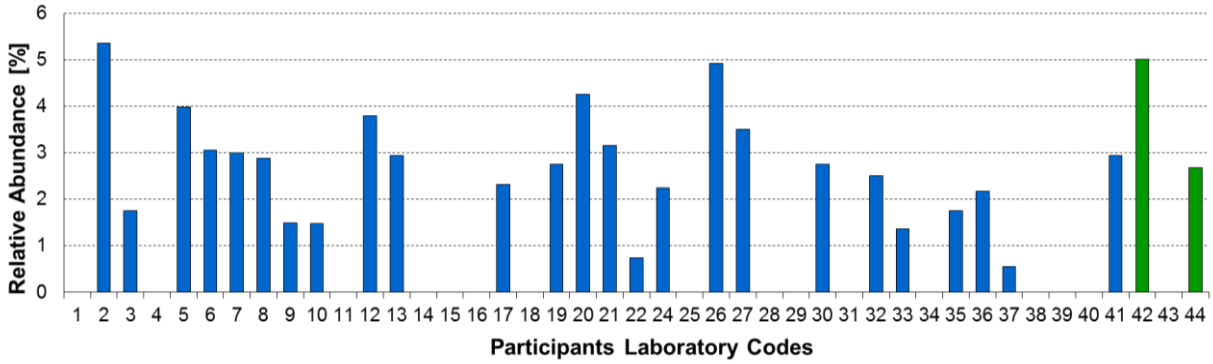


Figure 12. The relative abundance of ***Gomphonema parvulum* var. *parvulum* f. *parvulum*** determined by each participant in the ‘Stream’ sample. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

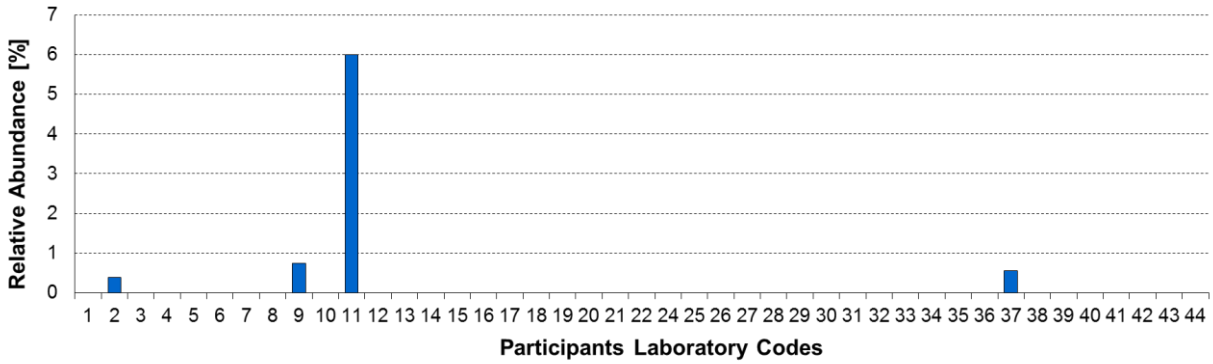


Figure 13. The relative abundance of ***Gomphonema parvulum* var. *parvulum* f. *saprophilum*** determined by each participant in the ‘Stream’ sample. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

Overall, the identification of *Gomphonema parvulum* var. *parvulum* did not seem to pose any problems to most of the participants in the intercalibration exercise. However, one participant mistook *G. parvulum* for *G. exilissimum*. Most valves of *G. parvulum* var. *parvulum* are wider than valves from *G. exilissimum* (Table 7). Additionally, small valves of *G. parvulum* var. *parvulum* are more distinctly elliptic-club-like in shape compared to the more lanceolate shape of *G. exilissimum* (Hofmann et al. 2013; Plate 2). Additionally, the valve ends of *G. exilissimum* are often slightly bent (Hofmann et al. 2013). With 7-20 striae per 10 µm, the striae density of *G. parvulum* var. *parvulum* is very variable, while *G. exilissimum* has a smaller range (12-14 striae/10 µm) (Hofmann et al. 2013). The two taxa can be distinguished relatively well by their length to width ratio (Jüttner et al. 2013; Table 7). It is important to separate these two taxa, as their (weighted) indicator values differ distinctly in the German PHYLIB-software that assesses the water quality (Table 10), and identification affects the inferred ecological status class of the sample (Chapter 4.5).

Table 7. Characteristics to compare *Gomphonema parvulum* var. *parvulum* from *G. exilissimum* (**Hofmann et al. 2013**; *Jüttner et al. 2013).

Taxon	**Length (µm)**	**Width (µm)**	**Striae/10 µm**	**Length/Width**
G. parvulum var. *parvulum*	10-36	5-8	7-20	< 3.9
G. exilissimum	20-38	4.5-6	12-14	3.9-6.8*

As required, most participants differentiated the forms of *Gomphonema parvulum* var. *parvulum*. The results of the auditors and participants suggest that the 'Stream' sample mainly contained the form *parvulum*. Morphologically, the forms *parvulum* and *saprophilum* can only be distinguished by valve width according to Hofmann et al. (2013)(Table 8).

Table 8. Valve characteristics that differentiate *Gomphonema parvulum* var. *parvulum* f. *parvulum* from *Gomphonema parvulum* var. *parvulum* f. *saprophilum* (**Hofmann et al. 2013)**.

Taxon	**Length (µm)**	**Width (µm)**	**Striae/10 µm**
G. parvulum var. *parvulum*	10-36	5-8	7-20
forma *parvulum*		5-6.5	
forma *saprophilum*		6-8	

Abarca et al. (2014) corrected the species diagnose of *Gomphonema parvulum* var. *parvulum* and *G. parvulum* var. *parvulum* f. *saprophilum* and changed the latter to species level, i.e. to *Gomphonema saprophilum* (Lange-Bertalot & Reichardt) Abarca, R. Jahn, J. Zimmermann & Enke. With the omission of the form *saprophilum* and the transitions of the other varieties to species level (*G. exilissimum*, *G. lagenula* and *G. parvulius*), the variety and form *parvulum* have become superfluous. Consequently, the above taxa are called *Gomphonema parvulum*, *G. saprophilum* and *G. exilissimum* according to Abarca et al. (2014) (Table 9). Following a slightly different new concept (Levkov et al. 2016) the characteristics of former *G. parvulum* var. *parvulum* f. *saprophilum* according to Lange-Bertalot (1993) and following Hofmann et al. (2013)(Table 8) is allocated to the range of *G. parvulum*. This recent taxonomic correction could be the reason for participants not differentiating the forms of *G. parvulum* var. *parvulum*.

Gomphonema parvulum may also be mistaken for *G. varioreduncum* Jüttner, Ector, E. Reichardt, Van de Vijver & E.J. Cox, *G. lagenula* Kützing and *G. parvulius* (Lange-Bertalot & E. Reichardt) Lange-Bertalot & E. Reichardt. *G. varioreduncum* is characterised by typically bent ends and is common in

moderately acidic pH waters (Jüttner et al. 2013). Valves of *G. lagenula* are not as heteropolar as *Gomphonema parvulum*, while *G. parvulius* is commonly smaller and thinner.

Table 9. Important characteristics for the identification of *Gomphonema parvulum*, *G. saprophilum* and *G. exilissimum* according to **Jüttner et al. (2013), Abarca et al. (2014) and Lange-Bertalot et al. (2017)**.

Taxon	**Length (µm)**	**Width (µm)**	**Striae/ 10 µm**	**Comment**
G. parvulum	22-29	5.0-7.5	12-20	Valves lanceolate, linearly lanceolate or ovally heteropolar, ends shortly rostrate
G. saprophilum	22-27	6.0-8.0	12-20	Valves club-like lanceolate, shape somewhat rhomboid, ends only shortly protracted, **head poles are wider compared to *G. parvulum***
G. exilissimum	20-39	4.3-6.0	12-16	Valves thinly lanceolate and naviculoid, ends slightly and shortly protracted, sometimes slightly bent

Table 10. Saprobic values (**S**), trophic values (**T**) and weighting (**G**) of the *Gomphonema parvulum*-aggregate for lotic systems and lakes as listed in the PHYLIB software (version 5.3, December 2015).

Taxon	**Lotic Systems**				**Lakes**		
	S	**G**	**TI**	**G**	**TI_{North}**	**TI_{South}**	**G**
G. parvulum	-	-	-	-	-	-	-
G. parvulum var. *parv.* f. *parvulum*	-	-	3.6	2.0	2.95	-	-
G. parvulum var. *parv.* f. *saprophilum*	-	-	3.6	2.0	2.95	-	-
G. exilissimum	-	-	0.7	2.0	0.98	-	-

We recommend using the taxonomic concept of Lange-Bertalot et al. (2017) (summarized in Table 9) when identifying and differentiating the species *Gomphonema parvulum*, *G. saprophilum* and *G. exilissimum*. Lange-Bertalot et al. (2017) is the supplemented, taxonomically revised and translated (German to English) edition of Hofmann et al. (2013) and presents the revised taxonomy of the three species according to Jüttner et al. (2013) and Abarca et al. (2014). According to Lange-Bertalot et al. (2017), *Gomphonema parvulum* and *G. saprophilum* are mainly differentiated by their valve width (as also in Hofmann et al. 2013) and by the wider head poles of *G. saprophilum* compared to *G. parvulum* (Table 9).

In the German PHYLIB-software for assessing the water quality (version 5.3, December 2015) *Gomphonema parvulum* and *G. saprophilum* have identical indicator values and weights (Table 10). However, it is important to enter the names *G. parvulum* var. *parvulum* f. *parvulum* (German data processing number (DV-no.) 6158) and *G. parvulum* var. *parvulum f. saprophilum* (DV-no. 16535) into the current software. The name *G. parvulum* (DV-no. 16572) has no trophic- or saprobic value (or weighting) listed in the PHYLIB-software (Table 10), and therefore the relative abundances would not be incorporated into the assessment. *Gomphonema saprophilum* is not (yet) listed in the PHYLIB-software (version 5.3, December 2015).

To reduce the discrepancy of an assessment with the PHYLIB-training set that was based on the taxonomic knowledge of 2003 and earlier (Mauch et al. 2003, from 2011) and the current taxonomic concept (according to Lange-Bertalot et al. 2017), we recommend a recount of the training set using the recent taxonomic knowledge.

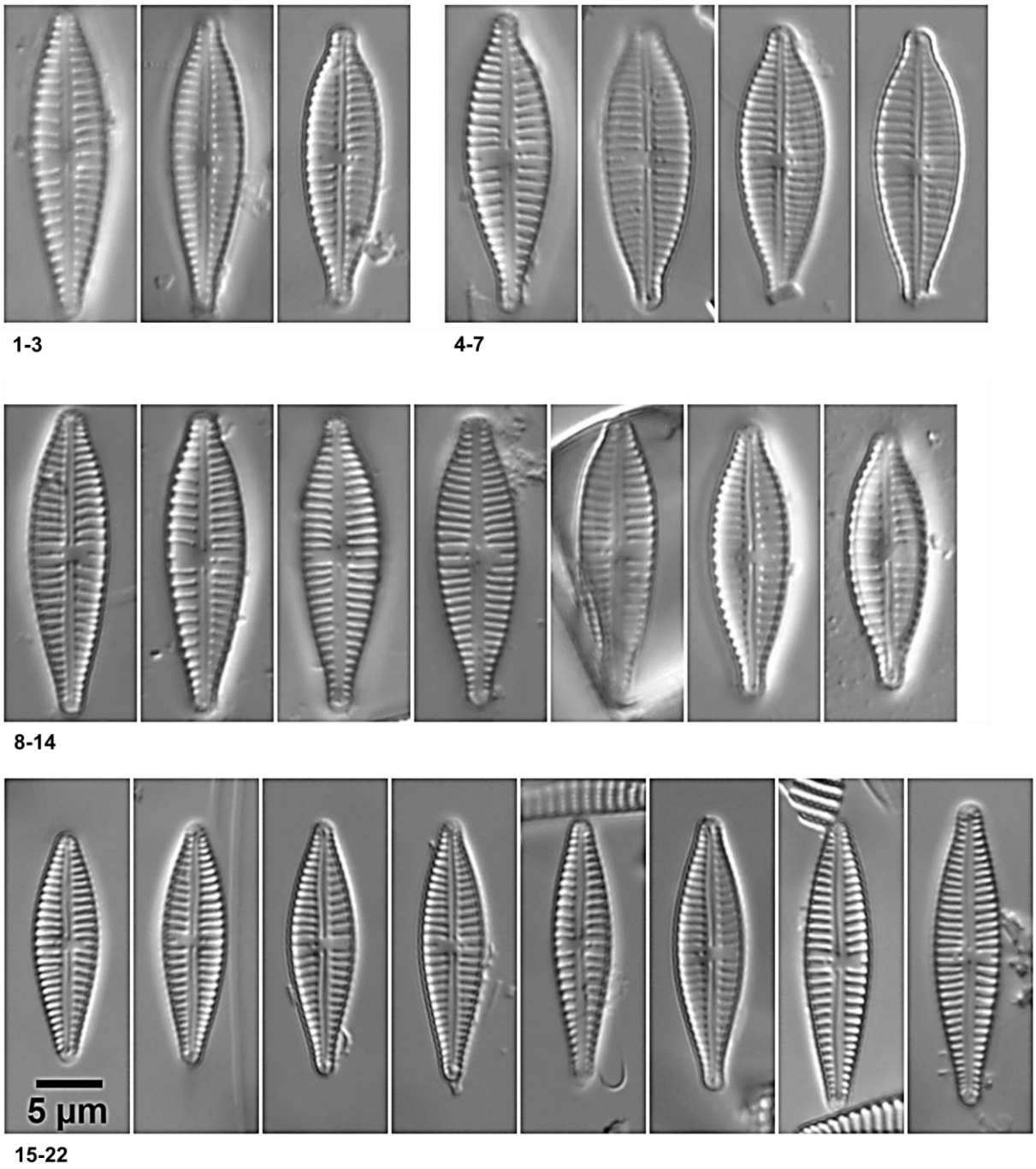


Plate 2. Valves of the ***Gomphonema parvulum***-aggregate (**1-14**) according to the taxonomic concept of Krammer & Lange-Bertalot (1986-2004) and Hofmann et al. (2013) from the ‘Stream’ sample and, for comparison, ***G. exilissimum*** (**15-22**) from the ‘Lake’ sample. (**1-3**)**:** *Gomphonema parvulum* var. *parvulum* **f. *parvulum*** (due to a valve width of 5-6 µm), (**4-7**)**:** *G. parvulum* var. *parvulum* **f. *saprophilum*** (valve width: 6.5-8 µm), (**8-14**)**:** *G. parvulum* var. *parvulum* f. *parvulum* or f. *saprophilum* (valve width: 6-6.5 µm).

Note: According to the new taxonomic concept of Levkov et al. (2016), the valves **1-14** are exclusively *Gomphonema parvulum* (Kützing) Kützing.

4.3.3 *Navicula cryptocephala*

Navicula cryptocephala was one of the dominant diatom species in the ‘Stream’ sample (Fig. 14). The auditors determined the relative abundance of *Navicula cryptocephala* (var. *cryptocephala*) to be 8.8 %, 8.5 % and 6.8 % (average: 8.0 %). The participants determined the relative abundance of *N. cryptocephala* (var. *cryptocephala*) to range from 5.0 % to 28.9 % (average: 9.9 %). Two participants (laboratory codes 6 and 14) documented some uncertainty about the taxonomic allocation by listing *Navicula* cf. *cryptocephala* with 4.6 % and 7.4 %, respectively. In contrast to most participants, three participants did not detect any *Navicula cryptocephala* (var. *cryptocephala*) and one participant detected obviously too high abundances (Fig. 14), suggesting that they had problems with the identification. Hofmann et al. (2013) list several species that can be mistaken for *N. cryptocephala*, of which only *N. veneta* was determined in low abundances by several participants and auditors (Fig. 15). The comparison of the counting results for *N.* (cf.) *veneta* and *Navicula* (cf.) *cryptocephala* (var. *cryptocephala*) suggest that they have been mistaken for each other by several participants (compare Fig. 14 & 15).

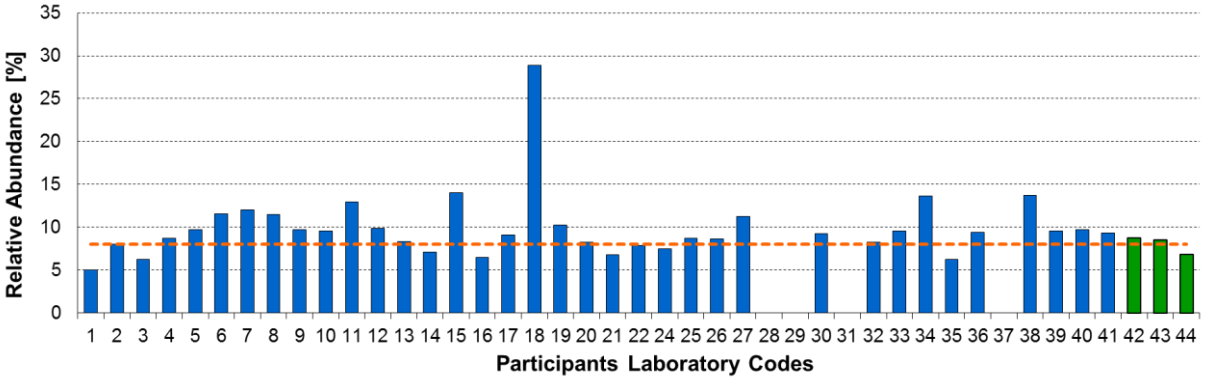


Figure 14. The relative abundance of ***Navicula cryptocephala, N. cryptocephala var. cryptocephala*** and ***N.* cf. *cryptocephala*** determined by each participant in the 'Stream' sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

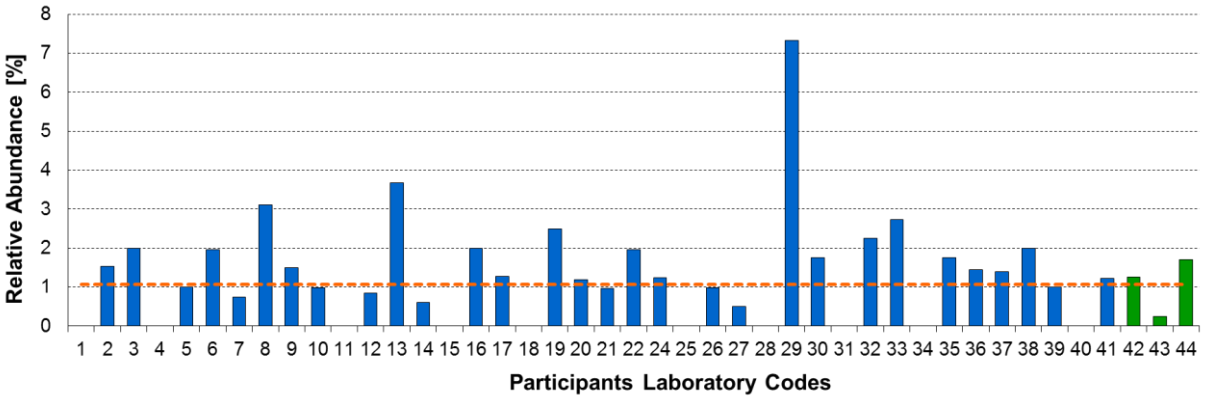


Figure 15. The relative abundance of ***Navicula veneta*** and ***N.* cf. *veneta*** determined by each participant in the ‘Stream’ sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

Navicula cryptocephala is usually larger and has a higher striae density than *N. veneta* (Table 11). Additionally, both species can be distinguished with certainty using shape and striae orientation. Striae are distinctly more strongly radial in *N. cryptocephala* compared to *N. veneta* (Plate 3). The central area of *N. veneta* is relatively small and is commonly only made of two shortened central striae on each side. In contrast, *N. cryptocephala* has a medium-sized, round to elliptical central area (Plate 3).

For a definite identification of *N. cryptocephala* and its distinction to similar taxa (e. g. *N. veneta*), all characteristics (Plate 3) that are provided in Table 11 and Hofmann et al. (2013) need to be taken into account.

Table 11. Characteristics for the identification of *Navicula cryptocephala* in comparison to *N. veneta*. Source: Hofmann et al. (2013).

Taxon	**Length (µm)**	**Width (µm)**	**Striae/10 µm**	**Comment**
N. cryptocephala	20-40	5-7	14-18	Striae strongly radial
N. veneta	13-30	4.4-6	13.5-15	Striae weakly radial

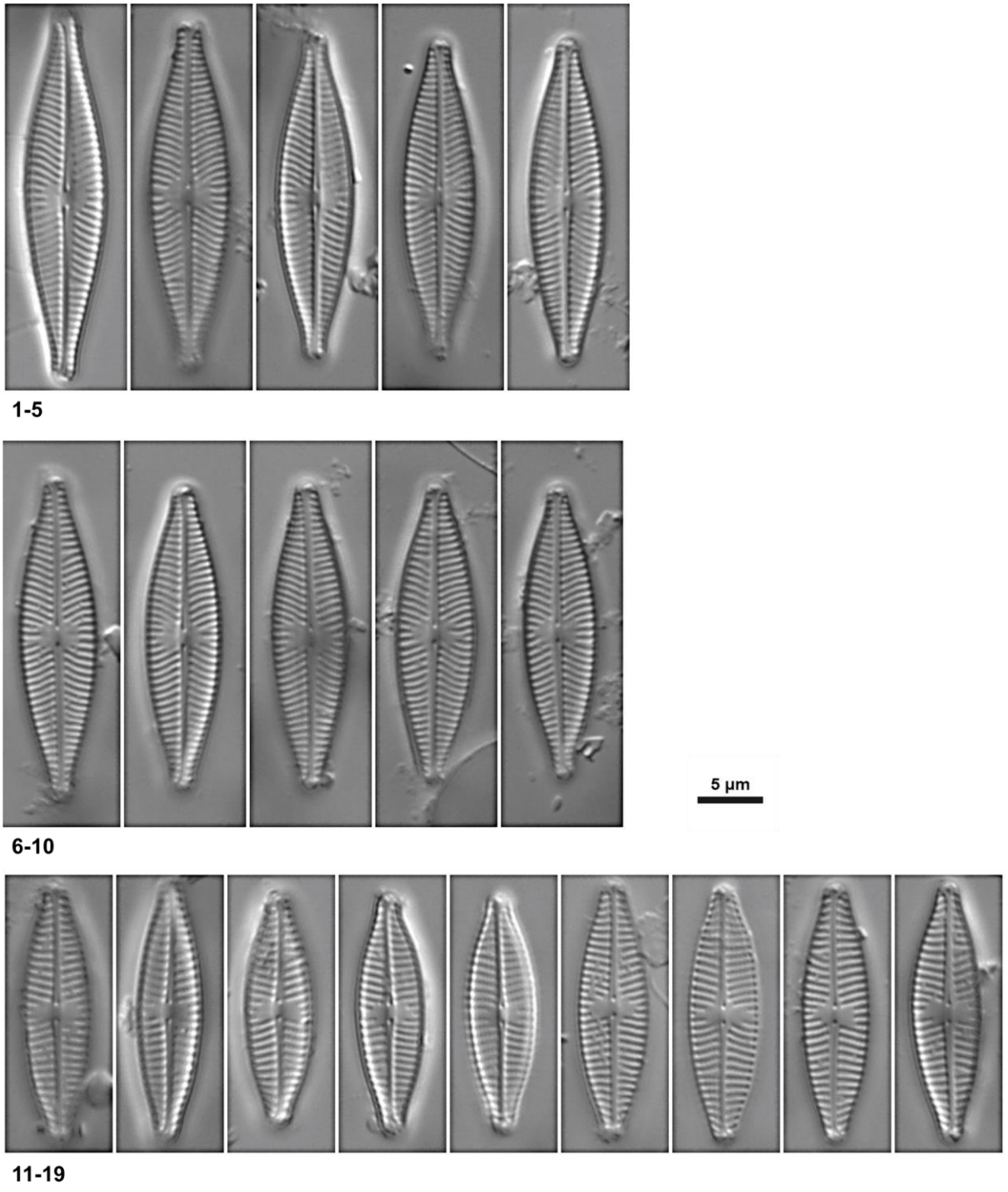


Plate 3. Comparison of *Navicula cryptocephala* **(1-10)** and *N. veneta* **(11-19)** from the ‘Stream’ sample.

4.3.4 *Nitzschia paleacea*

Nitzschia paleacea was one of the abundant diatom species in the ‘Stream’ sample (Fig. 16). The auditors determined *Nitzschia paleacea* with relative abundances of 6.0 %, 4.3 % and 3.2 % (average: 4.5 %). The participants determined the relative abundance of *Nitzschia paleacea* to range from 0.2 % to 10.1 % (average: 3.6 %; n=33). Seven participants did not detect any *Nitzschia paleacea*. These results suggest that identifying *Nitzschia paleacea* at all and in appropriate abundances posed problems to participants, i.e. distinguishing this species clearly from other taxa was problematic (Fig. 16). Only one participant (Laboratory Code 19) indicated some uncertainty about the taxonomic allocation by listing *N.* cf. *paleacea* with 0.25 %.

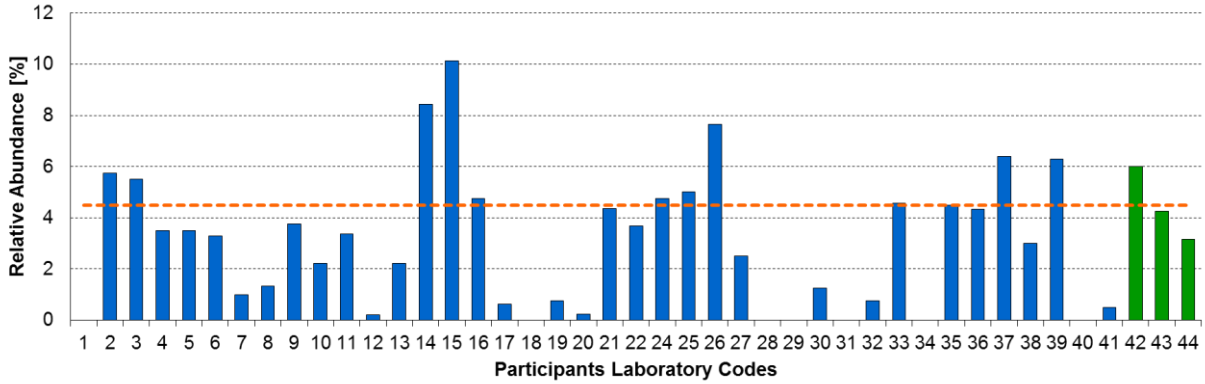


Figure 16. Sum of the relative abundance of ***Nitzschia paleacea*** and ***N.* cf. *paleacea*** determined by each participant in the ‘Stream’ sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

According to Krammer & Lange-Bertalot (1999) and Hofmann et al. (2013), *Nitzschia paleacea* may be mistaken for *N. archibaldii*, *N. palea*, *N. gracilis* and *N. graciliformis*. The latter was either not determined or excluded from the count according to protocol by the participants, as this species is planktonic.

Nitzschia archibaldii was determined with a relative abundance of 3.2 % to 3.5 % (average: 3.4 %) by the auditors in the ‘Stream’ sample and with zero (19 participants) to 5.7 % by the participants (Fig. 17). Seven participants did not identify either *N. archibaldii* or *N. paleacea* (Fig. 16 & 17) suggesting some overall problems with identifying taxa in the *Nitzschia* genus. Also, three participants identified distinctly above average abundances of *N. paleacea*, but no *N. archibaldii*-valves, suggesting a misidentification of *N. archibaldii* with *N. paleacea* (Fig. 16 & 17).

The *Nitzschia palea*-aggregate (see detailed discussion in the next Chapter 4.3.5) can also be mistaken for *N. paleacea* and were identified with relative abundances of 7.5 %, 7.8 % and 4.4 % by the auditors in the ‘Stream’ sample (Fig. 19, Chapter 4.3.5). Some participant results indicate problems with distinguishing these *Nitzschia*-species. On average, the auditors identified *Nitzschia paleacea* with relative abundances of 4.5 %, *N. archibaldii* with 3.4 % and the *Nitzschia palea*-aggregate with 6.6 %. In contrast, one participant only identified *N. paleacea* with 10.1 % of the three taxa, while three participants mainly identified the *Nitzschia palea*-aggregate with relative abundances of >15 % (Fig. 16-18).

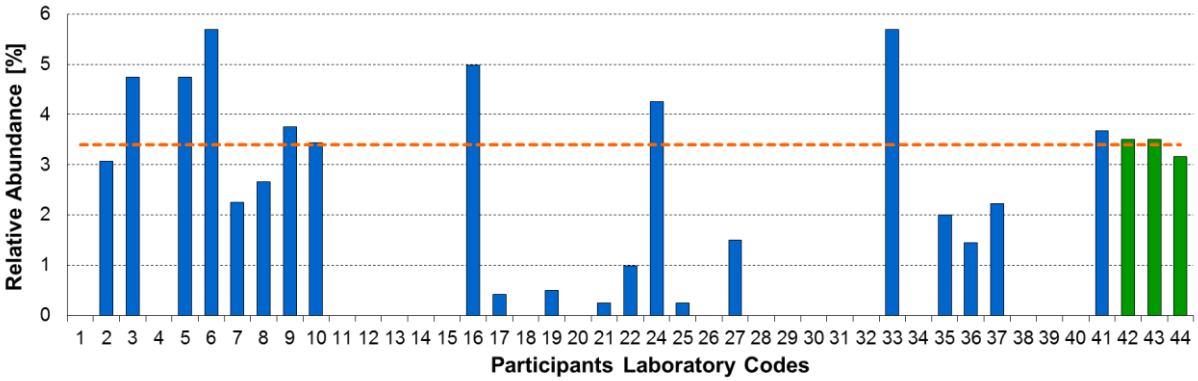


Figure 17. The relative abundance of ***Nitzschia archibaldii*** determined by each participant in the ‘Stream’ sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

Nitzschia gracilis also occurred in the ‘Stream’ sample with low relative abundances (Fig. 18). Two auditors determined this species with a relative abundance of 2.3 % and 1.7 % and 17 participants with 0.24 % to 2.5 % (Fig. 18). Two participants (laboratory codes 20 and 32) labelled the valves with “cf.“.

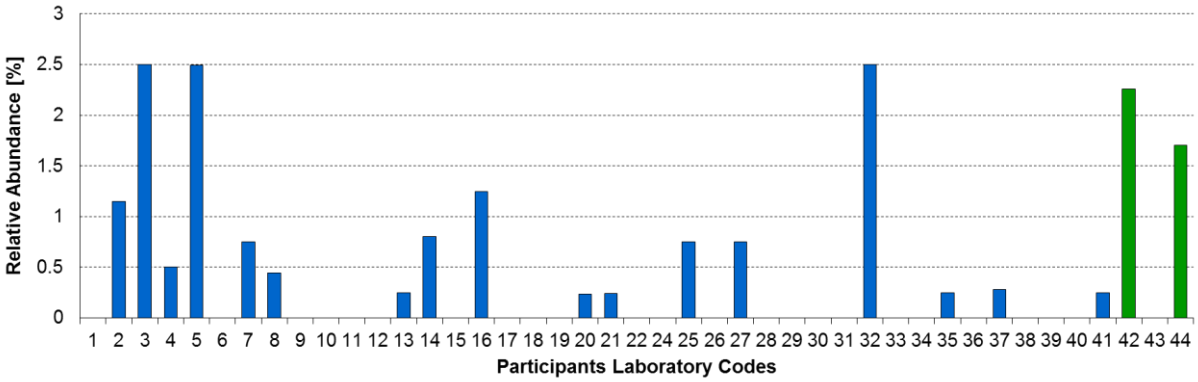


Figure 18. Sum of the relative abundance of ***Nitzschia gracilis*** and ***Nitzschia* cf. *gracilis*** determined by each participant in the ‘Stream’ sample. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

According to the depicted counting results (Fig. 16-18) many participants had difficulties with identifying *Nitzschia*-species. This assorts well with the results from the first German intercalibration exercise (Dreßler et al. 2014). In any case, all characteristics need to be considered for a definite identification. One characteristic is the presence (or absence) of a central node (“gap”), which is visible by the greater distance between the two middle fibulae compared to the remaining fibulae. This gap is present in *N. paleacea* (Table 12). In contrast, the middle fibulae of *N. archibaldii*, *N. palea* and *N. gracilis* are equidistant to the remaining fibulae, i.e. they have no “gap”. Other important characteristics include the valve length and width, fibulae density and (if visible) the striae density. As several characteristic measurement-ranges often overlap among species (Table 12, Plate 4), it is essential to also consider the valve shape.

Table 12. Selected characteristics for the identification of *Nitzschia paleacea*, *N. archibaldii*, *N. palea*, *N. gracilis* and *N. graciliformis*. Sources: Krammer & Lange-Bertalot (1999), Hofmann et al. (2013).

Taxon	**Length (µm)**	**Width (µm)**	**Fibulae/ 10 µm**	**Striae/10 µm**	**Comment**
N. paleacea	8-55	1.5-4	14-19	n.a. (SEM: 44-55)	**with gap**, ends (acutely rounded) gradually narrowed
N. archibaldii	15-40	2-3	14-19	n.a. (SEM: 46-55)	**without gap**, tapering towards acutely rounded, sometimes weakly capitate ends
N. palea	15-70	2.5-5	9-17	28-40	**without gap**, wedge-shaped, acutely rounded ends
N. gracilis	30-110	2.5-4	12-18	38-42	**without gap**, ends gradually narrowed or extended rather abruptly rostrate
N. graciliformis	58-150	2-2.5	16-21	n.a. (SEM: 45-60)	**with gap**, ends first gradually narrowed and then strongly protracted rostrate

n.a. not visible using light microscopy, SEM = visible using scanning electron microscopy

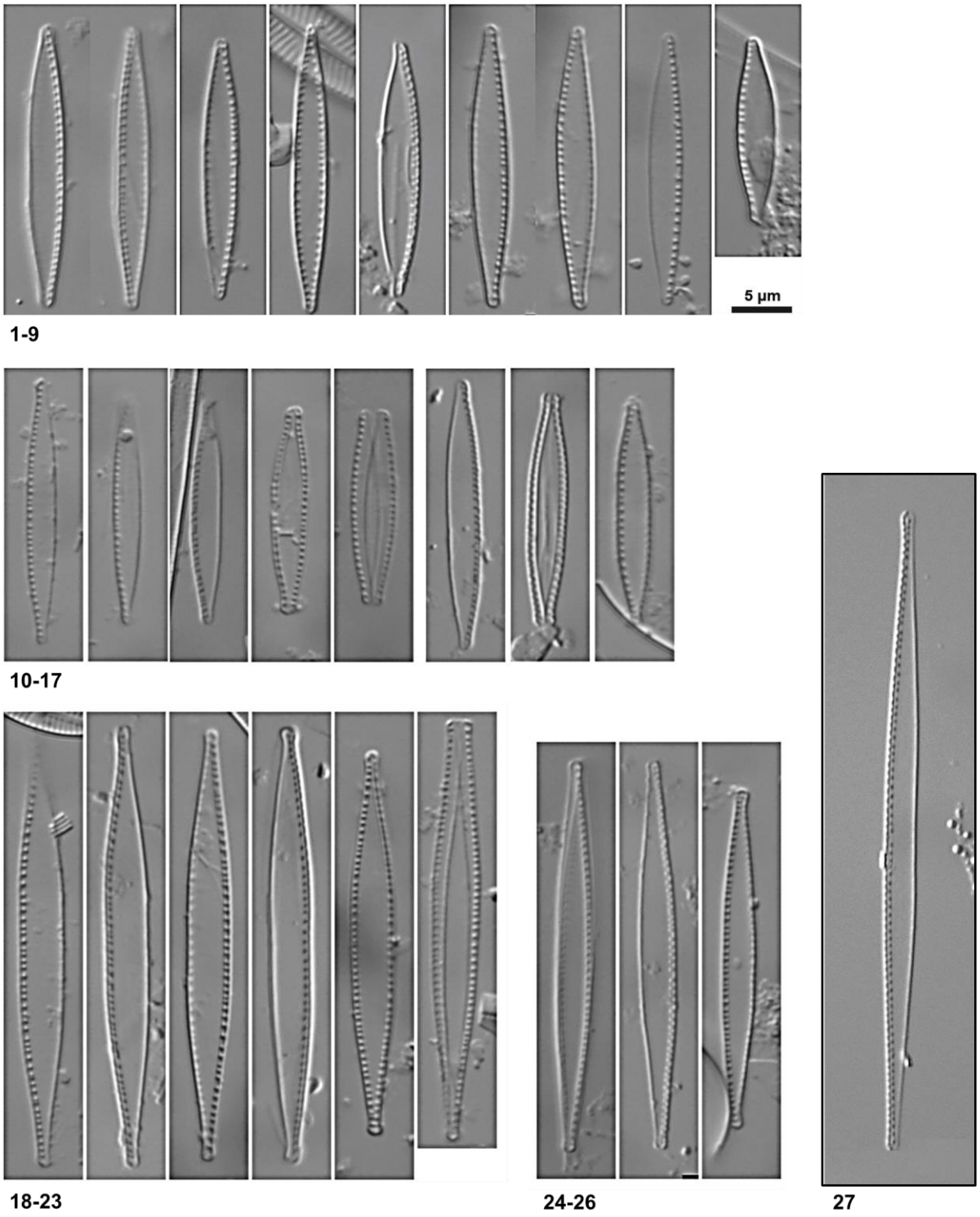


Plate 4. Comparison of *Nitzschia paleacea* with similar taxa from the ‘Stream’ sample. **1-9: *N. paleacea***, **10-17: *N. archibaldii* or *N. palea* var. *debilis*** due to valve width of 2.5-3.0 µm, length of 18.5-27.5 µm and 14-18 fibulae in 10 µm; shape corresponds more with *N. archibaldii* in Fig. 10-12 and more with *N. palea* var. *debilis* in Fig. 15-17 or with *N. lacuum* in Fig. 13 and 14; **18-23:** Characteristics (width: 3.2-3.9 µm, length: 37.0-41.0 µm, 13-17 fibulae/10 µm) correspond to ***N. palea* var. *tenuirostris*** and ***N. gracilis***, shape is more similar to ***N. palea* var. *tenuirostris***; **24-26: *N. archibaldii*** due to valve width (2.7-3.0 µm), length (22.0-35.0 µm) and fibulae density (15-17/10 µm); However, characteristics also match ***N. palea* var. *tenuirostris*** and, with a valve length > 30 µm (i.e. Fig. 24 and 25), also match ***N. gracilis***. **27: *N. graciliformis*** (valve not from a sample of this intercalibration exercise). Also compare to Figures on Plate 5.

4.3.5 *Nitzschia palea*-aggregate

In the ‘Stream’ sample the *Nitzschia palea*-aggregate includes *N. palea*, *N. palea* var. *debilis*, *N. palea* var. *palea, N. palea* var. *tenuirostris*, *N*. cf. *palea, N. palea* cf. var. *debilis*, *N. palea* cf. var. *palea* and *N. palea* cf. var. *tenuirostris*. The auditors identified the *Nitzschia palea*-aggregate with relative abundances ranging from 4.4-7.8 % (average: 6.6 %) (Fig. 19). The participants determined this aggregate with 3.2 % to 23.2 % (average: 9.3 %, n=36). The high variability of identified abundances and the fact that four participants did not detect any valves from the *Nitzschia palea*-aggregate (Fig. 19) indicate difficulties with the identification of this aggregate.

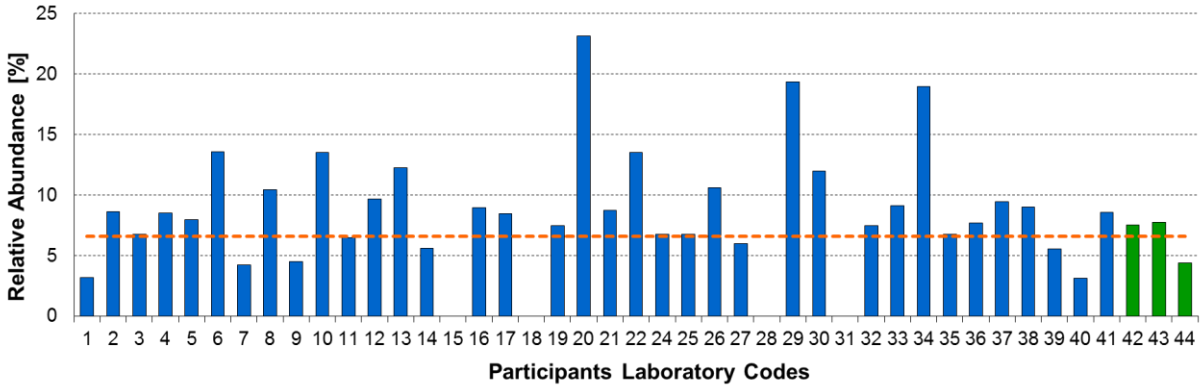


Figure 19. The relative abundance of ***Nitzschia* (cf.) *palea*-aggregate** determined by each participant in the ‘Stream’ sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

Nitzschia palea can be mistaken for *N. gracilis* and *N. archibaldii* (both detailed in the previous Chapter 4.3.4) as well as *N. pumila*, *N. pura*, *N. fruticosa*, and *N. intermedia* (Krammer & Lange-Bertalot 1999, Hofmann et al. 2013), all of which have no central node. *N. pumila*, *N. pura* and *N. fruticosa* were not determined by the auditors or participants in the ‘Stream’ sample.

Nitzschia intermedia was identified by two auditors with relative abundances of 0.5 % and 2.4 %, i.e. apparently this species was present in the ‘Stream’ sample. This species was also identified by 13 participants with 0.5 % to 2.4 % (Fig. 20).

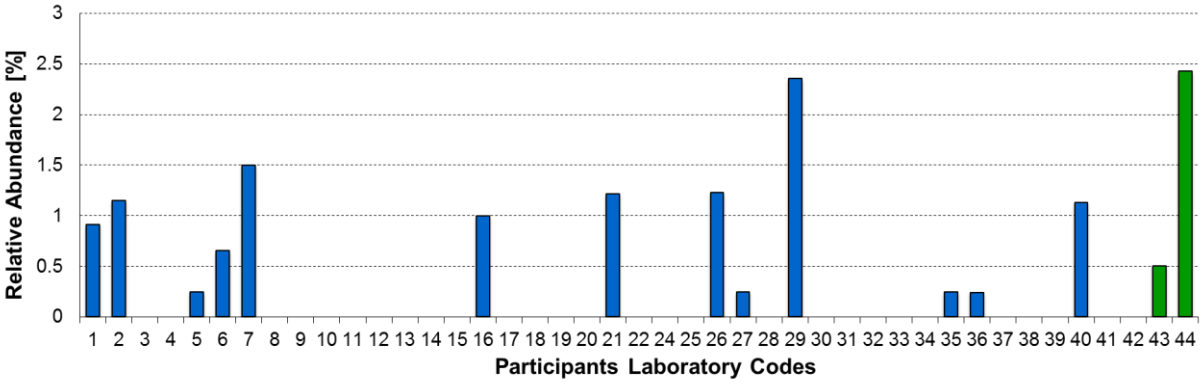


Figure 20. The relative abundance of ***Nitzschia intermedia*** determined by each participant in the ‘Stream’ sample. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

Species with a central node that *Nitzschia palea* may be mistaken for are *N. paleacea* (details in Chapter 4.3.4) and *N. capitellata* (Krammer & Lange-Bertalot 1999). The latter was determined with relative abundances of 0.5, 0.25 and 0.5 % by the auditors (Fig. 21). Most participant results indicate no problems with the identification of *N. capitellata*. An exception is the 8 % relative abundance of *N. capitellata* by one participant suggesting confusion with other *Nitzschia*-species (Fig. 21). For example, this participant did not identify any valves from the *Nitzschia palea*-aggregate or *N. archibaldii* (Fig. 17 and 19).

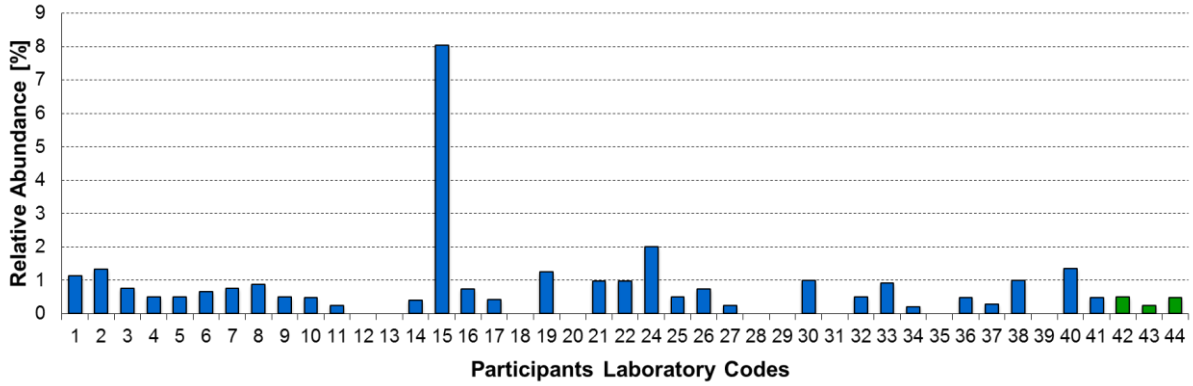


Figure 21. The relative abundance of ***Nitzschia capitellata (*var. *capitellata)*** determined by each participant in the ‘Stream’ sample. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

Six participants listed only *Nitzschia palea* and none of its varieties. Therefore, they did not differentiate *Nitzschia palea* to variety level (Fig. 22-25), despite the postulation by the instruction protocol and as necessary for an exact water quality assessment by PHYLIB (Schaumburg et al. 2011c & 2012, see Table 14). One participant (Laboratory Code 6) labelled the relative abundances (0.2 %) of the *Nitzschia palea*-aggregate with “cf.“ and another participant identified *N. palea* var. *debilis* and *N. palea* var. *palea* in addition to *N. palea*. Six participants and one auditor did not list any *N. palea* var. *palea* for the ‘Stream’ sample, but other *N. palea* varieties, suggesting that their listed *Nitzschia palea* probably referred to *N. palea* var. *palea* (Fig. 22-25).

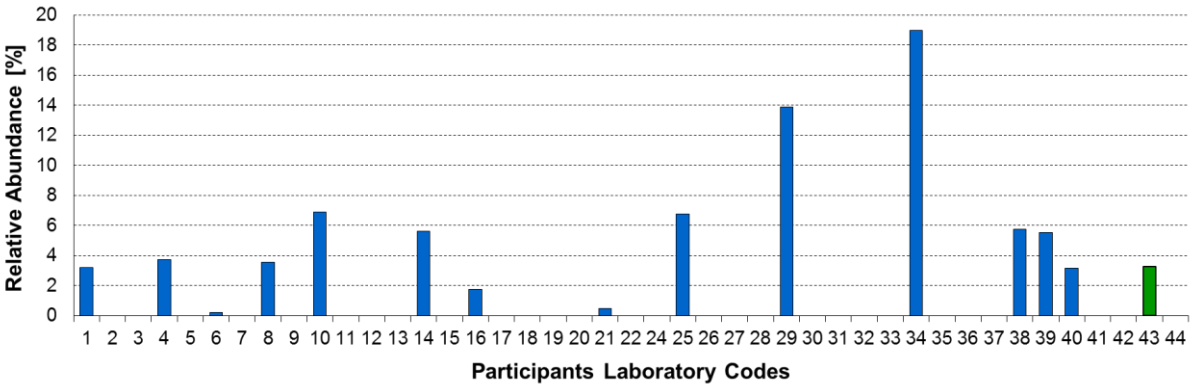


Figure 22. The relative abundance of ***Nitzschia* (cf.) *palea*** (listed without differentiation of varieties) determined by each participant in the ‘Stream’ sample. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

Two auditors identified *Nitzschia palea* var. *palea* with relative abundances of 3.3% and 3.7 % and 24 of the 40 participants of the intercalibration exercise with 0.4 % to 8.0 % (average: 3.6 %, n=24)(Fig. 23).

Two participants (laboratory codes 19 and 37) labelled their abundances for 0.25 % and 4.2 % with “cf.“. *Nitzschia palea* var. *debilis* was identified with relative abundances of 3.5 % and 1.5 % by two auditors and with 0.24 % to 13.5 % (average: 4.1 %) by 23 participants (Fig. 24). The remaining 17 participants did not list this variety. *Nitzschia palea* var. *tenuirostris* was determined with 0.8 %, 3.0 % and 0.7 % (average: 1.5 %) by the three auditors and with 0.24 % to 9.3 % (average 2.9 %) relative abundances by 23 participants in the ‘Stream’ sample (Fig. 25).

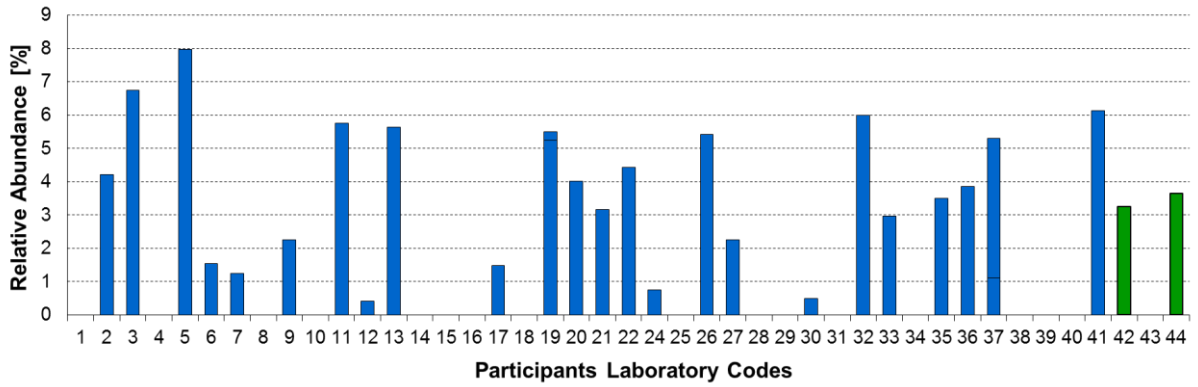


Figure 23. The relative abundance of ***Nitzschia palea* (cf.) var. *palea*** determined by each participant in the ‘Stream’ sample. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

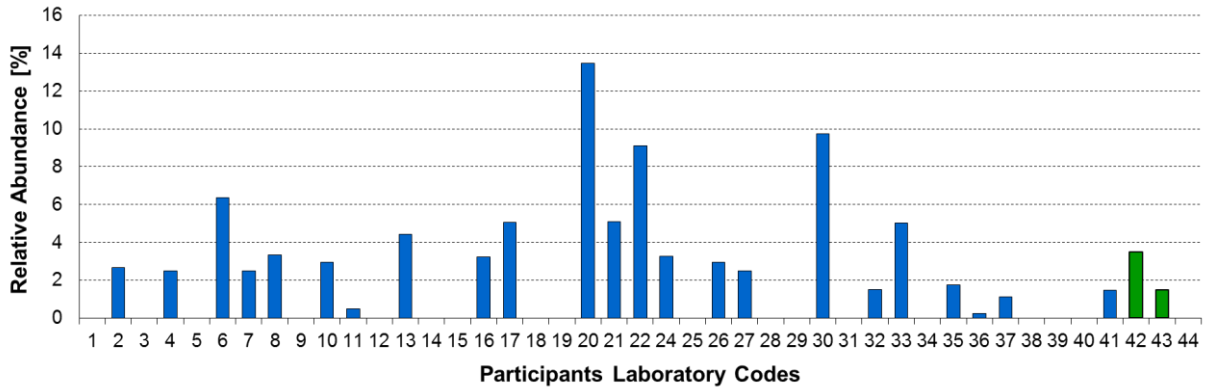


Figure 24. The relative abundance of ***Nitzschia palea* (cf.) var. *debilis*** determined by each participant in the ‘Stream’ sample. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

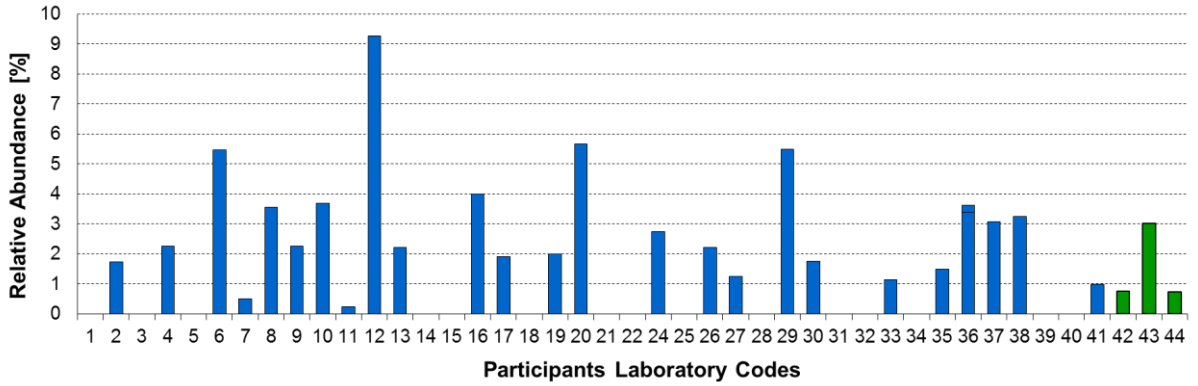


Figure 25. The relative abundance of ***Nitzschia palea* (cf.) var. *tenuirostris*** determined by each participant in the ‘Stream’ sample. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

As shown above, *Nitzschia palea* may be mistaken for other *Nitzschia*-species. *Nitzschia palea* usually has a linear-lanceolate to linear, seldom a lanceolate shape (Hofmann et al. 2013). Typical are their wedge-shaped narrowed, acutely rounded ends (Table 13). These characteristics need to be considered together with their given width, length, fibulae density and striae density for distinguishing them from similar species. *Nitzschia paleacea* (Chapter 4.3.4) and *Nitzschia capitellata* can usually be distinguished by the presence of their central node (“gap” between the middle fibulae) from *N. palea*. *Nitzschia intermedia* is usually well distinguished from *N. palea* by their low striae density and on average bigger size (often >5µm wide and at least 40 µm long) (Table 13).

The distinctions of the varieties provided in Hofmann et al. (2013; Table 13) are rather coarse: *N. palea* var. *debilis* is narrower compared to *N. palea* var. *palea* and linear –lanceolate, with a fibulae- and striae density near the high range of the species. *N. palea* var. *tenuirostris* has a valve width in the small to medium range and more or less strongly protracted valve ends. Valves within the medium to large width-range without the specifics of the other varieties should be allocated to the nominate variety *palea*.

N. palea var. *debilis* is very similar to *N. archibaldii*, which is on average narrower (Hofmann et al. 2013, Table 13). Trobajo et al. (2009) measured a valve-width of 2.8-5.1 µm from 25 clone cultures of *N. palea* from various freshwater-biotopes world-wide and a valve width > 3.1 µm for *N. palea* var. *debilis*. This valve width would enable an unambiguous distinction to *N. archibaldii* (2-3 µm). Additionally, *N. archibaldii* has on average a higher fibulae density (14-19 fibulae/10 µm) compared to *N. palea* var. *debilis* (9-17 fibulae/10 µm).

Differentiating *N. palea* var. *tenuirostris* from *N. gracilis* is also very difficult (Hofmann et al. 2013, see also Plate 5) and remains ambiguous even after examining the type material (personal correspondence: Bart Van De Vijver). In Sweden, the two species are currently separated based on the visibility of the striae for their water monitoring. If the striae are visible (provided the microscope has a high resolution), the valve is rather *N. palea* var. *tenuirostris* than *N. gracilis.*

Table 13. Characteristics to compare *Nitzschia palea* from similar *Nitzschia*-species and to differentiate the varieties of *N. palea*. Sources: Krammer & Lange-Bertalot (1999), Hofmann et al. (2013). *Trobajo et al. 2009 (explanations see text).

Taxon	**Length (µm)**	**Width (µm)**	**Fibulae/ 10 µm**	**Striae/10 µm**	**Comment**
N. palea	15-70	2.5-5 (2.8-5?)*	9-17	28-40	**without gap**, wedge-shaped, acutely rounded ends
N. paleacea	8-55	1.5-4	14-19	n.a. (SEM: 44-55)	**with gap**, ends (acutely rounded) gradually narrowed
N. capitellata	20-70	3.5-6.5	10-18	35-40	**with gap**, ends wedge-shaped and often capitate, usually constricted valve-middle
N. archibaldii	15-40	2-3	14-19	n.a. (SEM: 46-55)	**without gap**, tapering towards acutely rounded, sometimes weakly capitate ends
N. gracilis	30-110	2.5-4	12-18	38-42	**without gap**, ends gradually narrowed or extended rather abruptly rostrate
N. intermedia	40-200	4-7	7-13	20-33	**without gap**, striae easily distinguishable and appearing dotted
N. palea var. *palea*		medium to large valve width, without specifics of other varieties			
N. palea var. *debilis*		narrower than *N. palea* var. *palea* and linear-lanceolate, fibulae- and striae density within high range			
N. palea var. *tenuirostris*		small to medium valve width, more or less strongly protracted ends			

n.a. not visible using light microscopy, SEM = visible using scanning electron microscopy.

Finally, we recommend to obligatory consider all characteristics (valve dimensions and shape) when identifying *Nitzschia palea* and its varieties. The partly large difficulties to unambiguously allocate these taxa, may be due to the sometimes ambiguous taxa description in the identification literature. Still, hardly any participants labelled relative abundances of the taxa with “cf.” or “aff.”. We recommend to designate “cf.” or “aff.” to ambiguously identified taxa and to document these valves in writing and photographically. Despite the discussed difficulties of unambiguous distinction of the varieties and the separation of *N. palea* from similar *Nitzschia*-species, an identification of these taxa to the highest possible taxonomic level is essential for the water quality assessment (see Table 14).

Table 14. Saprobic values (**S**), trophic values (**TI**) and weighting (**G**) of the *Nitzschia palea*-aggregate for lotic systems and lakes as listed in the PHYLIB software (version 5.3, December 2015).

Taxon	**Lotic Systems**				**Lakes**		
	S	**G**	**TI**	**G**	**TI_{North}**	**TI_{South}**	**G**
N. palea	-	-	-	-	-	-	-
N. palea var. *debilis*	-	-	2.3	1.0	-	-	-
N. palea var. *palea*	-	-	3.3	3.0	3.05	-	-
N. palea var. *tenuirostris*	-	-	-	-	-	-	-
N. paleacea	2.7	3.0	2.3	2.0	3.5	5.0	3.0
N. capitellata var. *capitellata*	3.4	2.0	3.8	5.0	7.29	-	-
N. archibaldii	1.9	3.0	2.0	2.0	-	-	-
N. gracilis	1.3	4.0	2.5	2.0	-	-	-
N. intermedia			2.9	2.0	5.74	5.0	3.0

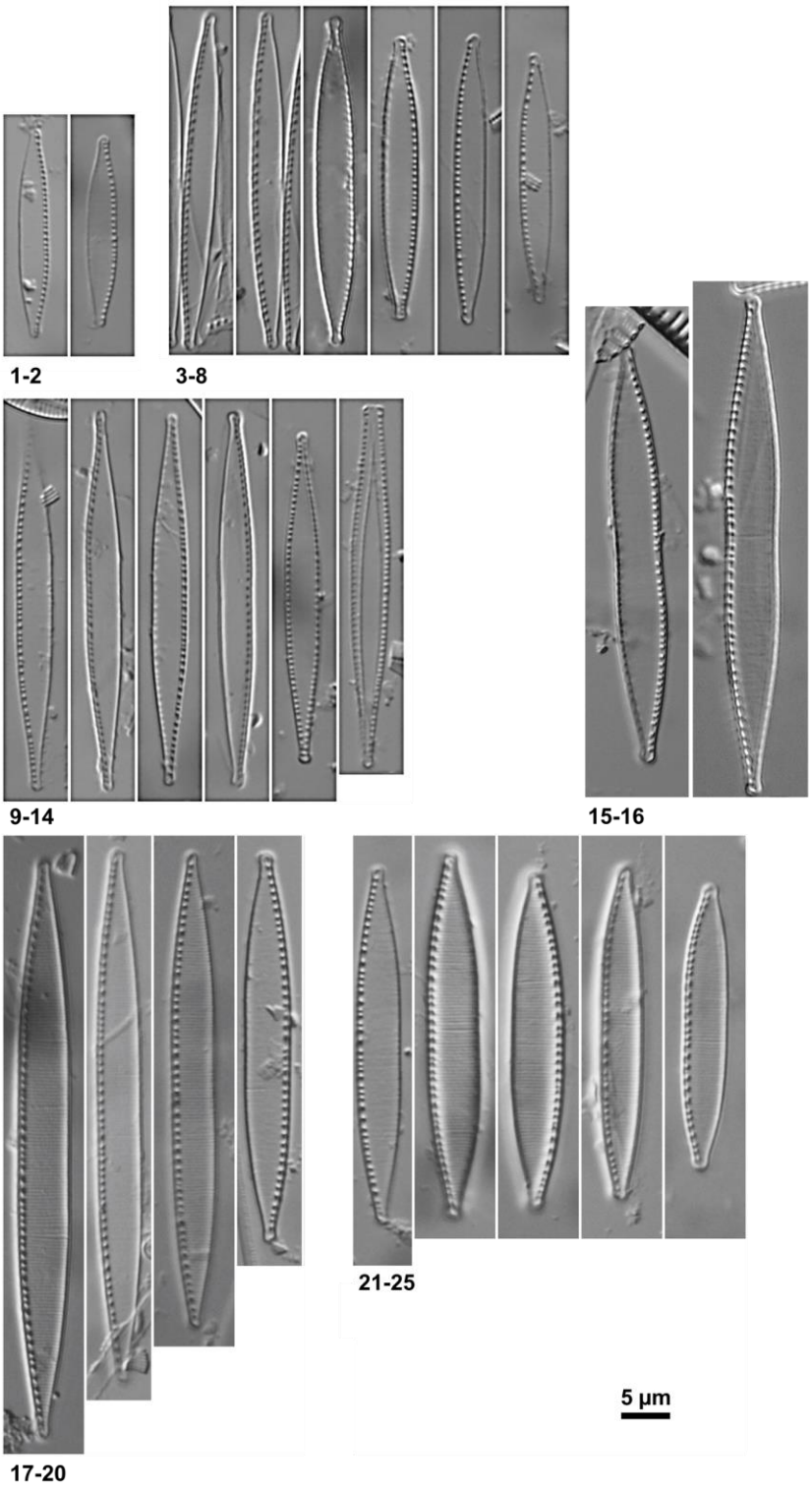


Plate 5. Comparison of *Nitzschia palea* and varieties with similar *Nitzschia*-species from the ‘Stream’ sample. **1-2: *N. palea* var. *debilis*** due to valve-dimensions (width: 3.2-3.4 µm, length: 21.0-23.0 µm, fibulae: 16-17/10 µm), **3-8: *N. palea var. palea*** (width: 3.5-4.0 µm, length: 28.0-36.5 µm, 10-15 fibulae/10µm), **9-14:** Characteristics (width: 3.2-3.9 µm, length: 37.0-41.0 µm, 13-17 fibulae/10 µm) correspond to both ***N. palea* var. *tenuirostris* and *N. gracilis***, shape is more similar to ***N. palea* var. *tenuirostris***, **15-16: *N. capitellata*** (valve not from the ‘Stream’ sample), **17-20: *N. intermedia***, Fig. 18 and Fig. 20 also show characteristics of *N. palea var. palea*, as striae density is relatively high, **21-25: *N*. (cf.) *palea* var. *palea*** due to valve length (< 40 µm), fibulae- (12-13/10 µm) and striae- (29-30/10µm; except Fig. 21 with >> 30) density. 22-23 with cf., as valve width > 5µm. Also compare to Figures on Plate 4.

4.3.6 *Planothidium lanceolatum* and *P. frequentissimum*

The auditors determined the *Planothidium lanceolatum-frequentissimum*-complex with relative abundances ranging from 3.7 - 4.3 % (average: 3.9 %) in the ‘Stream’ sample (Fig. 26). This complex included *Planothidium frequentissimum* (var. *frequentissimum*), *P. lanceolatum*, *Achnanthes lanceolata* ssp. *lanceolata*, *Achnanthes lanceolata*-aggregates, *Achnanthes lanceolata* ssp. *frequentissima* var. *rostratum*, *Planothidium frequentissimum* var. *minus* and *Planothidium frequentissimum* var. *magnum*. Most participants (37) found this complex with 2.3 % to 7.6 % (average: 4.1 %, n=37). Three participants did not detect any valves from this *Planothidium*-complex (Fig. 26). Therefore, most participants did not seem to have problems to allocate valves to this complex.

For the *Planothidium lanceolatum-frequentissimum*-complex, only one participant listed the outdated names: *Achnanthes lanceolata*-aggregate (DV-no. 6244) with 3.0 % relative abundance (Fig. 27) and *Achnanthes lanceolata* ssp. *frequentissima* var. *rostratum* with 1.5 % (Fig. 28). Therefore, the subspecies *lanceolata* (now called *P. lanceolatum*) and *frequentissima* (now *P. frequentissimum*) were not differentiated to a sufficient taxonomic resolution and allocated to an incorrect subspecies as ssp./var. *rostratum* (now *P. rostratum*).

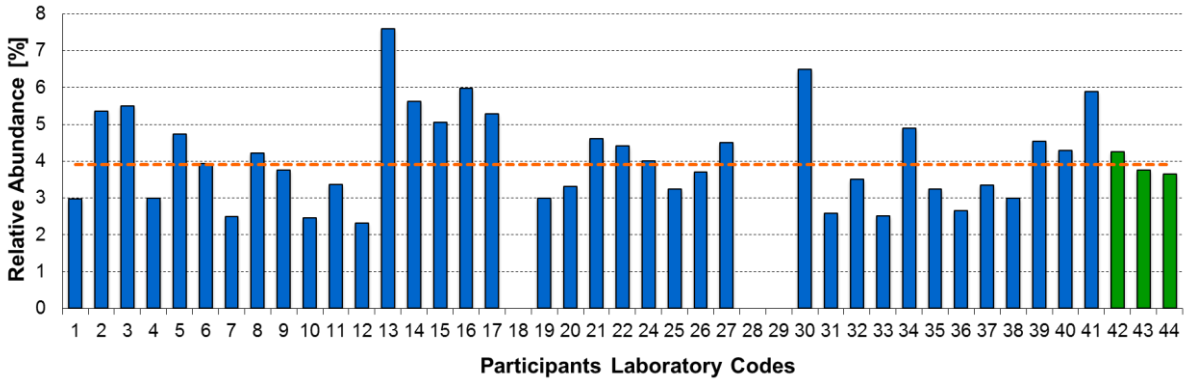


Figure 26. The relative abundance of ***Planothidium lanceolatum-frequentissimum*-complex** determined by each participant in the ‘Stream’ sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

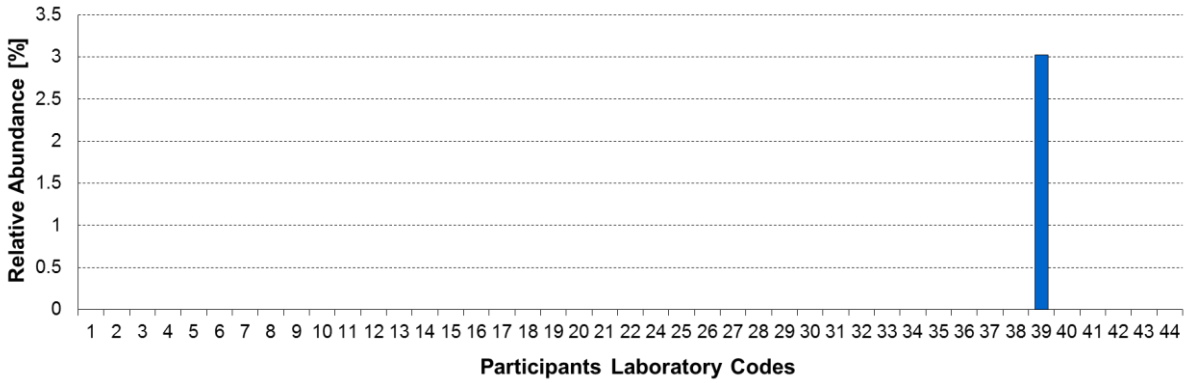


Figure 27. The relative abundance of ***Achnanthes lanceolata*-aggregate** (DV-no. 6244) determined by one participant (blue bar) in the ‘Stream’ sample.

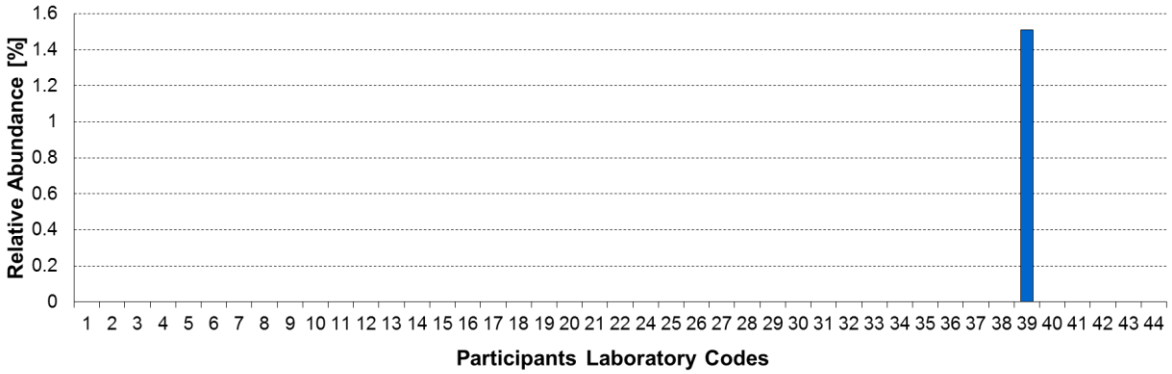


Figure 28. The relative abundance of ***Achnanthes lanceolata* ssp. *frequentissima* var. *rostrata*** determined by one participant (blue bar) in the ‘Stream’ sample.

The auditors exclusively identified *Planothidium lanceolatum* (1.0 %, 1.3 % and 0.7 % relative abundances) and *P. frequentissimum* (3.3 %, 2.5 % and 2.9 %) from this complex, as most participants did too (Fig. 29 and 30). Some participants (laboratory codes 1, 31 and 40) only identified *P. lanceolatum* and no *P. frequentissimum*, suggesting some misidentifications (compare Fig. 29 and 30). One participant (Laboratory Code 14) only listed the outdated taxa name *Achnanthes lanceolata* ssp. *lanceolata* for *P. lanceolatum*.

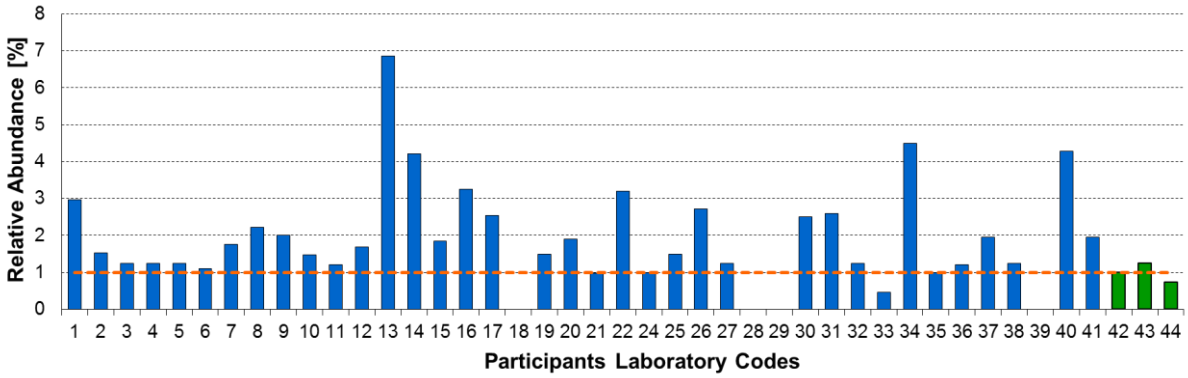


Figure 29. The relative abundance of ***Planothidium lanceolatum*** and ***Achnanthes lanceolata* ssp. *lanceolata*** determined by each participant in the ‘Stream’ sample. Blue bars: participants, green bars: auditors. Orange horizontal dashed line = average of auditors.

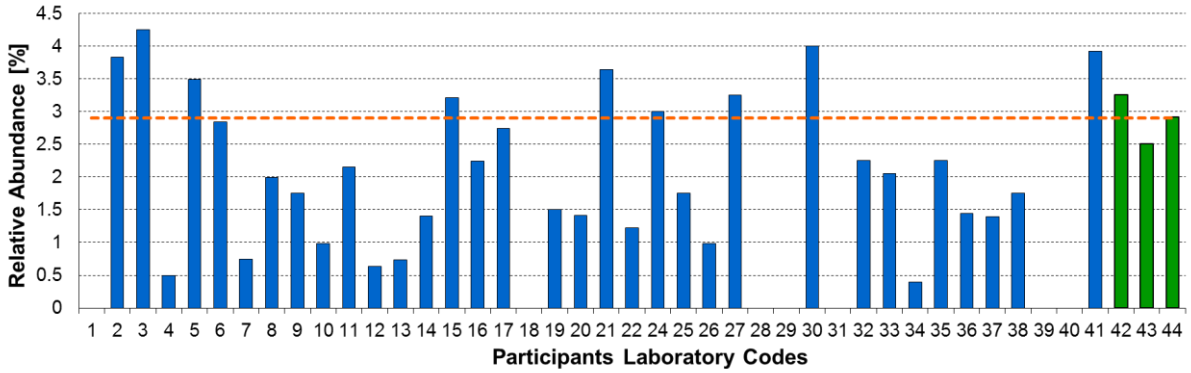


Figure 30. The relative abundance of ***Planothidium frequentissimum*** **(var. *frequentissimum*)** determined by each participant in the ‘Stream’ sample. Blue bars: participants, green bars: auditors. Orange horizontal dashed line = average of auditors.

Furthermore, one participant identified *Planothidium frequentissimum* var. *magnum* (1.3 % relative abundance) and one participant determined *Planothidium frequentissimum* var. *minus* with 0.5 % (Fig. 31 and 32).

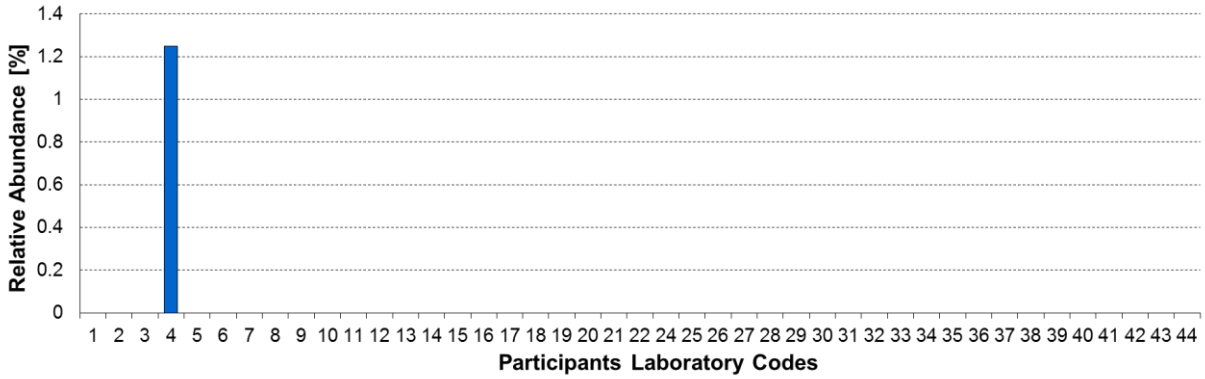


Figure 31. The relative abundance of ***Planothidium frequentissimum*** **var. *magnum*** determined by one participant (blue bar) in the ‘Stream’ sample.

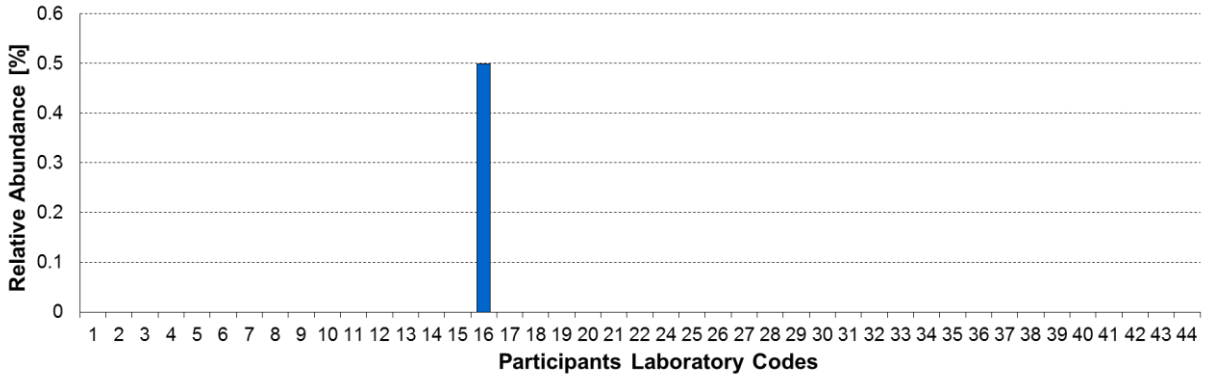


Figure 32. The relative abundance of ***Planothidium frequentissimum*** **var. *minus*** determined by one participant (blue bar) in the ‘Stream’ sample.

Planothidium lanceolatum and *P. frequentissimum* were the most abundant taxa from this complex. Therefore, their distinction is discussed in the following. Similar to the here presented ‘Stream’

sample, both species often co-occur in assemblages. Still, they have different indicator values in the PHYLIB-software (Table 16), leading to different water quality assessments with different abundances of each species. Valves of *P. lanceolatum* are more elliptical with broader rounded ends compared to *P. frequentissimum* (Table 15, Plate 6). Despite overlapping ranges, the dimensions should still be considered for the distinction, as *P. lanceolatum*-valves are on average longer than *P. frequentissimum* valves. The araphid valves can be distinguished by their “spot” located on one side of the central area. This “spot” is a depression in *P. lanceolatum* valves (Plate 6: Fig. 13) and appears to have a blurred delimitation using light microscopy. In contrast, the “spot“ in *P. frequentissimum*-valves is formed by a cave (Plate 6: Fig. 12), with an edge that appears as arched lines (horseshoe-shaped) in the light microscope and thus appears distinctly delimited (Table 15, Plate 6).

Planothidium lanceolatum and *P. frequentissimum* may have been mistaken for *Planothidium rostratum* and *Achnanthes lanceolata* var. *rostrata* (Fig. 28), which represent two different taxa (Lange-Bertalot 1993; Bak & Lange-Bertalot 2014), but are still listed as one species (*P. rostratum*) in Hofmann et al. (2013). However, the rostrate ends of *P. rostratum* (and also of *A. lanceolata* var. *rostrata*) separate this species well from *Planothidium lanceolatum* and *P. frequentissimum*.

According to the recent taxonomic knowledge, the varieties *Planothidium frequentissimum* var. *magnum* (Laboratory Code 4) and *P. frequentissimum* var. *minus* (Laboratory Code 16) should be separated from P. *frequentissimum* var. *frequentissimum*. The indicator values of the varieties differ in the PHYLIB-software that assesses the water quality (Table 16). *P. frequentissimum* var. *minus* may be separated from the other varieties due to their round-elliptical valve shape and also their relatively short length (Table 15). Substantially more difficult to impossible is the differentiation of the other varieties (*frequentissimum* and *magnum)* from each other.

Other taxa that may be mistaken for taxa from this complex are, for example, *P. dubium*, *P. biporomum* and *P. incuriatum* (see N’Guessan et al. 2014; Wetzel et al. 2013).

Finally, we recommend to use all characteristics listed in Hofmann et al. (2013) and also the valve shape to differentiate *Planothidium lanceolatum* and *P. frequentissimum.* This is particularly important for separating the raphe valves. If a definite allocation of the raphe valve is impossible, the valves should be treated the same way as valves in girdle band views, i.e. after the count, these raphe valves should be allocated to all possibly fitting taxa according to their relative abundance. In the PHYLIB-software, the abundances of *P. frequentissimum* only contribute to the water quality assessments, if the varieties are differentiated (Table 15 and 16). As the differentiation of the varieties is often difficult based on the here presented characteristics, valves that can not unambiguously be allocated to a variety, should be listed as *P. frequentissimum* (DV-no. 36209).

The PHYLIB-software version 5.3 from December 2015 does not list any trophic values for the varieties of *Planothidium frequentissimum* for lotic systems, probably inadvertently. In contrast, version 5.3 from February 2016 lists them again (as previous versions have)(Table 16). We recommend to document such changes in the data set in detail, when revising the PHYLIB software, e.g. in the software version documentation file.

Table 15. Characteristics for the identification of *Planothidium lanceolatum*, *P. frequentissimum* and *P. rostratum*. Sources: Straub (1985), Hofmann et al. (2013). Taxa are listed with their German data processing number (dv-number) (Mauch et al., 2003, version 2011).

Taxon	Length (µm)	Width (µm)	Striae/ 10 µm	Comment
P. lanceolatum (26048)	6- ~40	4.5-10	10-15 (raphe valve)	Blurred delimitation of "spot" on araphid valve, shape rather elliptical with widely rounded
P. frequentissimum(36209)	4-30	3.5-7	13-20	Two arched lines clearly delimit horseshoe-formed "spot" on araphid valve, shape more strongly lanceolate than *P. lanceolatum*
P. frequentissimum var. *magnum*[1)] (26046)	12-25.5	4.3-5.9	14-17	
P. frequentissimum var. *minus*[2)] (26047)	6-10	3.5-5	13-20	Round-elliptical shape
P. rostratum (26051)			10-13.5	Rostrate protracted ends, horseshoe-shaped "spot" on araphid valve

[1)] described as *Achnanthes rostrata* var. *magna*, [2)] described as *Achnanthes rostrata* var. *minor.*

Table 16. Saprobic values (**S**), trophic values (**TI**) and weighting (**G**) of *Planothidium lanceolatum*, *P. frequentissimum* (and varieties) and *P. rostratum* for lotic systems and lakes as listed in the PHYLIB software (version 5.3 from December 2015 and *from February 2016). For more information see text. Taxa are listed with their German data processing number (dv-number) (Mauch et al., 2003, version 2011).

Taxon	Lotic Systems				Lakes		
	S	G	TI	G	TI_{north}	TI_{south}	G
P. lanceolatum (26048)	-	-	3.3	3.0	1.15	-	-
P. frequentissimum (36209)	-	-	-	-	-	-	-
P. freq. var. *frequentissimum* (16606)	-	-	2.8*	3.0*	2.28	-	-
P. freq. var. *magnum* (26046)	-	-	2.8*	3.0*	-	-	-
P. freq. var. *minus* (26047)	-	-	2.8*	3.0*	-	-	-
P. rostratum (26051)	-	-	-	-	-	-	-

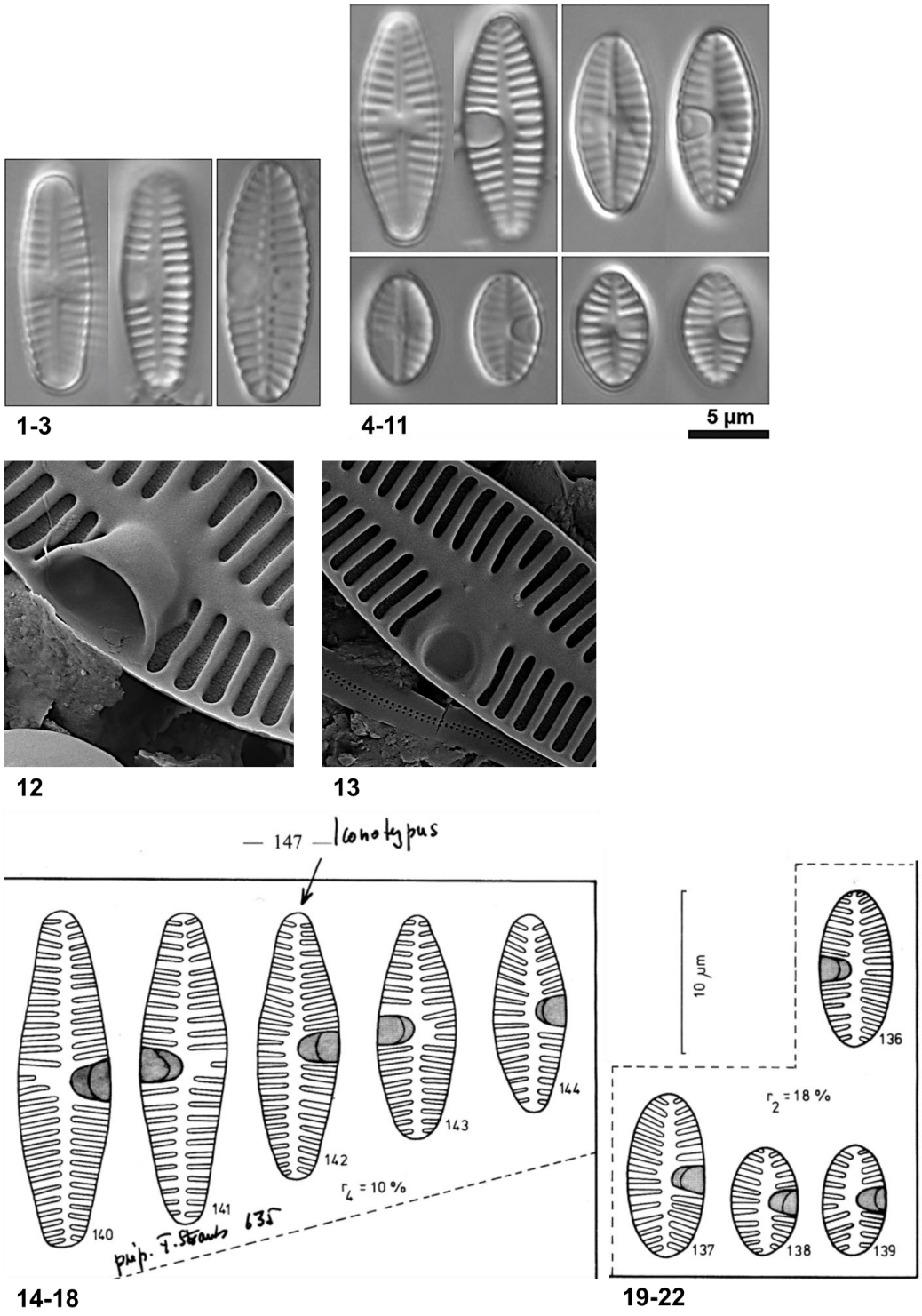


Plate 6. Comparison of *Planothidium lanceolatum* and *P. frequentissimum* from the ‘Stream’ sample (Fig. 1-13), as well as drawings from the original description (Straub 1985) for the differentiation of the varieties *magnum* and *minus* of *P. frequentissimum* (Fig. 14-22). **1-3:** *P. lanceolatum*: (1) raphe valve, (2-3) araphid valve with characteristic “spot“ (blurred delimitation), **4-11:** *P. frequentissimum*: left = raphe valve, right = araphid valve, “spot“ on araphid valves distinctly delimited, **12-13:** SEM-pictures (by B. van de Vijver; given scale does not apply here), (12) *P. frequentissimum*: “spot“ is formed by a cave, (13) *P. lanceolatum*: “spot“ is a depression, **14-18:** *P. frequentissimum* var. *magnum* depicted in Straub (1985), **19-22:** *P. frequentissimum* var. *minus* depicted in Straub (1985).

4.4 Details of taxonomic problems in the ‘Lake’ sample

4.4.1 *Achnanthidium minutissimum*-aggregate

The identification of *Achnanthidium minutissimum* and similar taxa often posed problems, as already noted in the first German intercalibration exercise for benthic diatoms (Dreßler et al. 2014). This species and similar taxa occurred in both the ‘Lake’ sample and the ‘Stream’ sample. Here, we focus on the ‘Lake’ sample, as the species was more abundant there.

In the ‘Lake’ sample, several taxa of the *Achnanthidium minutissimum*-aggregate and similar taxa were identified by the auditors and participants of this intercalibration exercise. Here, the *Achnanthidium minutissimum*-aggregate includes: *A. minutissimum*, *A. minutissimum* (cf.) var. *minutissimum*, *A. minutissimum* (cf.) var. *jackii*, *A. lineare*, *A.* (cf.) *caledonicum*, *A.* (cf.) *eutrophilum*, *A. affine* and *A. sublinearis* (Fig. 33). Additionally, *Achnanthidium pyrenaicum*, *A. saprophilum* and *A. straubianum* were determined occasionally by some participants. Three participants did not determine any valves of the genera *Achnanthidium* or *Achnanthes* in the ‘Lake’ sample (Fig. 33).

The auditors determined the *Achnanthidium minutissimum*-aggregate with relative abundances ranging from 14.6-17.8 % (Fig. 33). Most participants (37) found the aggregate to range from 7.0 % to 24.2 % (average: 15.7 %, n=37)(Fig. 33). Noticeable, four participants determined distinctly below average abundances. One of these participant additionally identified valves from the genus *Achnanthidium* with 5.5 %, and three participants additionally identified valves from the genera *Achnanthidium* and *Achnanthes* with 8.6 %, 8.1 % and 9.8 %. Therefore, these four participants and the three participants that did not identify any valves from the aggregate (Fig. 33) seem to have fundamental difficulties to identify and differentiate species of the genera *Achnanthidium* and *Achnanthes*.

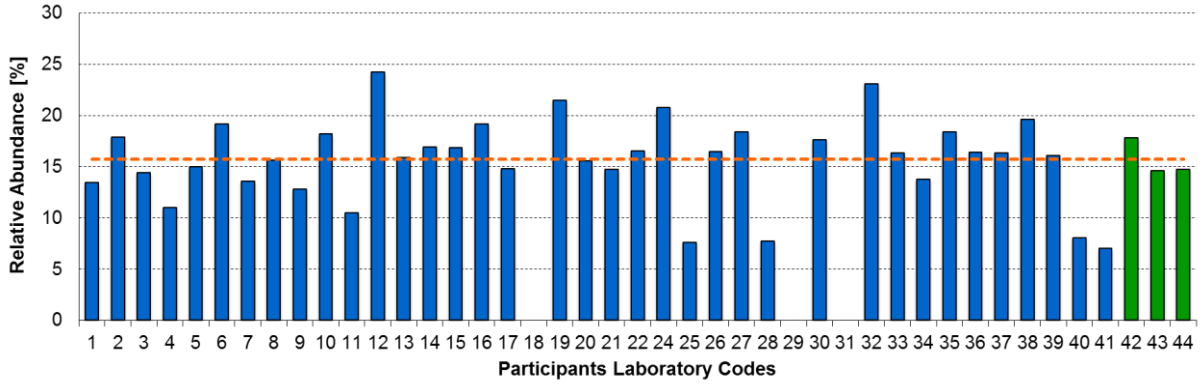


Figure 33. The relative abundance of ***Achnanthidium minutissimum*-aggregate** determined by each participant in the ‘Lake’ sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

Of the *Achnanthidium minutissimum*-aggregate nine participants listed *Achnanthidium minutissimum* (without further differentiation of the varieties) (Fig. 34), of which five participants did not list any varieties in addition to the aggregate (Fig. 34, 35 & 37).

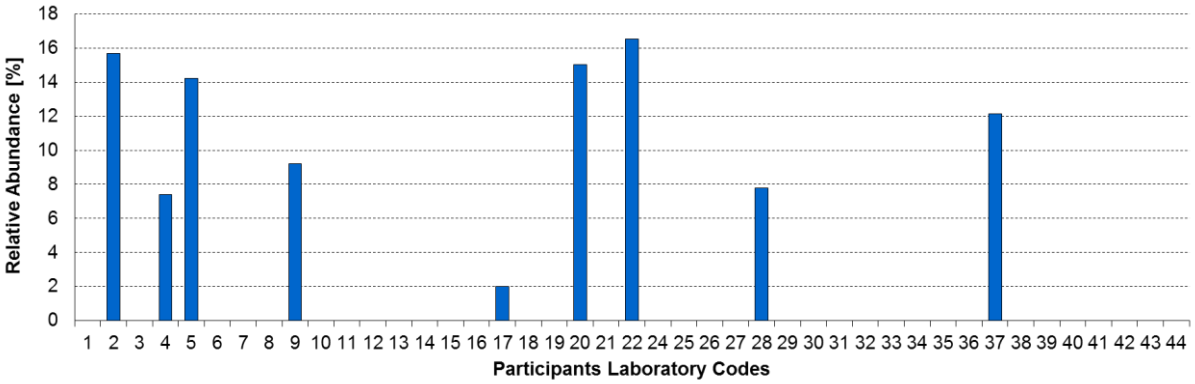


Figure 34. The relative abundance of ***Achnanthidium minutissimum*** determined by each participant in the ‘Lake’ sample. Blue bars: participants.

Within the *Achnanthidium minutissimum*-aggregate the auditors identified *Achnanthidium minutissimum* var. *minutissimum* with the highest relative abundances of 15.4 %, 8.8 % and 11.1 % in the ‘Lake’ sample (Fig. 35). Of the 40 participants, 30 also determined the relative abundance of *A. minutissimum* var. *minutissimum* to range from 1.6 % to 23.1 % (average 14.3 %, n=30). One participant (Laboratory Code 36) additionally listed *A. minutissimum* cf. var. *minutissimum* with a relative abundance of 2.8 %.

All auditors identified the relative abundance of *Achnanthidium lineare* to be 2.4 %, 3.4 % and 1.8 %. Only five participants also identified this species (Fig. 36). Two auditors and several participants determined *Achnanthidium minutissimum* (cf.) var. *jackii* with mainly low relative abundances (<1.6 %; once: 4.7 %) in the ‘Lake’ sample (Fig. 37). Ambiguity during the identification was indicated with “cf.“ by one auditor (Laboratory Code 43) and two participants (laboratory codes 2 and 30). Additionally, two auditors and eight participants determined *A. eutrophilum* in the ‘Lake’ sample (Fig. 38), of which one auditor (Laboratory Code 43) labelled this taxon with “cf.“. The same auditors and 12 participants listed *A. caledonicum*. One auditor (Laboratory code 43) and five participants labelled this taxon with “cf.“ (Fig. 39). Also, two participants identified *A. affine* with 6.3 % and 0.2 %, respectively (Fig. 40) and two other participants determined *A. sublineare* with 0.39 % (each) in the ‘Lake’ sample (Fig. 41).

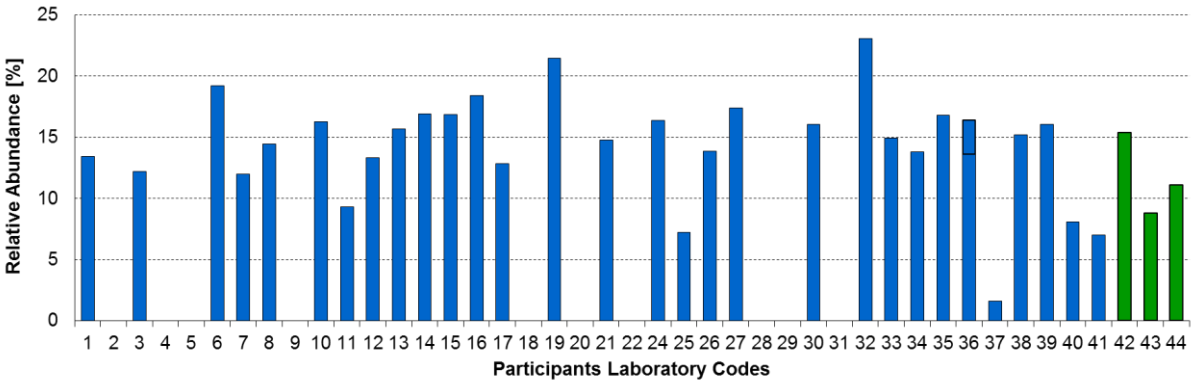


Figure 35. The relative abundance of ***Achnanthidium minutissimum* (cf.) var. *minutissimum*** determined by each participant in the ‘Lake’ sample. Blue bars: participants, green bars: auditors.

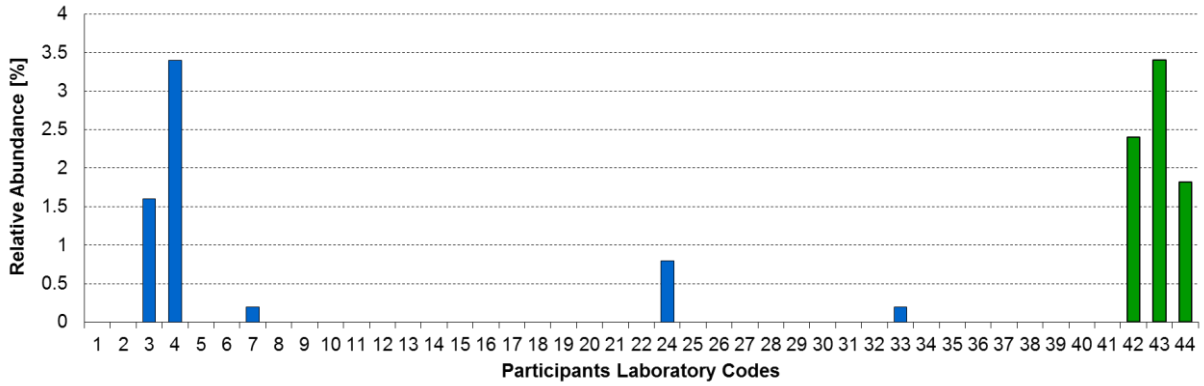


Figure 36. The relative abundance of ***Achnanthidium lineare*** determined by each participant in the ‘Lake’ sample. Blue bars: participants, green bars: auditors.

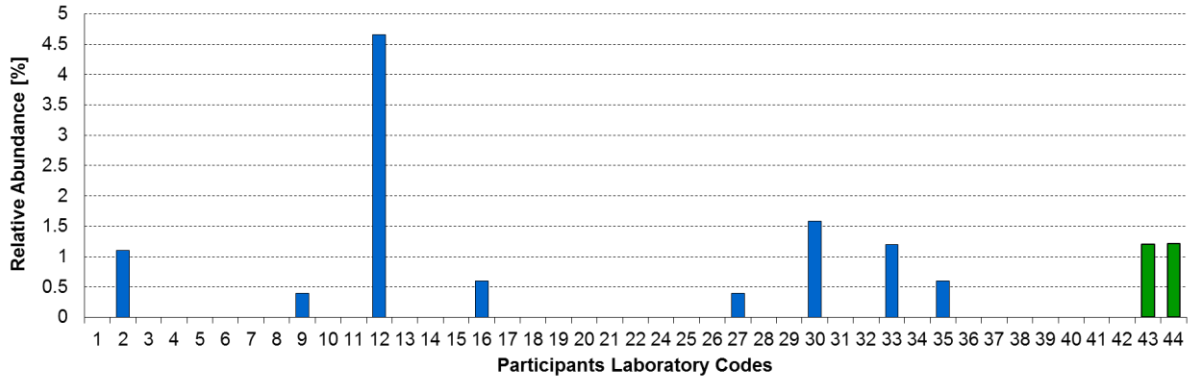


Figure 37. The relative abundance of ***Achnanthidium minutissimum* (cf.) var. *jackii*** determined by each participant in the ‘Lake’ sample. Blue bars: participants, green bars: auditors.

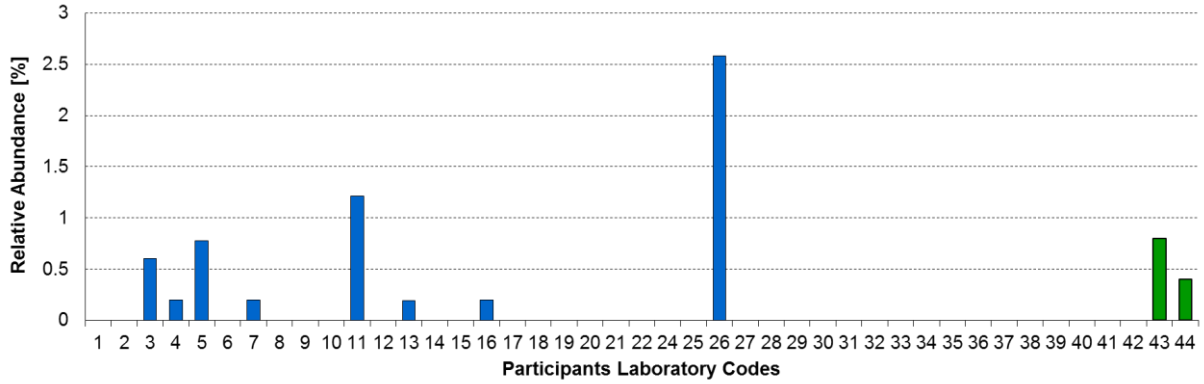


Figure 38. The relative abundance of ***Achnanthidium* (cf.) *eutrophilum*** determined by each participant in the ‘Lake’ sample. Blue bars: participants, green bars: auditors.

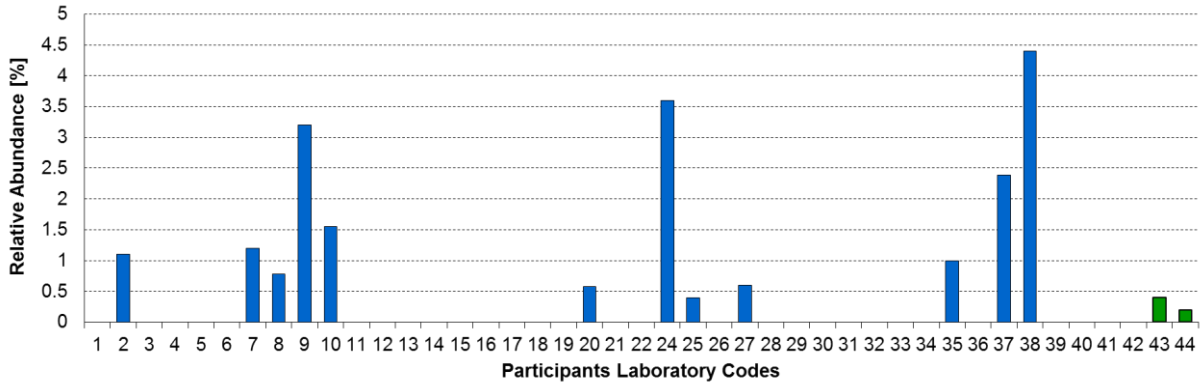


Figure 39. The relative abundance of ***Achnanthidium* (cf.) *caledonicum*** determined by each participant in the ‘Lake’ sample. Blue bars: participants, green bars: auditors.

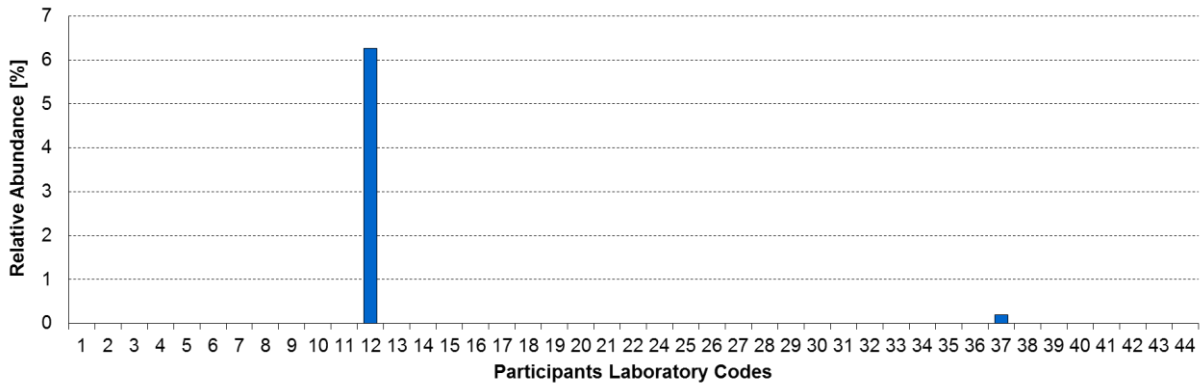


Figure 40. The relative abundance of ***Achnanthidium affine*** determined by each participant in the ‘Lake’ sample. Blue bars: participants.

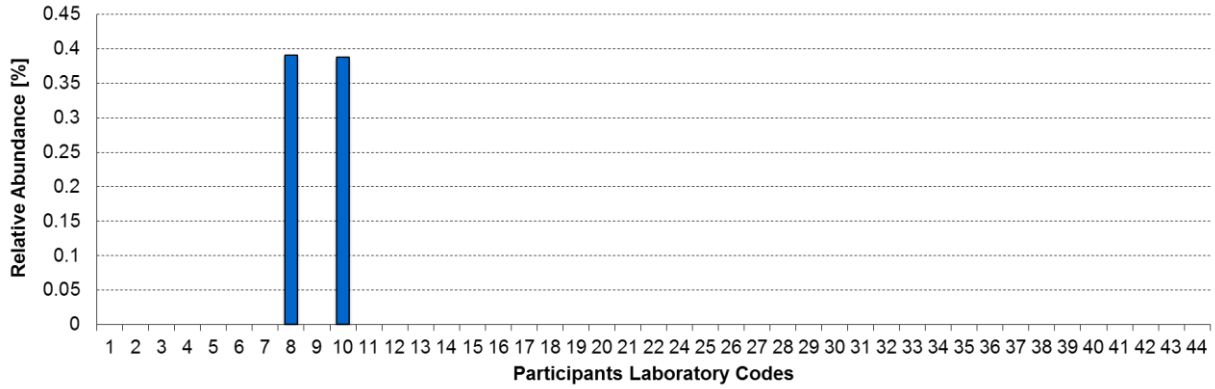


Figure 41. The relative abundance of ***Achnanthidium sublineare*** determined by each participant in the ‘Lake’ sample. Blue bars: participants.

Important characteristics of the *Achnanthidium minutissimum*-aggregate and other, occasionally determined taxa by the auditors and participants in the ‘Lake’ sample are summarized in Table 17.

- *Achnanthidium minutissimum* var. *jackii* (~26 striae/10µm) may be mainly distinguished from *A. minutissimum* var. *minutissimum* (~30 striae/10µm) by their striae density. Additionally, Morales et al. (2011) state that the presence of a fascia (crossband) in the central area on the raphe-valve distinguishes *A. minutissimum* var. *jackii.* However, according to Potapova &

Hamilton (2007) the fascia is not always present and also occurs occasionally on *A. minutissimum* var. *minutissimum* valves (Novais et al. 2015). Thus, the fascia is an ambiguous characteristic.

- The identification and distinction of *Achnanthidium lineare* and *A. sublineare* is important from an ecological view-point and therefore, for the water quality assessment. Both species typically occur in waters with very low nutrient levels (Van de Vijver et al. 2011). The valves of both species are often shorter and thinner (*A. lineare* only on average) and *A. sublineare* has a higher striae density compared to *A. minutissimum* var. *minutissimum* (Table 17).
- *Achnanthidium caledonicum* has more linear valves with broadly rounded capitate ends compared to *A. minutissimum* var. *minutissimum* (Table 17). However, valve dimensions and shape overlap widely between the two species.
- *Achnanthidium affine* can be distinguished from *A. minutissimum* var. *minutissimum* mainly by their lower striae density. Valves of *A. affine* are on average wider and the raphe valve has a characteristic, butterfly-shaped crossband (fascia).
- *Achnanthidium saprophilum* has a more compact valve shape (mainly shorter) and more broadly rounded ends compared to *A. minutissimum* var. *minutissimum*. Valve length, width and striae density of both taxa overlap greatly (Table 17). Therefore, often a certain differentiation is difficult.

Note that the names *Achnanthidium minutissimum* var. *minutissimum* and *Achnanthidium minutissimum* var. *jackii* are outdated according to current taxonomic concepts that use *Achnanthidium minutissimum* and *Achnanthidium jackii* instead (Lange-Bertalot et al. 2017). However, we used the former names here according to the German standard identification literature (Hofmann et al. 2011 & 2013) and PHYLIB-software.

Table 17. Characteristics for the identification of *Achnanthidium minutissimum* var. *minutissimum* in comparison to similar taxa, as well as other species determined by the participants of the intercalibration exercise in the 'Lake' sample. R-valve = raphe valve, Sources: Hofmann et al. (2013) and others (see below).

Taxon	**Length (µm)**	**Width (µm)**	**Striae/10 µm**	**Comments**
A. minutissimum var. *minutissimum*	5-25	2.5-4	~30	Striae in the central area (CA) irregularly shortened (one- or both-sided), R-valve: rarely a fascia [1)]
A. minutissimum var. *jackii*	5-25	2.5-4	~26	[2)] R-valve: CA usually with fascia
A. lineare [3)]	9-13.5	2.2-2.8	28-32	Valves with parallel margins, widely rounded ends, not rostrate; R-valve: CA with rectangular fascia
A. eutrophilum	4-19	3-5	23-27	Rhombic shape, length to width ratio relatively large
A. caledonicum	10-35	2.7-3.8	~30	Broadly rounded, capitate ends
A. affinis	8-30	3.5-5	Mid: 22-24, Ends: 30	R-valve with a butterfly-shaped fascia
A. sublineare [3)]	7.5-15	1.5-2.1	Mid: 33-34 Ends: ~36	Striae-density
A. pyrenaicum	6-35	3-6	R-valve: (15)20-27(40); araphid valve: Ø 18-24	Striae-density
A. saprophilum	8-15	3-4	R-valve: Mid: ~28 Ends: ~32	Broadly rounded ends
A. straubianum	6-10	3-4	25-29	

[1)] according to Novais et al. 2015; [2)] according to Potapova & Hamilton 2007, Morales et al. 2011; [3)] according to Van de Vijver et al. 2011

Characteristics to differentiate *Achnanthidium eutrophilum*, *A. pyrenaicum* and *A. straubianum* from *A. minutissimum* var. *minutissimum* are presented and discussed in detail in the report of the first German intercalibration exercise (Dreßler et al. 2014).

Overall, we support the recommendations of the first intercalibration exercise (Dreßler et al. 2014) on how to identify *Achnanthidium minutissimum* var. *minutissimum* and similar taxa. Again, we emphasize that both need to be incorporated, the valve dimensions and additional characteristics (shape, specific features), to identify all diatom objects to the highest possible level (as postulated). If an allocation to a species (or taxon) is ambiguous due to overlapping characteristics, we recommend to label the object accordingly (with "cf." or "aff.") and document it well (pictures, brief description of the problem).

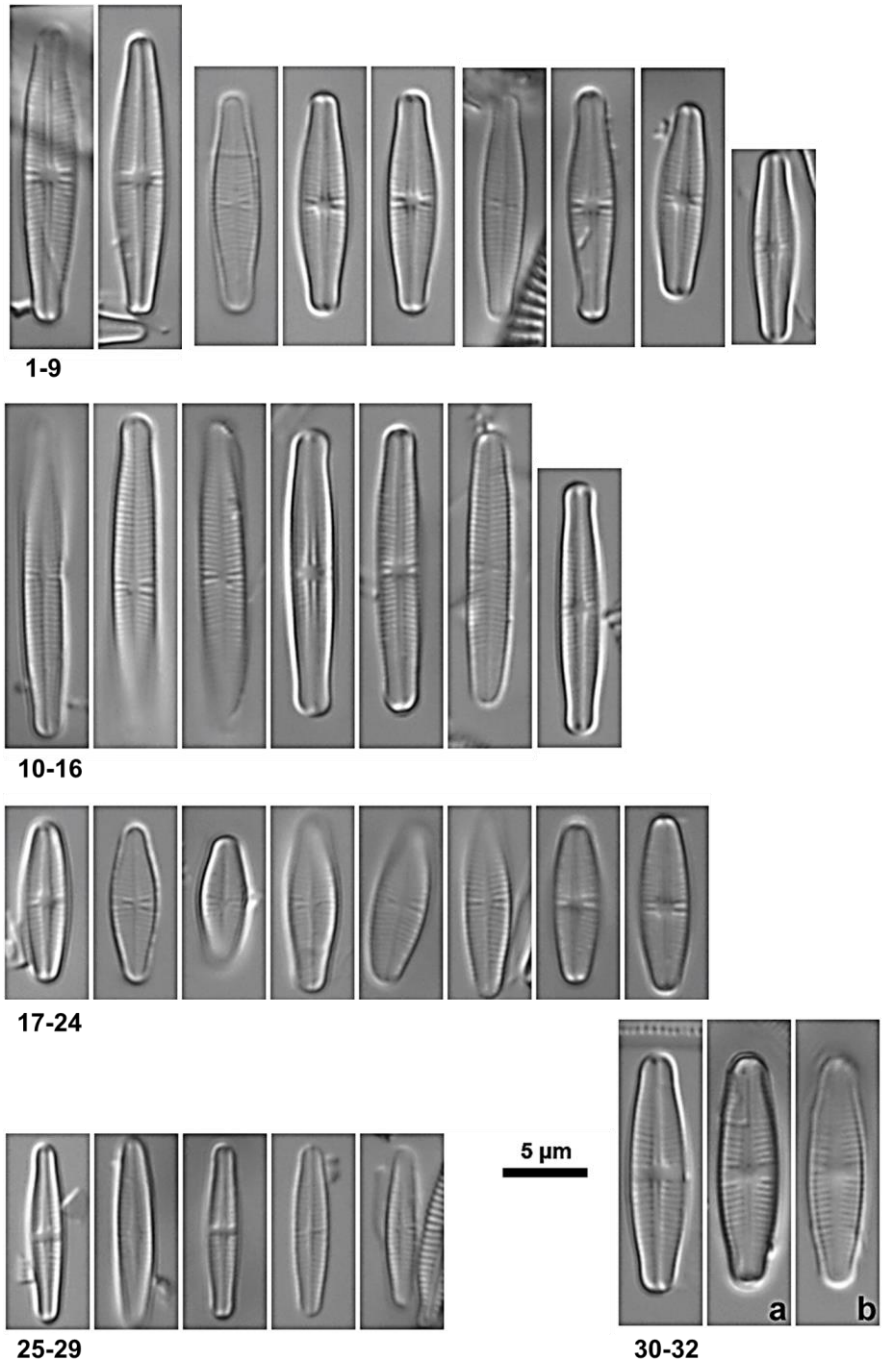


Plate 7. Comparison of *Achnanthidium minutissimum* with similar taxa from the ‘Lake’ sample (see Table 17). **1-9: *A. minutissimum***: 30 striae/10 µm, **10-16:** Dimensions (17.2-20.5 µm long, 2.4-2.7 µm wide, 28-30 striae/10 µm) correspond to ***A. minutissimum***, but the **shape** is rather **atypical**; valves are too large for *A. lineare* and too small for both *A. caledonicum* and *A. neomicrocephalum*, but valves are also similar to ‘particularly narrow valves’ (“besonders schmale Schalen") of “*Achnanthes minutissima*” in Krammer & Lange-Bertalot (2004; Plate 32: 27-30, p. 60 and p. 313, respectively), **17-24:** Dimensions (8.4-12.0 µm long, 2.7-3.3 µm wide, 28-30 striae/10 µm) correspond to ***A. minutissimum***; not *A. eutrophilum* or *A. saprophilum* despite fitting shapes, as some valve width are too low and the striae are too dense (for *A. eutrophilum*), **25-29: *Achnanthidium* sp.** (28-30 striae/10 µm), due to valve width (1.7-2.2 µm) that does **not** correspond to *A. minutissimum var. minutissimum*, *A. sublineare* with higher striae density, *A. lineare* with different shape and usually wider; similar to ‘particularly narrow valves’ (see above), **30-32: *A. jackii*** from the ‘Stream’ sample for comparison, 31 and 32 depict both valves of the same frustule: (a) raphe valve, (b) araphid valve; fascia (crossband) visible in the central area of both raphe valves (30 and 31).

4.4.2 *Cymbella vulgata* and other *Cymbella*-species

The auditors identified *Cymbella vulgata* as the second most common species in the 'Lake' sample. They determined the relative abundance of this species to be 13.0 %, 10.2 % and 8.7 % (average: 10.6 %). In contrast, only 19 of 40 participants listed any *Cymbella vulgata* and partly with distinctly below average abundances (Fig. 42), indicating severe difficulties with the identification of this species. Only one participant (Laboratory Code 26) indicated any ambiguity of the identification by labelling a relative abundance of 2.4 % with "cf.". One participant (Laboratory Code 21) listed *C. vulgata* var. *vulgata*. In the following, we only use the name *C. vulgata*.

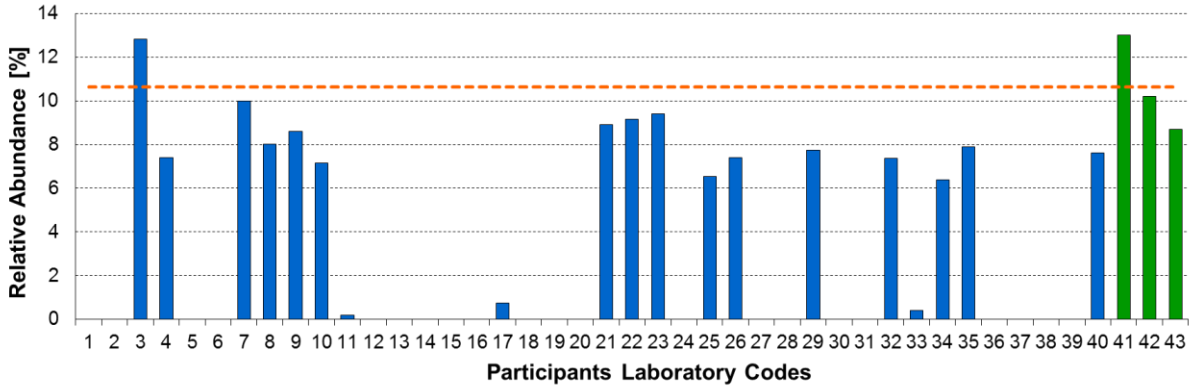


Figure 42. The relative abundance of ***Cymbella* (cf.) *vulgata* (var. *vulgata*)** determined by each participant in the 'Lake' sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

The certain identification of the genus *Cymbella* did not pose a problem to most participants of the intercalibration exercise. Only three participants identified the relative abundance of this genus to be distinctly below or above the auditor average abundance (Fig. 43).

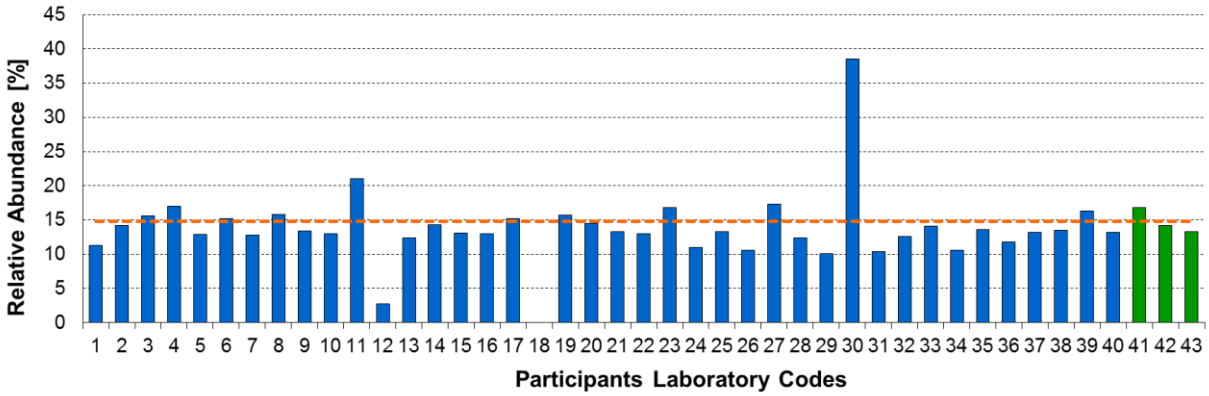


Figure 43. Sum of the relative abundance of all ***Cymbella*-taxa** determined by each participant in the 'Lake' sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

Several species can be mistaken for *Cymbella vulgata*, namely *C. excisa*, *C. excisiformis*, *C. parva*, *C. perparva*, *C. hungarica*, *C. maggiana* and *C. novazeelandiana* (Krammer 2002). The latter three species were either not found at all or only occasionally by the participants in the 'Lake' sample (Fig. 44).

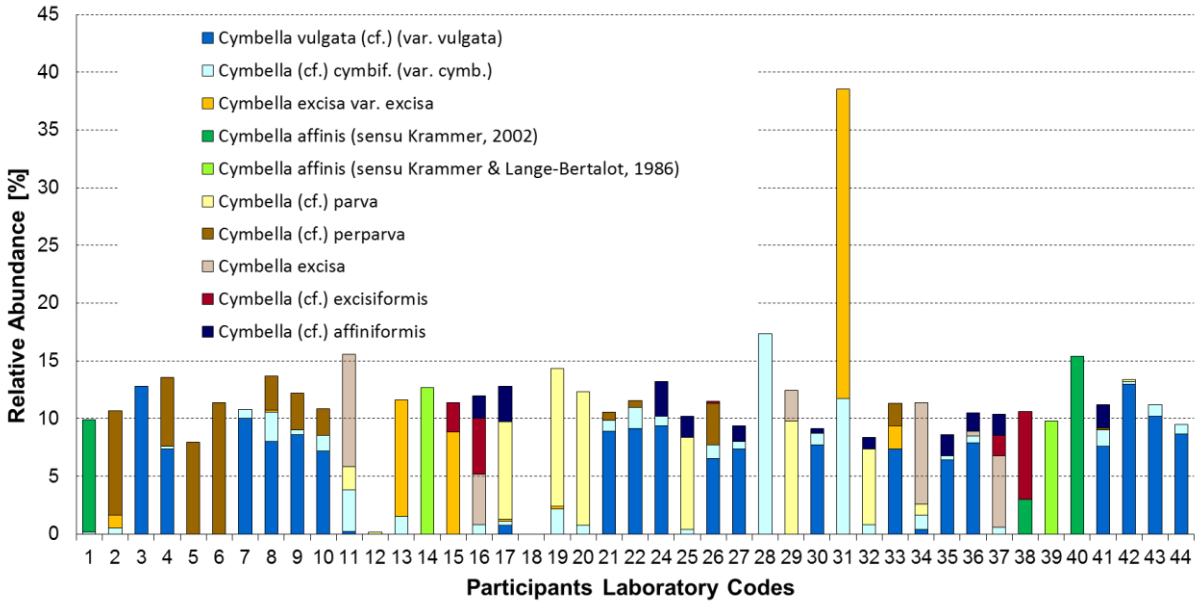


Figure 44. ***Cymbella*-taxa** that were identified with a relative abundance of at least 3 % by a participant or auditor in the ‘Lake’ sample. Blue: relative abundance of *Cymbella vulgata*, *cymbif*. = *cymbiformis*, participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

Next to *Cymbella vulgata*, the auditors additionally determined *C. cymbiformis* var. *cymbiformis* and *C. kappii* with low relative abundances (Fig. 45 and 46). In contrast to the auditors, participants apparently identified the latter as *C. perparva*. As *C. kappii* and *C. perparva* are hardly distinguishable using light microscopy, they are presented together in one figure (Fig. 45). Participants that determined the relative abundances of these species to be distinctly greater than 4 % (Fig. 45), likely misidentified the more abundant *C. vulgata*. Similarly, distinctly higher abundances than 1-2 % of *C. cymbiformis* (var. *cymbiformis*) in the ‘Lake’ sample also indicate possible misidentifications of *C. vulgata* (Fig. 46). Other *Cymbella*-taxa that participants identified with relative abundances of more than 3 % also likely indicate misidentifications. These taxa include *C. excisa* (var. *excica*), *C. excisiformis*, *C. parva*, *C. affinis* and *C. affiniformis* (Fig. 44). Despite these apparent difficulties of identifying and differentiating *Cymbella*-taxa, especially *C. vulgata*, hardly any identifications were labelled with cf., i.e. listed as determined with ambiguity or listed at genus level (*Cymbella* sp.) (Fig. 47).

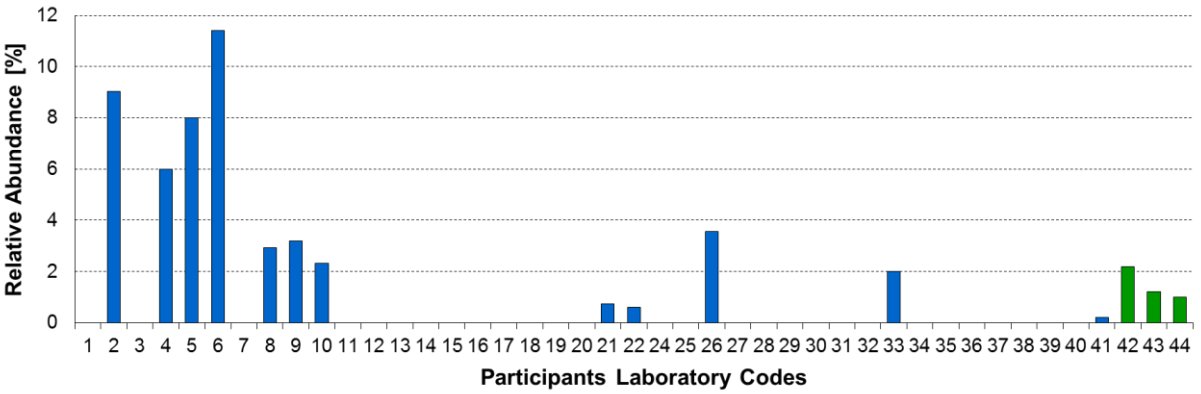


Figure 45. The relative abundance of ***Cymbella* (cf.) *perparva*** (blue) and ***C. kappii*** (green, exclusively identified by the auditors) determined by each participant in the ‘Lake’ sample.

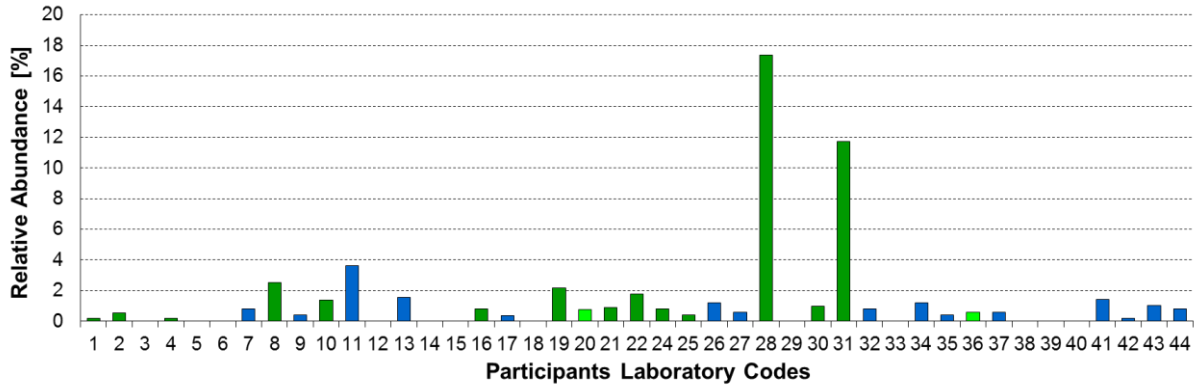


Figure 46. The relative abundance of ***Cymbella* (cf.) *cymbiformis* (var.) *cymbiformis*** determined by each participant in the ‘Lake’ sample. Blue: *C. cymbiformis* var. *cymbiformis*, green: *C. cymbiformis*, light green: *C.* cf. *cymbiformis*. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

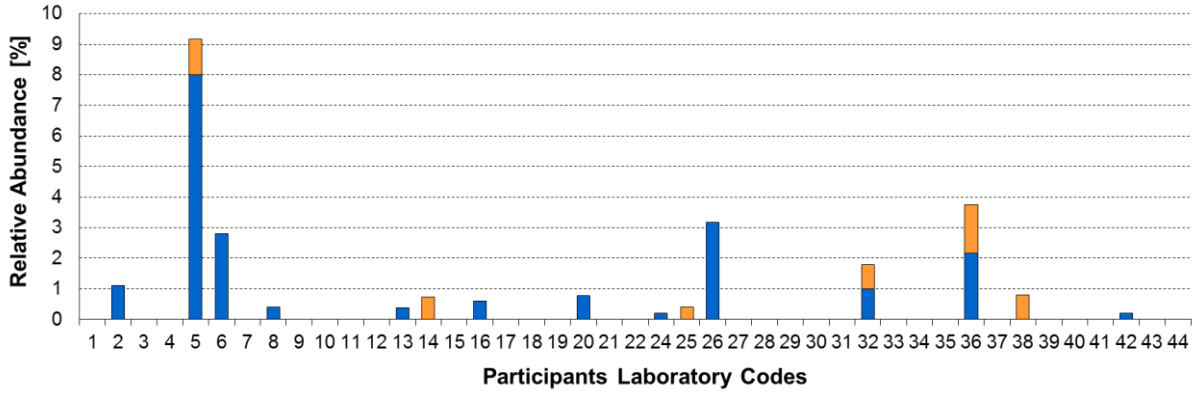


Figure 47. The relative abundance of ambiguously determined species from the genus *Cymbella* (***Cymbella* cf.**, blue) as well as ***Cymbella* sp.** (not further differentiated, orange) determined by each participant in the ‘Lake’ sample. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

Similar to the other, already presented and difficult to identify taxa/aggregates, for a definite identification of the *Cymbella*-species all characteristics listed in the identification literature need to be taken into account. According to the appropriate identification keys, especially the areolae density (points / 10µm), the presence and number of stigmata, valve width and valve shape are necessary characteristics to identify the correct groups (Krammer 2002, Hofmann et al. 2013). Other important characteristics to distinguish the species include the length to width ratio, the shape of the valve ends and the shape of the proximal raphe ends.

When using all listed characteristics (Table 18), *Cymbella vulgata* should be identifiable without ambiguity, especially when taking the size and shape of a valve into account, as well as the areolae density (20-24(25) points / 10 µm), the length to width ratio and the presence of one ventral stigma. *Cymbella vulgata* can be distinguished from *C. excisa* and *C. excisiformis* by the areolae density and shape of the valve ends. *C. affinis* and *C. affiniformis* have more than one stigma (2-4 and two stigmata, respectively), a higher areolae density and a smaller length to width ratio compared to *C. vulgata*. *Cymbella parva*, *C. perparva* and *C. kappii* also have a higher areolae density and a smaller length to width ratio compared to *C. vulgata*. *Cymbella cymbiformis*, that apparently was mistaken for *C. vulgata* by only two participants (Fig. 46), is usually bigger than *C. vulgata* and has distinctly coarser areolae (Table 18).

As detailed by the authors of Hofmann et al. (2013), they had difficulties to select the most common 700 diatom species presented in their book, particularly for certain genera (e.g. *Cymbella*). They point out that probably not all relevant species were included. For example, *Cymbella perparva* (or *C. kappii*), *C. affiniformis* and *C. hungarica* are not included in Hofmann et al. (2013). Accordingly, Krammer (2002) should additionally be used to identify *Cymbella*-species (see also Schaumburg et al. 2011c, 2012). Species that can only be identified ambiguously, should be labelled ("cf.", "aff.", *Cymbella* sp.) and documented (pictures) accordingly.

Table 18. Selected characteristics for the identification of *Cymbella vulgata* in comparison to similar taxa and to other *Cymbella*-taxa that were determined by participants and auditors in the 'Lake' sample. Sources: Hofmann et al. (2013) and Krammer (2002).

Taxon	**Length (µm)**	**Width (µm)**	**Length/ Width**	**Striae/ 10 µm**	**Areolae/ 10 µm**	**Comment**
C. vulgata	20-56	7.8-10.7/ 12.7	up to 5.2	mid: 8-12 ends: up to 14	20-24(25)	1 ventral stigma, ends rounded but not protracted
C. excisa	17-41	6-10.7	3.1-3.8	mid: 9-13 ends: 12-14	25-32	1 ventral stigma, distinct dorsiventral shape, ends protracted
C. excisiformis	18-44	6-9	4.2-5.3	mid: 9-11 ends: up to 16	24-30	1 ventral stigma, distinct dorsiventral shape, ends protracted
C. affinis (sensu Krammer 2002)	17-34	7.5-9.5	up to 4.0	mid (dorsally): 10-13 mid (ventrally): 13-15 ends: up to 17	27-32	2-4 ventral stigmata, ends rostrate
C. affiniformis	23-34	7.4-8.7	up to 3.9	mid: 10-12 ends: 14-15	28-30	2 ventral stigmata, ends protracted
C. parva	15-47	7-10	up to 4.5	mid: 9-11 ends: up to 13	28-30	1 ventral stigma, ends bluntly rounded, not protracted
C. perparva	22-46	6.4-8.7	up to 4.8	mid: 9-12 ends: ~12	25-29	1-3 ventral stigmata
C. kappii	22-58	7-10.5	up to 4.8	mid: 8-12 ends: 11-15	22-24	2-4 ventral stigmata
C. cymbiformis	40-105	13-17	up to 6.0	mid: 7-10 ends: up to 15	16-20	1-2(3) ventral stigmata

The relative abundance of *Cymbella vulgata* and *C. perparva/kappii* (on average determined by the auditors to be 12.1 %) do not contribute to the water quality assessment of the ‘Lake’ sample when using the German PHYLIB-method, because these species have no trophic indicator values (Table 19). This reduces the reliability of the inferred assessment (see Chapter 4.5). *Cymbella cymbiformis* only contributes to the assessment, if entered as *C. cymbiformis var. cymbiformis* (German DV-no. 6979). Therefore, the relative abundance from the 16 participants that listed *C.* (cf.) *cymbiformis* (DV-no. 36033) instead, do not contribute to the assessment when using the PHYLIB-method (Fig. 46). This emphasizes the necessity to recount the PHYLIB training set according to the current taxonomy to ideally generate indicator values for each species.

Table 19. Saprobic values (**S**), trophic values (**TI**) and weighting (**G**) of *Cymbella vulgata*, similar taxa and taxa that were identified by participants and auditors in the ‘Lake’ sample, for lotic systems and lakes as listed in the PHYLIB software (version 5.3, December 2015).

Taxon	**Lotic Systems**				**Lakes**		
	S	**G**	**TI**	**G**	**TI_{North}**	**TI_{South}**	**G**
C. vulgata	-	-	-	-	-	-	-
C. excisa	-	-	-	-	-	-	-
C. excisa var. *excisa*	-	-	-	-	-	4.1	2.0
C. excisiformis	-	-	-	-	-	2.4	1.0
C. affinis (sensu Krammer 2002)	1.0	5.0	1.6	2.0	0.48	1.5	3.0
C. affiniformis	1.2	4.0	0.7	4.0	1.09	-	-
C. parva	-	-	-	-	-	-	-
C. perparva	-	-	-	-	-	-	-
C. kappii	-	-	-	-	-	-	-
C. cymbiformis	-	-	-	-	-	-	-
C. cymbiformis var. *cymbiformis*	1.0	5.0	1.8	3.0	0.71	1.3	2.0

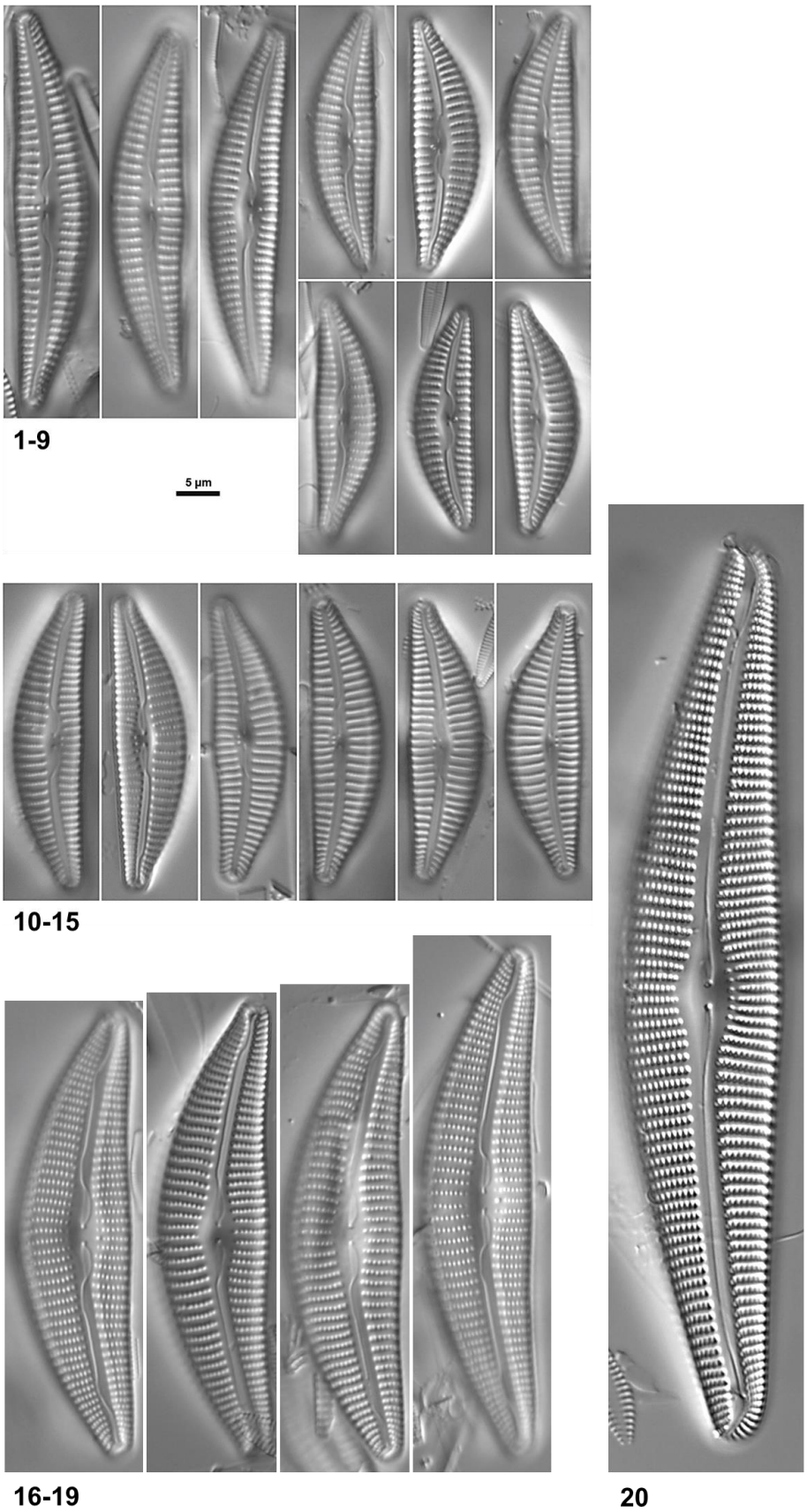


Plate 8. *Cymbella vulgata* and other *Cymbella* species in comparison from the ‘Lake’ sample. **1-9:** *C. vulgata*, **10-15:** *C. perparva* or *C. kappii*; areolae density (25-30 areolae / 10 µm) is characteristic of *C. perparva*, while the width of Fig. 13-15 are characteristic of *C. kappii*, **16-19:** *C. cymbiformis*, **20:** *C. helvetica*.

4.4.3 *Encyonopsis minuta* and *E. subminuta*

Encyonopsis minuta and *E. subminuta* were abundant in the 'Lake' sample. The auditors identified the relative abundance of *E. minuta* to be 8.2 %, 11.4 % and 8.3 % (average: 9.3 %) and of *E. subminuta* to be 6.8 %, 4.0 % and 3.6 % (average: 4.8 %; Fig. 48 and 49). Eight participants did not identify any *E. minuta* and nine participants did not determine any *E. subminuta* in the 'Lake' sample. The remaining participants determined the relative abundance of *E. minuta* to range from 0.6 % to 20.5 % (average 11.5 %, n=32; Fig. 48) and of *Encyonopsis subminuta* to range from 0.6 % to 13.6 % (average 5.0 %, n=31; Fig. 49). Only the participants with laboratory codes 4, 26 and 36 (*E. minuta*) and codes 32 and 38 (*E. subminuta*) labelled the identification of *Encyonopsis*-species as ambiguous ("cf."). The lack of determining either *Encyonopsis*-species and the sometimes distinct deviations from the average of the auditor relative abundances (Fig. 48 & 49) clearly demonstrate identification difficulties.

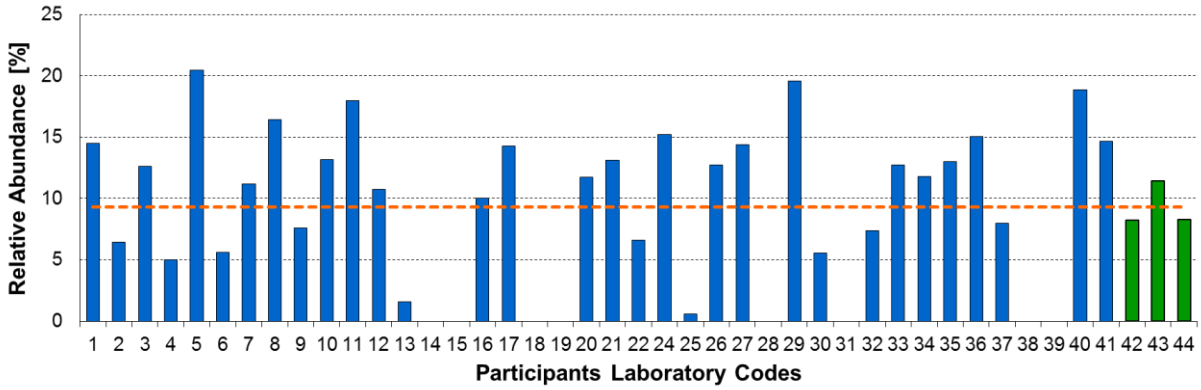


Figure 48. The relative abundance of ***Encyonopsis* (cf.) *minuta*** determined by each participant in the 'Lake' sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

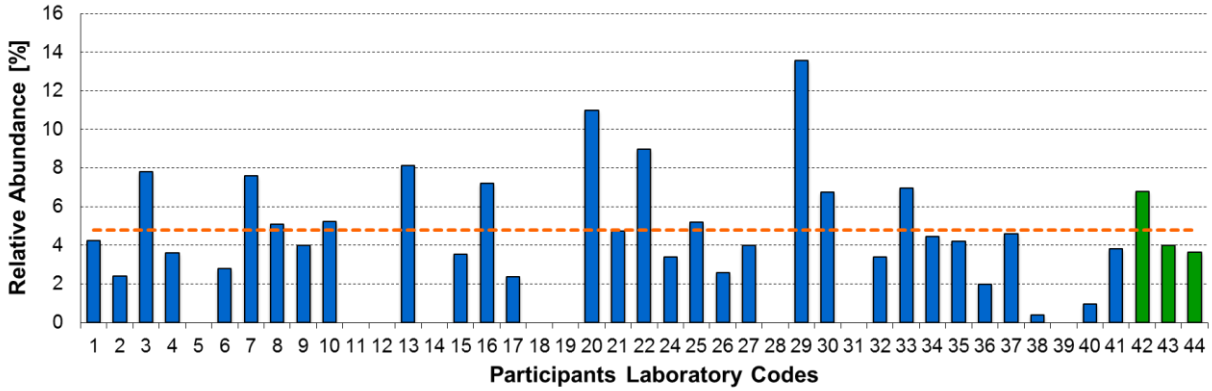


Figure 49. The relative abundance of ***Encyonopsis* (cf.) *subminuta*** determined by each participant in the 'Lake' sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

Difficulties identifying *Encyonopsis*-species were already apparent during the first German intercalibration exercise, particularly the exact allocation to the species level within the *Encyonopsis* genus (Dreßler et al. 2014). Similarly, most participants of this intercalibration exercise recognised the genus *Encyonopsis* (Fig. 50). Two participants determined *Cymbella microcephala* according to

Krammer & Lange-Bertalot (1986–2004) (Fig. 51), which may correspond to *Encyonopsis minuta*, *E. subminuta* or other *Encyonopsis*-species according to recent identification literature. These *Encyonopsis*-species are now independent species. Two other participants did not identify any *Encyonopsis*-species nor *C. microcephala*, while one participant determined distinctly above average abundances of *Encyonopsis*-species (Fig. 50 and 51).

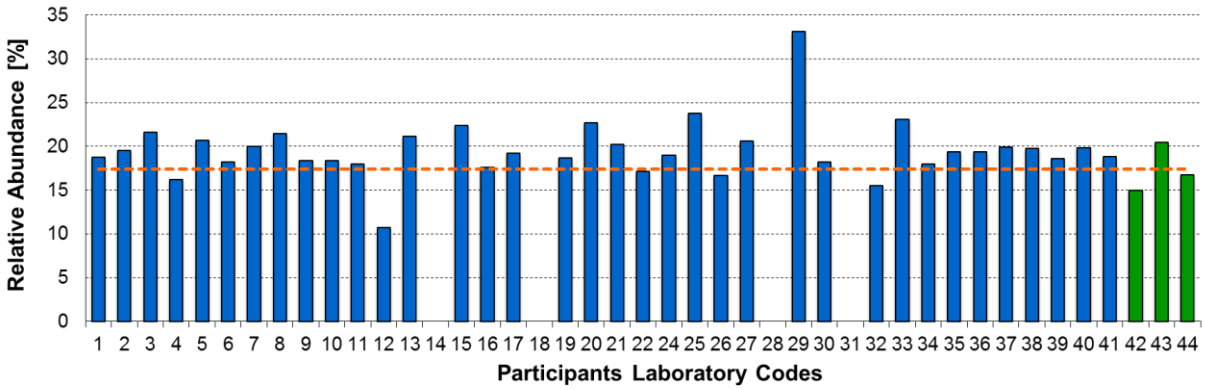


Figure 50. The relative abundance of ***Encyonopsis* sp.** and all **taxa within the genus** determined by each participant in the ‘Lake’ sample. Blue bars: participants, green bars: auditors. Orange horizontal dashed line = average of auditors.

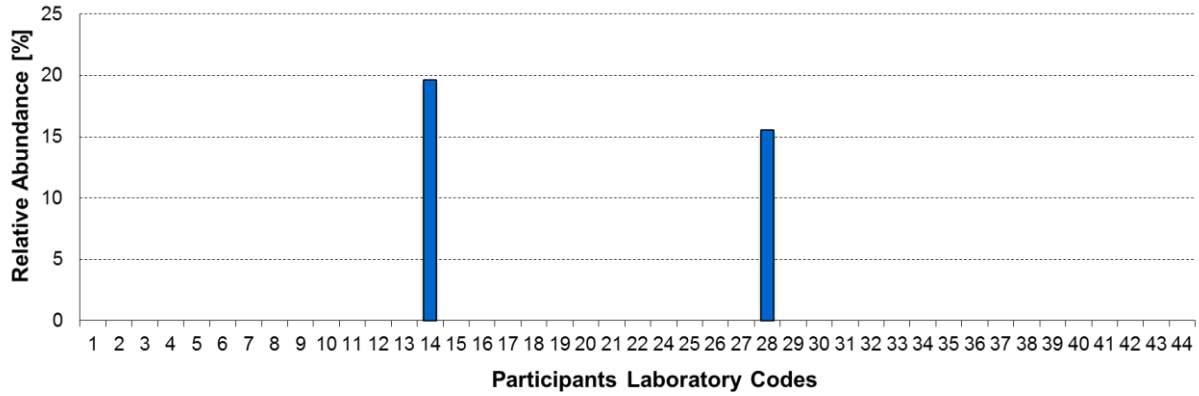


Figure 51. The relative abundance of ***Cymbella microcephala*** determined by each participant in the ‘Lake’ sample. Blue bars: participants.

Next to *E. minuta* and *E. subminuta*, participants and auditors of the intercalibration exercise identified additional *Encyonopsis*-species in the ‘Lake’ sample: *E. microcephala*, *E. krammeri*, *E. thumensis*, *E. eifelana*, *E. rostrata* and *E. subfonticola*, sometimes determined with ambiguity (labelled with “cf.“). Only one of the three auditors identified *Encyonopsis microcephala* (with a relative abundance of 3.2 %). Similarly, 17 participants determined the relative abundance of *E.* (cf.) *microcephala* to range from 0.2 % (labelled with “cf.“) to 18.8 % (Fig. 52). Also, two of three auditors determined *E. krammeri* with low relative abundances in the ‘Lake’ sample (Fig. 53). Twelve participants also identified this species with low relative abundances and also with 6.4 % abundance and with 15.8 %, respectively (Fig. 53). Three participants (laboratory codes 3, 16 and 26) labelled the findings with “cf.“ (ambiguous identifications). One auditor determined the relative abundance of *E. thumensis* to be 4.8 % (Fig. 54). Two participants (laboratory codes 4 and 6) identified *E.* cf. *thumensis* with low abundances (Fig. 54). Additionally, few participants identified *E. eifelana* (laboratory codes 2 and 6 with 1.5 % and 8.0 %, respectively), *E. rostrata* (Laboratory Code 2; 7.0 %) and *E. subfonticola* (Laboratory Code 2; 1.6 %).

Overall, the often inconsistent counting results distinctly indicate great problems identifying *Encyonopsis*-species.

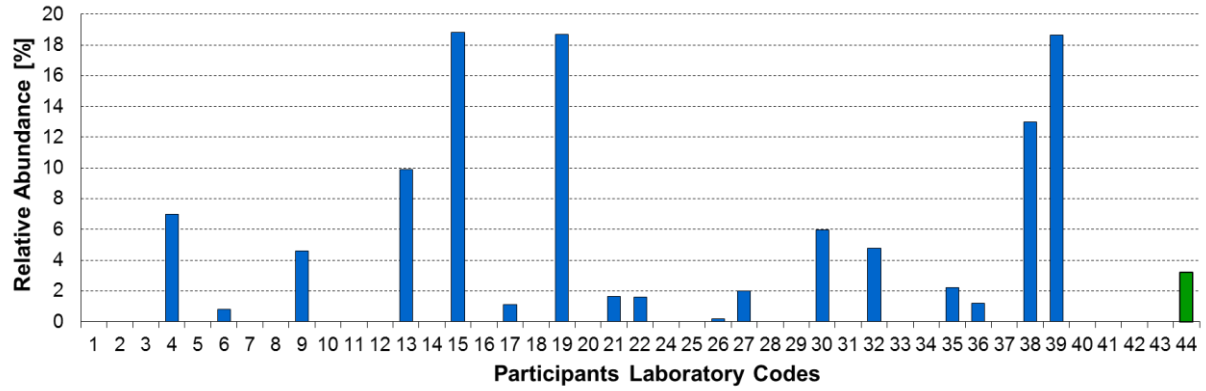


Figure 52. The relative abundance of ***Encyonopsis* (cf.) *microcephala*** determined by each participant in the ‘Lake’ sample. Blue bars: participants, green bar: auditor.

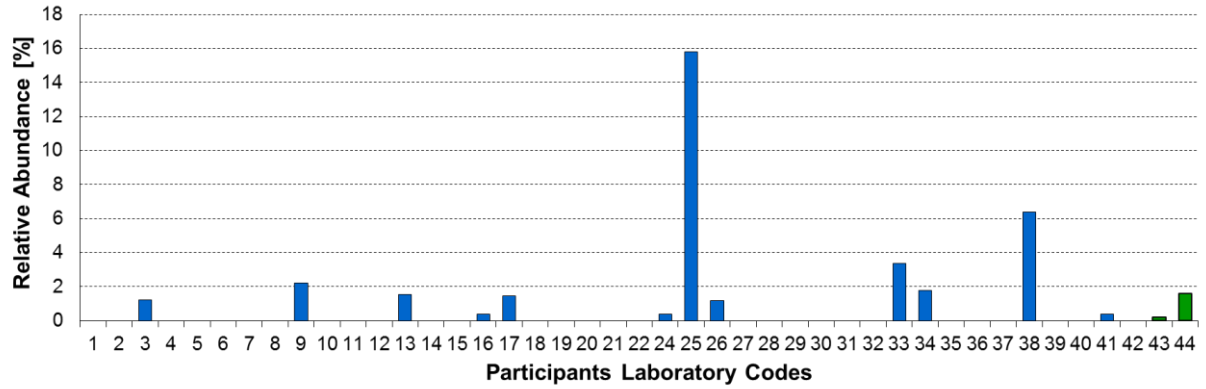


Figure 53. The relative abundance of ***Encyonopsis* (cf.) *krammeri*** determined by each participant in the ‘Lake’ sample. Blue bars: participants, green bars: auditors.

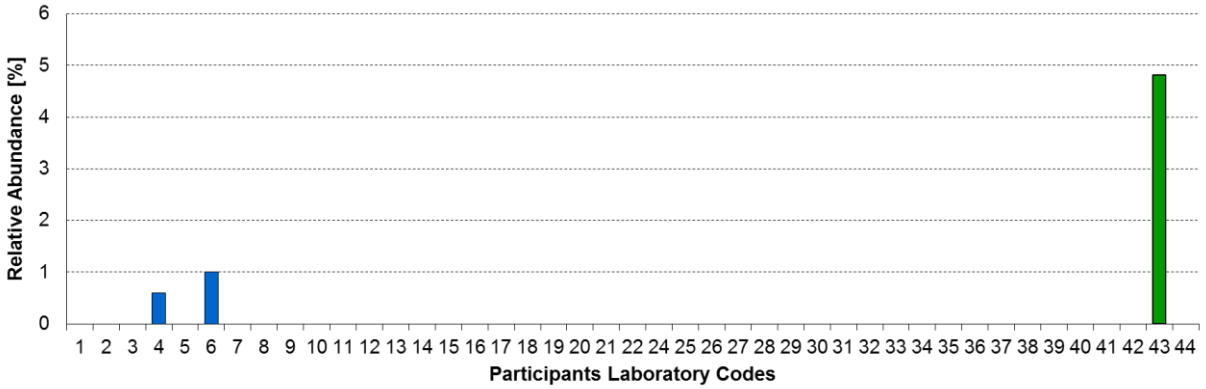


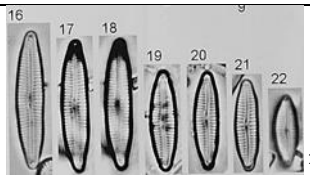
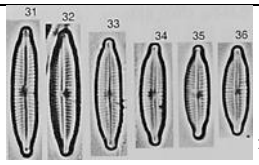
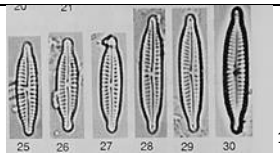
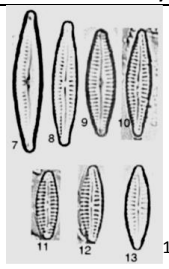
Figure 54. The relative abundance of ***Encyonopsis* (cf.) *thumensis*** determined by each participant in the ‘Lake’ sample. Blue bars: participants, green bar: auditor.

A detailed discussion of the taxonomic differentiation of *Encyonopsis*-species, especially of *E. minuta*, *E. subminuta*, *E. microcephala* and *E. krammeri,* is provided in the report of the first German intercalibration exercise of benthic diatoms (Dreßler et al. 2014). Also given are recommendations on

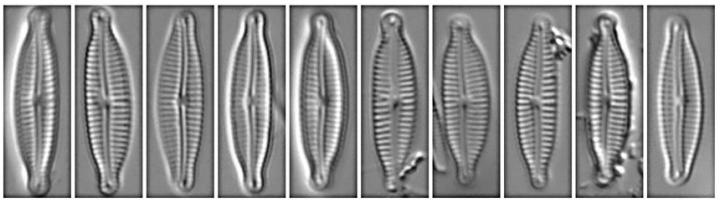
how to deal with these species when using the German PHYLIB-method for assessing the water quality of lakes and rivers (Dreßler et al. 2014). Important characteristics for the identification of the *Encyonopsis*-species that were identified by the participants and auditors in the ‘Lake’ sample are summarised in Table 20.

As already suggested in Dreßler et al. (2014), we recommend to take all characteristics into account that are provided in the identification literature to differentiate *Encyonopsis* species. Ideally Krammer (1997b) is used as identification literature, as the selection of *Encyonopsis*-species presented in Hofmann et al. (2013) are often not sufficient. If Krammer (1997b) is not available (volume is out of print), the diatom valves should be well documented (pictures). We recommend an exchange with colleagues that possess the volume.

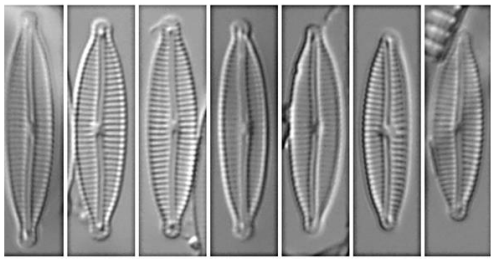
Table 20. Characteristics for the identification of *Encyonopsis minuta* and *E. subminuta* in comparison to similar taxa and other taxa that were identified by the participants and auditors in the ‘Lake’ sample. Sources: Hofmann et al. (2013) and Krammer (1997b).

Taxon	Length (µm)	Width (µm)	Length/ Width	Striae/ 10 µm	Comment
E. minuta	8-17	2.8-3.5	4.9	24-25	Valve width! Ends without shoulders
E. subminuta	10-25	3.4-4.5	max. ~6	23-26	Valve shape usually symmetrical, ends without shoulders
E. microcephala	10-23	3.5-4.2	5.4	23-25	Ends usually capitate with shoulders on both sides
E. krammeri	11.5-23	2.6-3.8	up to 7	(27)28-30(32)	Striae density!
E. thumensis	9.5-18	3.5-4	up to 4.5	23-26	 1)
E. eifelana	11-17	3.4-3.6	up to 4.7	23-24	 1)
E. rostrata	8-17	3-3.8	up to 4.3	19-21	 1) Striae density!
E. subfonticola	9-19	3-4	up to 4.8	19-22	 1) Striae density!

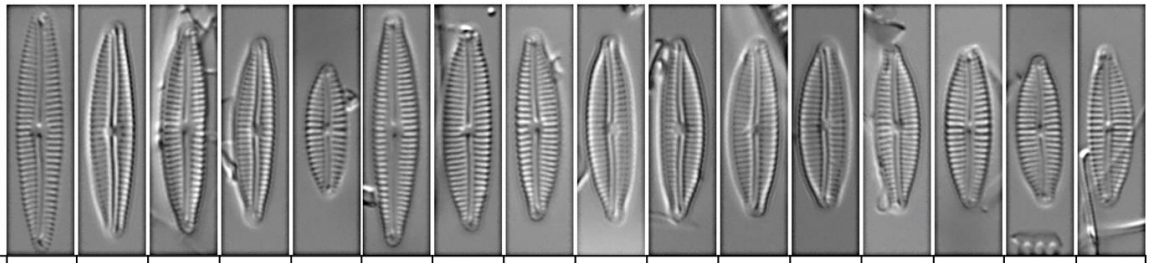
[1)] Figure sources: Krammer (1997b)



1-10



11-17 18-19



W	3.6	3.4	3.3	3.2	3.2	3.6	3.7	3.5	3.5	3.4	3.6	3.7	3.5	3.5	3.5	3.5
St	22.0	22.5	22.5	24.0	22.5	23.0	23.0	23.5	23.3	25.5	23.3	24.0	25.5	24.0	24.8	24.0

20-35

Plate 9. *Encyonopsis minuta, E. subminuta* and similar taxa in comparison from the ‘Lake’ sample. **1-10:** *E.* (cf.) *minuta* (width: 3.1-3.6; striae/10 µm: 23.3-24.8), with cf. for width >3.5µm, **11-17:** *E. subminuta* (width: 3.5-4.0; striae/10 µm: 23.5-25.0), **18-19:** *E.* cf. *minuta*, 18: Dimensions correspond to *E. minuta*, shape more similar to *E. subminuta*, 19: according to dimensions this valve could be *E. minuta*, *E. subminuta* and *E. thumensis*. However, shape does not really correspond well to either species, **20-35:** *Encyonopsis* sp., an unambiguous allocation of these valves is not possible, despite the incorporation of all identification characteristics (shape, width and striae density, see also Table 19), possible are: *E. subminuta*, *E. thumensis*, *E. minuta* or *E. krammeri*. W= valve width, St = number of striae / 10 µm of valve above numbers.

4.4.4 *Fragilaria (Pseudostaurosira) brevistriata*

Fragilaria brevistriata was one of the common species in the ‘Lake’ sample. The auditors determined the relative abundance of *Fragilaria brevistriata* to be 6.6 %, 7.0 % and 8.3 % (Fig. 55). The participants of the intercalibration exercise identified the relative abundance of *F. brevistriata* (also listed as *F. brevistriata* var. *brevistriata*, *Pseudostaurosira brevistriata* or *Staurosira* (cf.) *brevistriata*) to range from 0.8 % to 11.5 % (average 5.2 %, n = 36). Four participants did not determine any *F. brevistriata* in the ‘Lake’ sample (Fig. 55). This lack of identification and the relative abundances that distinctly deviate from the auditor average (Fig. 55), suggest difficulties with the identification of *F. brevistriata*.

Next to *Fragilaria brevistriata* the auditors and/or participants identified other small *Fragilaria*- taxa in the ‘Lake’ sample*: F. pinnata* var. *pinnata*/var. *lancettula*, *F. construens* (cf.) f. *venter*, *F. lapponica* and *F.* (cf.) *elliptica* (Fig. 56). The auditors determined the relative abundance of *F. pinnata* (var. *pinnata* and var. *lancettula*) to be 1.2 %, 2.6 % and 1.2 %. The participants identified the relative abundance of *F. pinnata* to range from 0.2 % to 5.7 % (average: 2.3 %)(Fig. 57). The lack of detection and the identification of relative abundances that distinctly deviate from auditor average abundances (Fig. 57) suggest some difficulties with the identification of *F. pinnata.*

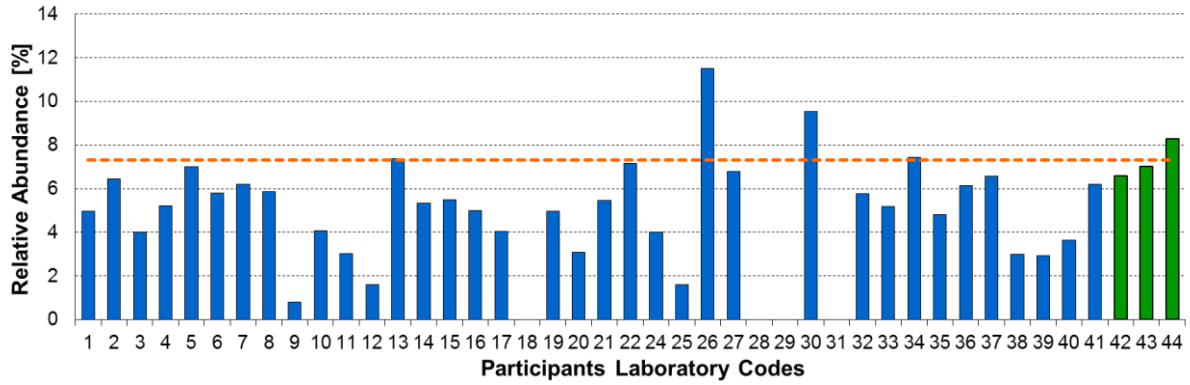


Figure 55. The relative abundance of ***Fragilaria* (cf.) *brevistriata* (var. *brevistriata*)** determined by each participant in the ‘Lake’ sample. Blue bars: participants, green bars: auditors. Orange horizontal dashed line = average of auditors.

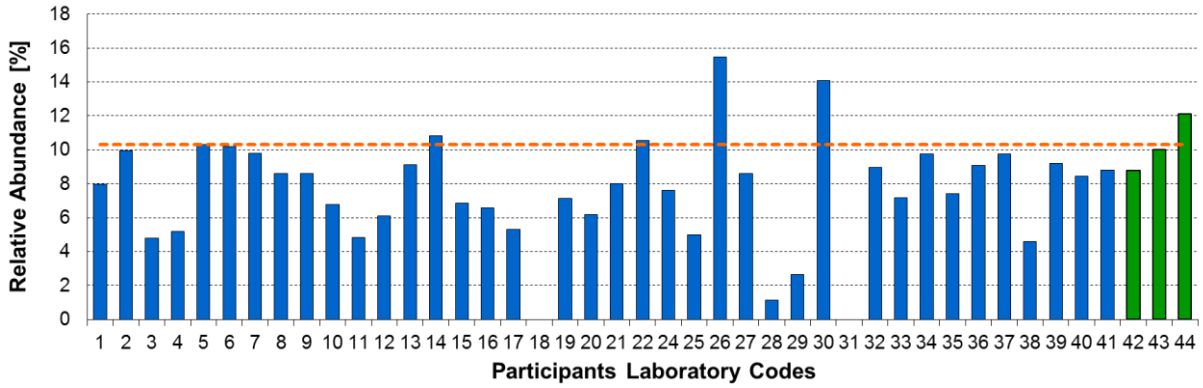


Figure 56. Sum of the relative abundance of **small *Fragilaria-species*** including *Fragilaria* (cf.) *brevistriata* (var. *brevistriata*), *F. pinnata* var. *pinnata*/var. *lancettula*, *F. construens* (cf.) f. *venter, F. lapponica, F.* (cf.) *elliptica* determined by each participant in the ‘Lake’ sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

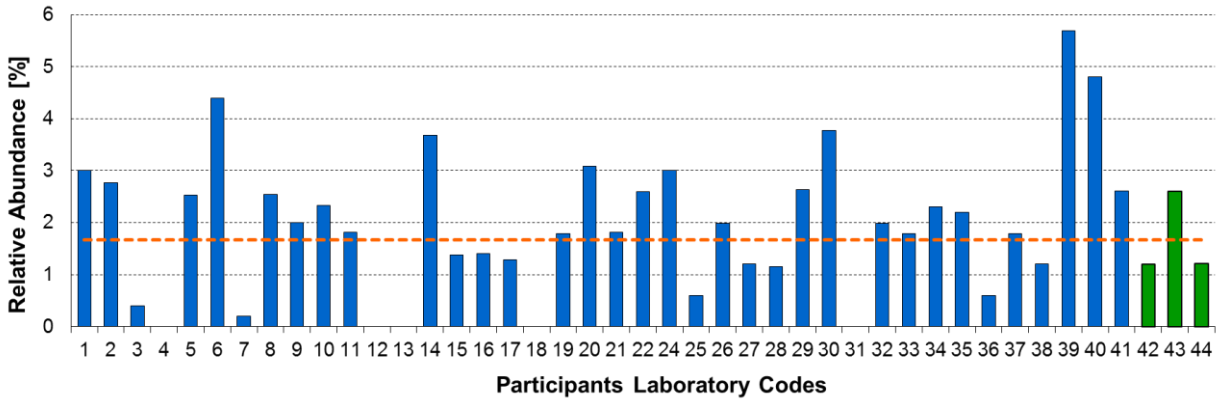


Figure 57. The relative abundance of ***Fragilaria pinnata* var. *pinnata*/var. *lancettula*** determined by each participant in the ‘Lake’ sample. Blue bars: participants, green bars: auditors. Orange horizontal dashed line = average of auditors.

The counting results are also relatively diverse for *Fragilaria construens* (cf.) f. *venter*, which was determined by the auditors with relative abundances of on average 1.3 % in the ‘Lake’ sample (Fig. 58). The participants determined the relative abundance of *Fragilaria construens* (cf.) f. *venter* to range from zero (15 participants) to 3.4 % (Fig. 58). Additionally, some participants identified *F. lapponica* and *F.* (cf.) *elliptica* with low abundances (Fig. 59 and 60).

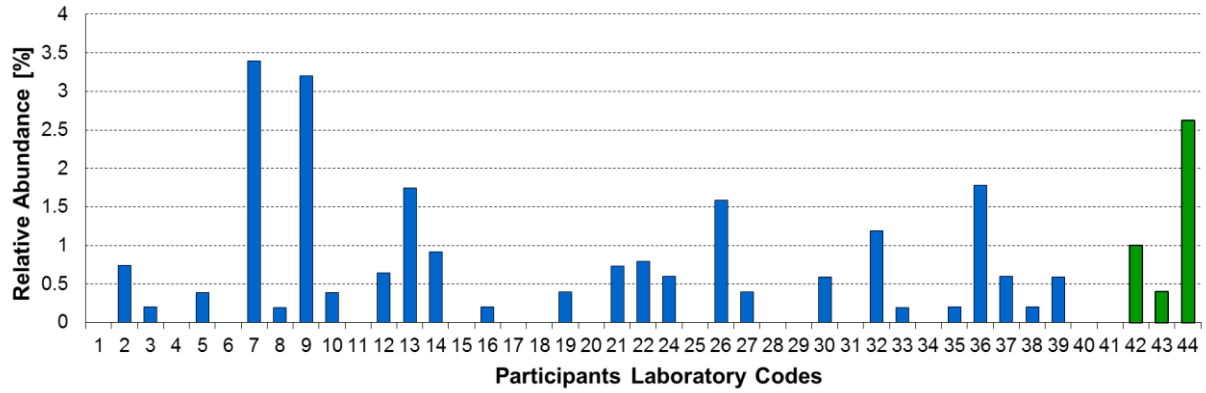


Figure 58. The relative abundance of ***Fragilaria construens* (cf.) f. *venter*** determined by each participant in the ‘Lake’ sample. Blue bars: participants, green bars: auditors.

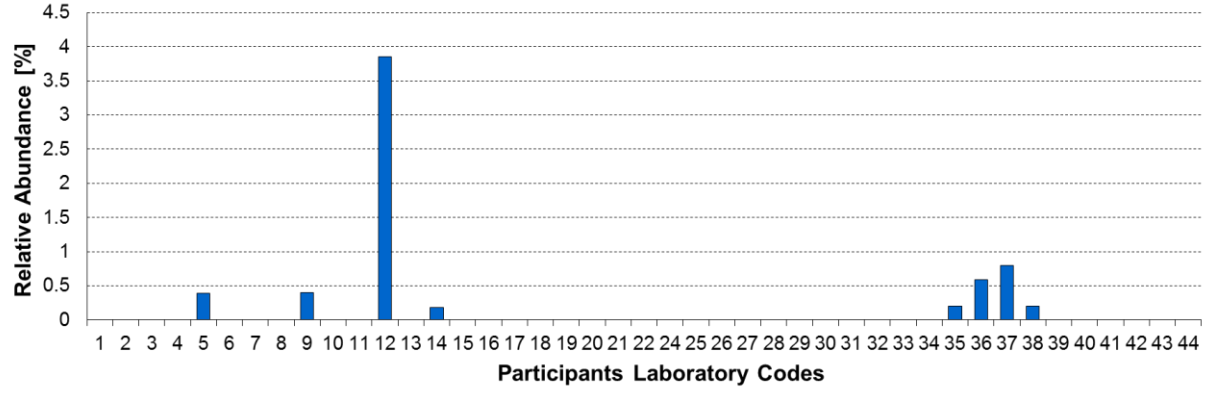


Figure 59. The relative abundance of ***Fragilaria lapponica*** determined by each participant in the ‘Lake’ sample. Blue bars: participants.

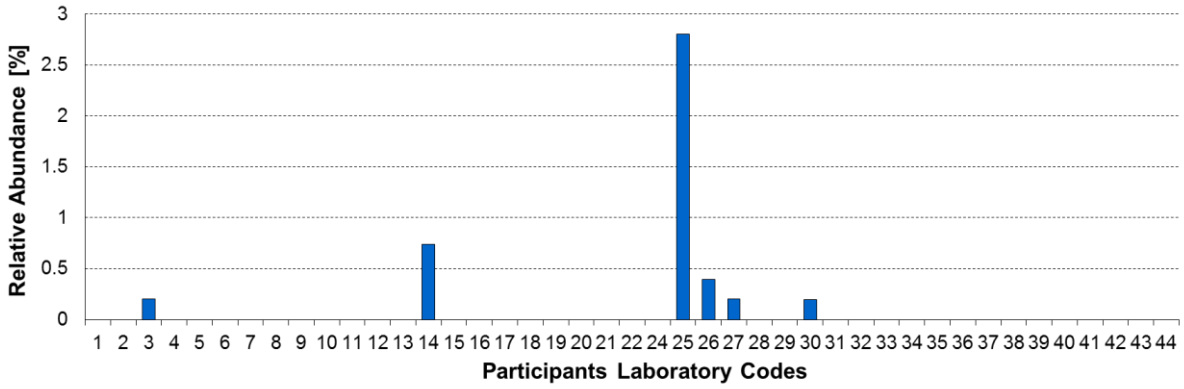


Figure 60. The relative abundance of ***Fragilaria* (cf.) *elliptica*** determined by each participant in the ‘Lake’ sample. Blue bars: participants.

The taxonomic distinction of *Fragilaria brevistriata* to the other, here mentioned similar taxa, is discussed in detail in the report of the first German intercalibration exercise (Dreßler et al. 2014) and also summarized in Table 21.

Table 21. Characteristics for the identification of *Fragilaria brevistriata* in comparison to similar taxa that were identified by the auditors and/or participants in the ‘Lake’ sample. Sources: Hofmann et al. (2013) and Krammer & Lange-Bertalot (1986-2004).

Taxon	**Length (µm)**	**Width (µm)**	**Striae/ 10 µm**	**Comment**
F. brevistriata	5-30 (more?)	3-7	12-17	Striae strongly shortened and marginal, extensive axial area
F. pinnata	3-35	2-8	5-12	Striae usually parallel
F. construens f. *venter*	4-9	3-6	19-21	Striae parallel, shape: elliptic, elliptic-lanceolate to rhombic
F. lapponica	10-30	3-6	6-10	Striae strongly shortened and marginal
*F. elliptica**	3-10 (more?)	2.8-6	11-16 (more?)	Striae dotted (areolae visible)

**Pseudostaurosira trainorii* according to recent taxonomy.

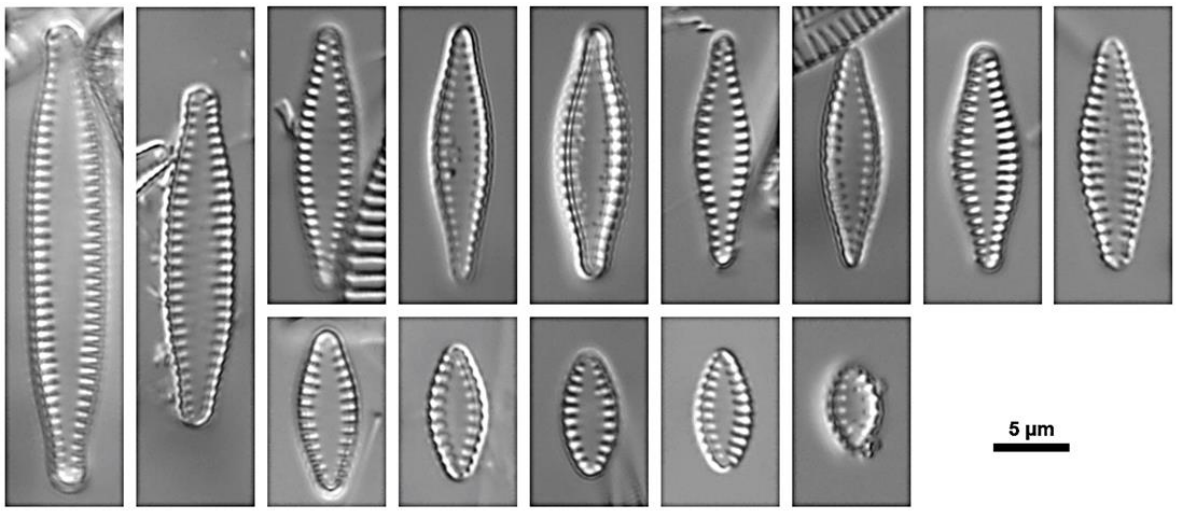
During the past years and decades, several taxa were split, recombined and renamed within the group of small fragilarioid diatoms (e.g. Williams & Round 1987, Morales 2001, Edlund et al. 2006, Morales et al. 2014 und 2015). These changes led to various names of the same taxon, depending on the identification literature or publication used and added uncertainty to the already existing taxonomic problems. Therefore, we recommend to use the now established allocation of *Fragilaria brevistriata* to *Pseudostaurosira* and of *F. pinnata* and *F. lapponica* to *Staurosirella* according to Hofmann et al. (2013). Also, the data listed in the German PHYLIB-software and the German DV-number register (Mauch et al. 2003, version 2011) need to be harmonized accordingly. For example, there are three different DV-no. listed for *F. brevistriata* (*F. brevistriata*: 36079, *F. brevistriata* var. *brevistriata*: 6388 and *Staurosira brevistriata*: 16616), and *Pseudostraurosira brevistriata* has no DV-number (Table 22). The differentiation of the “aggregate-species” *F. brevistriata* (DV-Nr. 36079) from the nominate variety *brevistriata* (DV-Nr. 6388) is superfluous, as no additional varieties exist or are part of the German PHYLIB-training set. Therefore, these changes would prevent that relative abundances of *F. brevistriata* do not contribute to the water quality assessment, when using the “wrong” DV-no. (Table 22; see also Chapter 4.5).

When using the German PHYLIB-method for the water quality assessment, it is still necessary to base the identification on Hofmann et al. (2013) and the supplementary identification literature given in the instruction protocols (Schaumburg et al. 2011c, 2012). Here, the taxonomy of these species mainly follow Krammer & Lange-Bertalot (1986-2004), as the training set of the PHYLIB-method was generated based on this older literature. Therefore, the use of the Hofmann et al. (2013)-taxonomy ensures the applicability of the PHYLB-method (Dreßler et al. 2014).

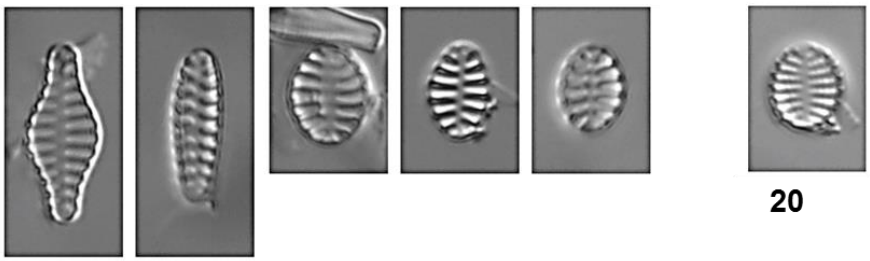
We recommend to record and document any taxa names that deviate from the DV-registry (Mauch et al. 2003, version from 2011) and the identification literature (or publications) that these names are based on, to enable a later allocation to the appropriate name.

Table 22. Saprobic values (**S**), trophic values (**TI**) and weighting (**G**) of *Fragilaria brevistriata* and similar taxa (including the various naming) that were identified by auditors and/or participants in the ‘Lake’ sample, for lotic systems and lakes in the PHYLIB software (version 5.3, December 2015).

Taxon	**DV-no.**	**Lotic Systems**				**Lakes**		
		S	**G**	**TI**	**G**	**TI_{North}**	**TI_{South}**	**G**
F. brevistriata	36079	-	-	-	-	-	-	-
F. brevistriata var. *brevistriata*	6388	1.3	4.0	3.0	1.0	2.81	-	-
Staurosira brevistriata	16616	1.3	4.0	3.0	1.0	2.81	-	-
Pseudostaurosira brevistriata	-	-	-	-	-	-	-	-
F. pinnata	36086	-	-	-	-	-	-	-
F. pinnata var. *pinnata*	6078	1.4	3.0	2.2	1.0	-	-	-
Staurosirella pinnata	-	-	-	-	-	-	-	-
F. construens f. *venter*	6828	-	-	2.3	2.0	-	-	-
Staurosira construens f. *venter*	-	-	-	-	-	-	-	-
F. lapponica	6403	-	-	-	-	2.5	-	-
Staurosirella lapponica	-	-	-	-	-	-	-	-
F. elliptica	6400	-	-	-	-	-	-	-



1-14



20

15-19

Plate 10. Comparison of *Fragilaria* (*Pseudostraurosira*) *brevistriata* and similar taxa from the ‘Lake’ sample. **1-14:** *F. brevistriata* Grunow according to Hofmann et al. (2013), 10-14: these valves may also be determined as *Pseudostaurosira elliptica* according to Edlund et al. (2006), **15-19:** *Fragilaria* (*Staurosirella*) *pinnata*, **20:** *Fragilaria elliptica* SCHUMANN (*Pseudostaurosira trainorii*).

4.4.5 *Navicula cryptotenella and N. cryptotenelloides*

The auditors determined the sum of the relative abundance of *Navicula cryptotenella* and *N. cryptotenelloides* to be 7.0 %, 9.0 % and 8.7 % in the 'Lake' sample (Fig. 61). The participants identified the relative abundance of both species to be on average 9.3 % (n = 38). Two participants did not detect these species. Therefore, *N. cryptotenella* and/or *N. cryptotenelloides* were identified by most participants, with some participants determining distinctly below average abundances (Fig. 61).

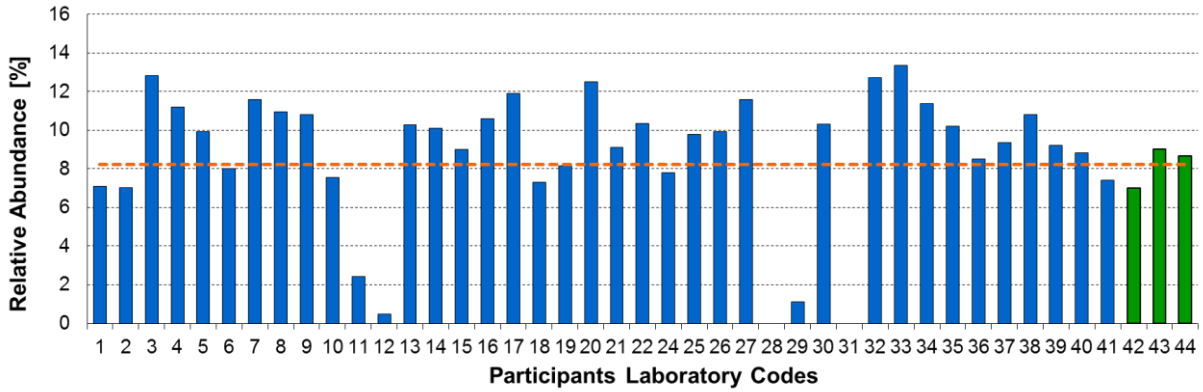


Figure 61. Sum of the relative abundance of ***Navicula* (cf.) *cryptotenella*** and ***N.* (cf.) *cryptotenelloides*** determined by each participant in the 'Lake' sample. Blue bars: participants (laboratory codes 1-41), green bars: auditors (laboratory codes 42-44). Orange horizontal dashed line = average of auditors.

The auditors determined the relative abundance of *Navicula cryptotenelloides* to be 6.2 %, 8.0 % and 7.5 % (Fig. 62). The participants determined the relative abundance of *N. cryptotenelloides* to be on average 7.9 % (n = 34), while six participants did not detect this species (Fig. 62). Some participants additionally listed low relative abundances of *N.* cf. *cryptotenelloides*, indicating an ambiguous identification (0.4 % by laboratory codes 26 and 33; 0.8 % by Laboratory Code 16; 2.4 % by Laboratory Code 36).

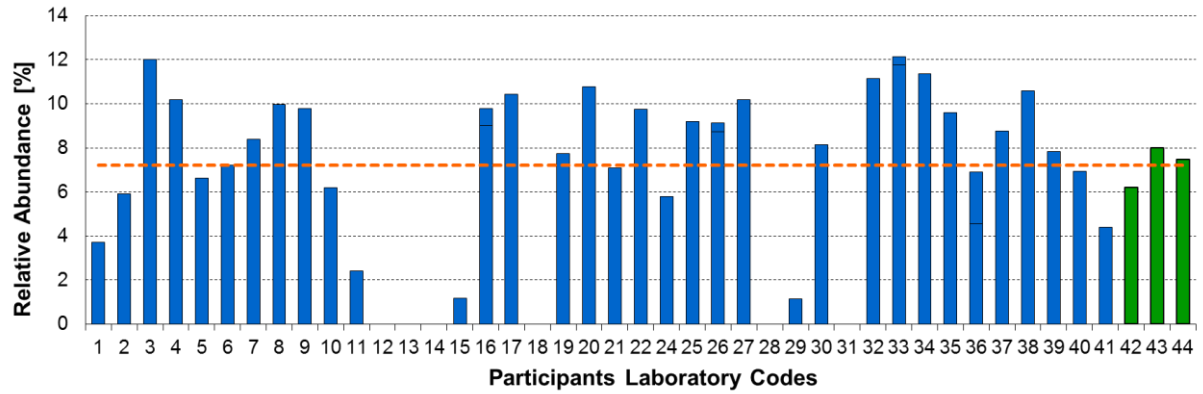


Figure 62. The relative abundance of ***Navicula* (cf.) *cryptotenelloides*** determined by each participant in the 'Lake' sample. Blue bars: participants, green bars: auditors. Orange horizontal dashed line = average of auditors.

Navicula cryptotenella only occurred with low relative abundances in the ‘Lake’ sample (Fig. 63). The auditors determined the relative abundances of *N. cryptotenella* to be 0.8 %, 1.0 % and 1.2 %, while the participants identified relative abundances of on average 2.2 %. The participant average was slightly higher than the auditor abundances due to distinctly higher abundances (7.3 % to 10.3 %) determined by four participants (Fig. 63). In contrast, four other participants did not record any *N. cryptotenella* (Fig. 63). One participant (Laboratory Code 36) recorded *N.* cf. *cryptotenella* with a relative abundance of 0.4 %.

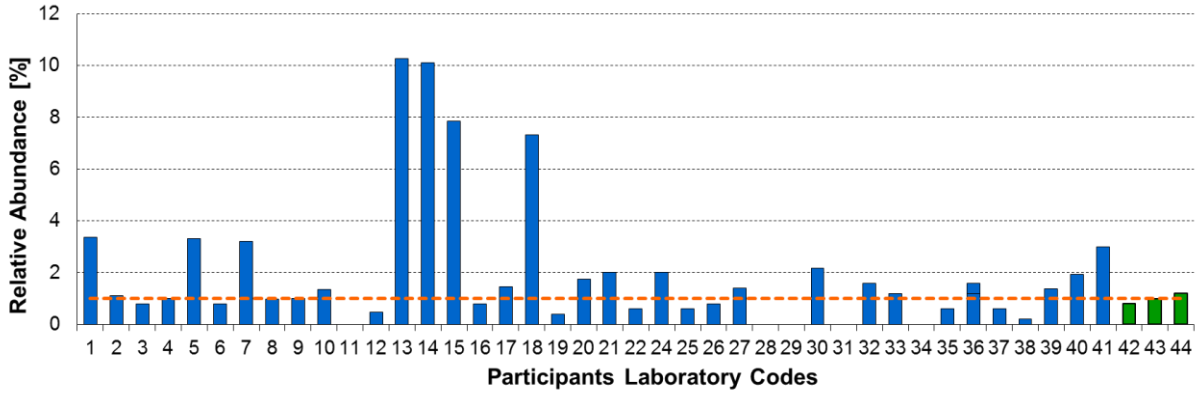


Figure 63. The relative abundance of ***Navicula* (cf.) *cryptotenella*** determined by each participant in the ‘Lake’ sample. Blue bars: participants, green bars: auditors. Orange horizontal dashed line = average of auditors.

The counting results of *N. cryptotenella* and *N. cryptotenelloides* demonstrate clear mistaking of both species (laboratory codes 13, 14, 15 and 18) and thus, suggest difficulties to distinguish the two species from each other. Additionally, these species may also be mistaken for *N. antonii*, *N. menisculus*, *N. upsaliensis* and *N. reichardtiana*. Of these species only *N. reichardtiana* was identified by the participants and auditors with relative abundances greater than 1 % in the ‘Lake’ sample (Fig. 64). The high relative abundances (6.2 %) of *N. reichardtiana* of one participant (Fig. 64) suggest a mistaking of *N. cryptotenelloides/cryptotenella*.

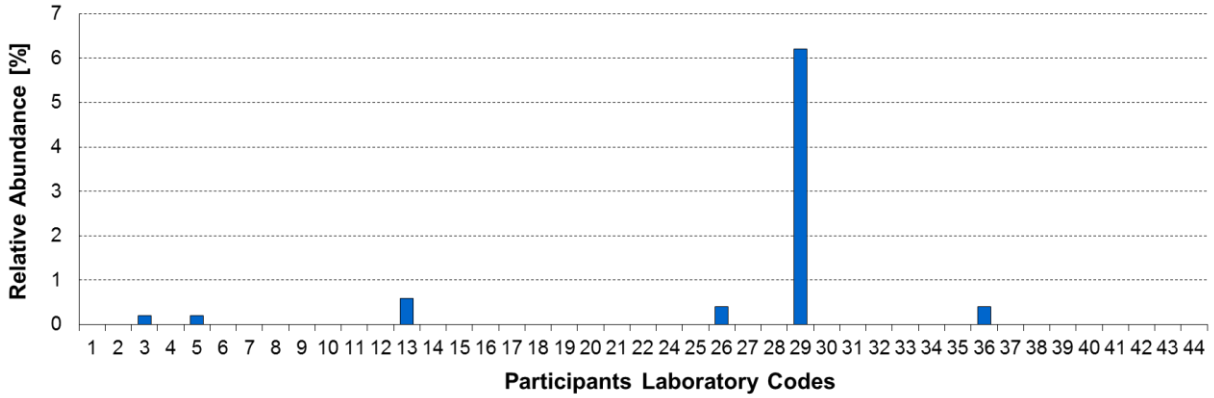


Figure 64. The relative abundance of ***Navicula reichardtiana*** determined by each participant in the ‘Lake’ sample. Blue bars: participants.

A detailed discussion of the taxonomic distinction of *N. cryptotenella* from *N. cryptotenelloides* and other, similar species is provided in the report of the first German intercalibration exercise (Dreßler et al. 2014, see also Dreßler et al. 2015). Additionally, detailed recommendations are presented on

how to deal with these species when using the German PHYLIB-method for water quality assessment (Dreßler et al. 2014). Complementary, essential characteristics to identify both species and to distinguish them from *N. reichardtiana* are provided in Table 23

Table 23. Characteristics for the identification of *Navicula cryptotenella* in comparison to *N. cryptotenelloides* and *N. reichardtiana*. The latter was also determined in the ‘Lake’ sample by several participants of the intercalibration exercise. Source: Hofmann et al. (2013).

Taxon	**Length (µm)**	**Width (µm)**	**Striae/ 10 µm**	**Areolae/ 10 µm**	**Comment**
N. cryptotenella	12-40	5-7	14-16	~38	Ends not protracted
N. cryptotenelloides	9-18	3.7-4.2	16-18	42-44	Ends not protracted
N. reichardtiana	12-22(26)	5-6	14-16	33-36	Ends protracted

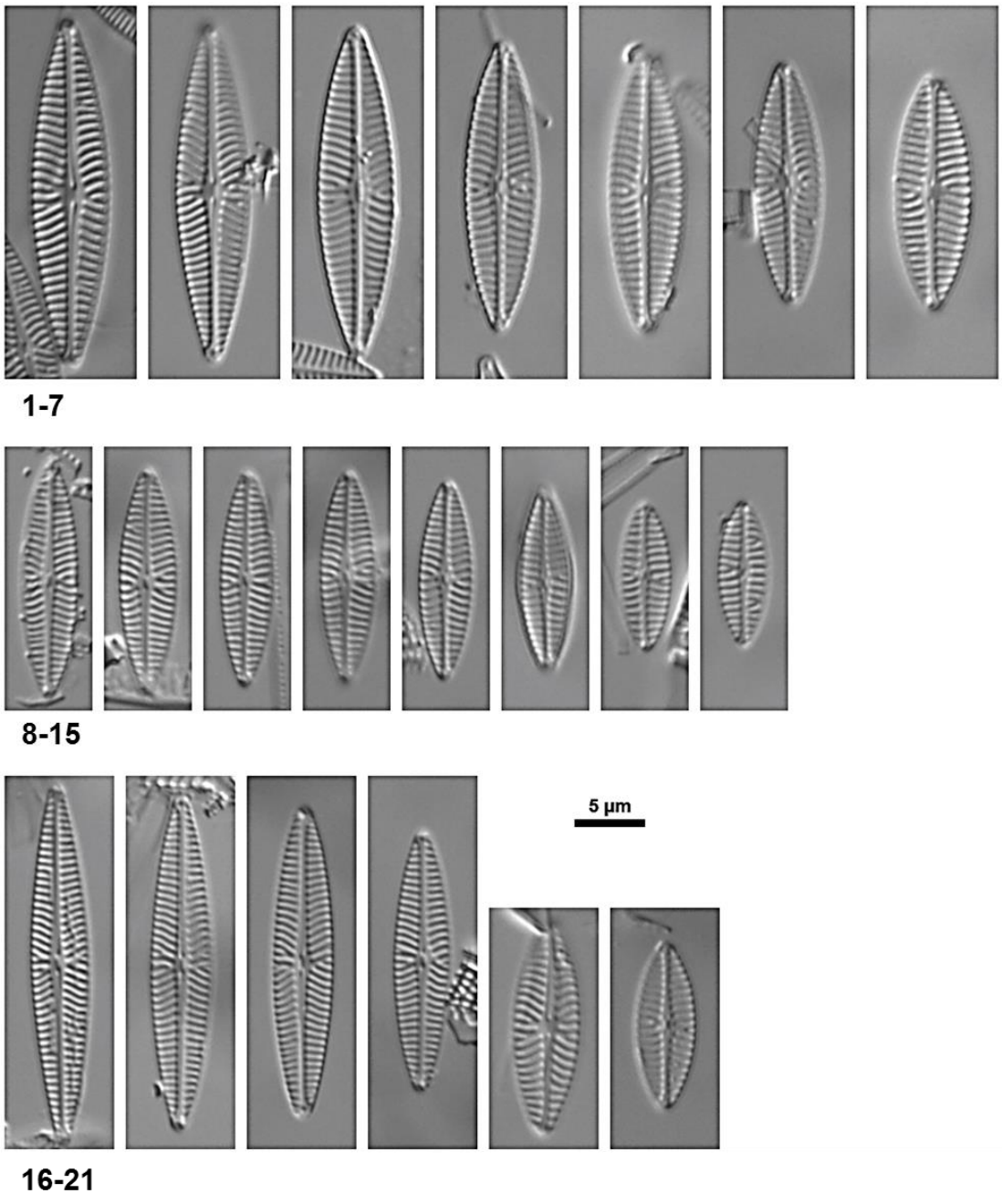


Plate 11. Comparison of *Navicula cryptotenella* and *N. cryptotenelloides* from the ‘Lake’ sample. **1-7: *N. cryptotenella*** due to valve characteristics (Table 23), **8-15: *N. cryptotenelloides*** according to characteristics presented in Table 23, **16-21: *Navicula* cf. *cryptotenella* or *N.* cf. *cryptotenelloides***. Allocation is ambiguous due to dimensions (valve length and width and/ or striae density). See also detailed discussion in Dreßler et al. (2014).

4.5 Effects of counting result variances on the ecological assessment with PHYLIB

In this chapter we assess if the differing counting results of the participants affected the ecological assessment of each site when using the German PHYLIB-method. Prior to the assessment, we calculated the number of taxa with indicator values (percentage of the number of all encountered taxa) and the sum of their relative abundances for each sample, i.e. the percentage of taxa that contributed to the ecological assessment. Subsequently, we calculated the diatom indices based on all counting results (from participants and auditors) using the PHYLIB-software version 5.3, December 2015.

4.5.1 ‘Stream’ sample

In the ‘Stream’ sample the participants and auditors identified a total of 374 taxa, i.e. 374 different names for the encountered taxa. Different taxa names that were used for the same taxon were not pooled. The goal of the analysis was to identify the effect of different naming (German DV-no.) on the water quality assessment, as the German DV-registry often offers several names for the same taxon. Additionally, we wanted to demonstrate that no assessment takes place at low taxonomic levels (genera, cf., aggregates).

We discovered that only 46.3 % of the 374 identified taxa names contributed to the water quality assessment with the German PHYLIB-method, as only those taxa (with these chosen taxa-names) have indicator values based on the training set of the PHYLIB-software (Fig. 65). In Figures 65-68 ‘indicator values’ or ‘indicative taxa’ only refer to the presence of a trophic value and weighting, not to the classification as reference or degradation species. Taxa names did not contribute to the water quality assessment for the following reasons (Fig. 65):

(1) Obligatory planktonic species (1.1 %). These taxa had to be excluded from the count according to the instruction protocol (Schaumburg et al. 2012).

(2) Taxa name listed in the PHYLIB software, but without indicator value (19.5 %).

(3) Taxa name not listed in the PHYLIB software or in the German DV-number register (DV-no.; Mauch et al. 2003, version 2011) (3.2 %).

(4) Insufficient level of taxonomic resolution. (A) Taxa names were entered on genus level (“spec.”), with “cf.“ or “aff.“ (17.4 %). (B) Taxa were identified to species level (or “species/varieties-aggregate” level). However, a higher taxonomic resolution would have been necessary for the assessment, i.e. the distinction of variety, subspecies or form (“aggregates“, 7.2 %).

(5) Outdated taxonomic concept (0.5 %). Taxa names could not be unambiguously allocated to a current taxon. And finally,

(6) Taxa name refers to an “outdated aggregate“ (4.8 %). These are species that had originally a number of varieties, subspecies or forms in the German DV-number register (DV-no.; Mauch et al. 2003, version 2011). With taxonomic revisions, all sub-species (var., ssp., f.) of these taxa became void so that only the nominate variety (or ssp. or f.) remained. In PHYLIB, only this nominate variety has listed indicator values, but both the nominate variety and the species name (with no indicator values) are listed. Species names that do not differentiate to a higher taxonomic level but that represent the correct taxonomic name according to the revised taxonomy were labelled as “outdated aggregate“ in this document.

Consequently, the relative abundance of taxa from the “outdated aggregate“ will not contribute to the water quality assessment, despite the presence of indicator values. For example, the German DV-number register (DV-no.; Mauch et al. 2003, version 2011) lists the species *Navicula cryptocephala* Kützing (DV-no. 36114) (referring to an “aggregate“, if not further differentiated) and its varieties *N. cryptocephala* var. *cryptocephala* Kützing (DV-no. 6010) and *N. cryptocephala* var. *veneta* (Kützing) Rabenhorst (DV-Nr. 6892). Rabenhorst had formerly assigned *N. veneta* Kützing as a variety of

N. cryptocephala. When this name was revoked, the so called “aggregate“ with the DV-no. 36114 became outdated and should be deleted from the DV-register. A deletion would also avoid future mistaking the taxon with the species *N. cryptocephala* (the currently correct name; i.e. DV-No. 6010, as also listed in Hofmann et al. 2013). This also holds true for taxa that always had only the species name and the corresponding nominate variety listed (but no other variety) in the DV-registry (e.g. *Fragilaria brevistriata*/*F. brevistriata* var. *brevistriata*, *F. virescens*/*F. virescens* var. *virescens*, *Navicula seminulum*/*N. seminulum* var. *seminulum*, *Sellaphora pupula*/*S. pupula* var. *pupula*). These “aggregates“ are also unnecessary and outdated.

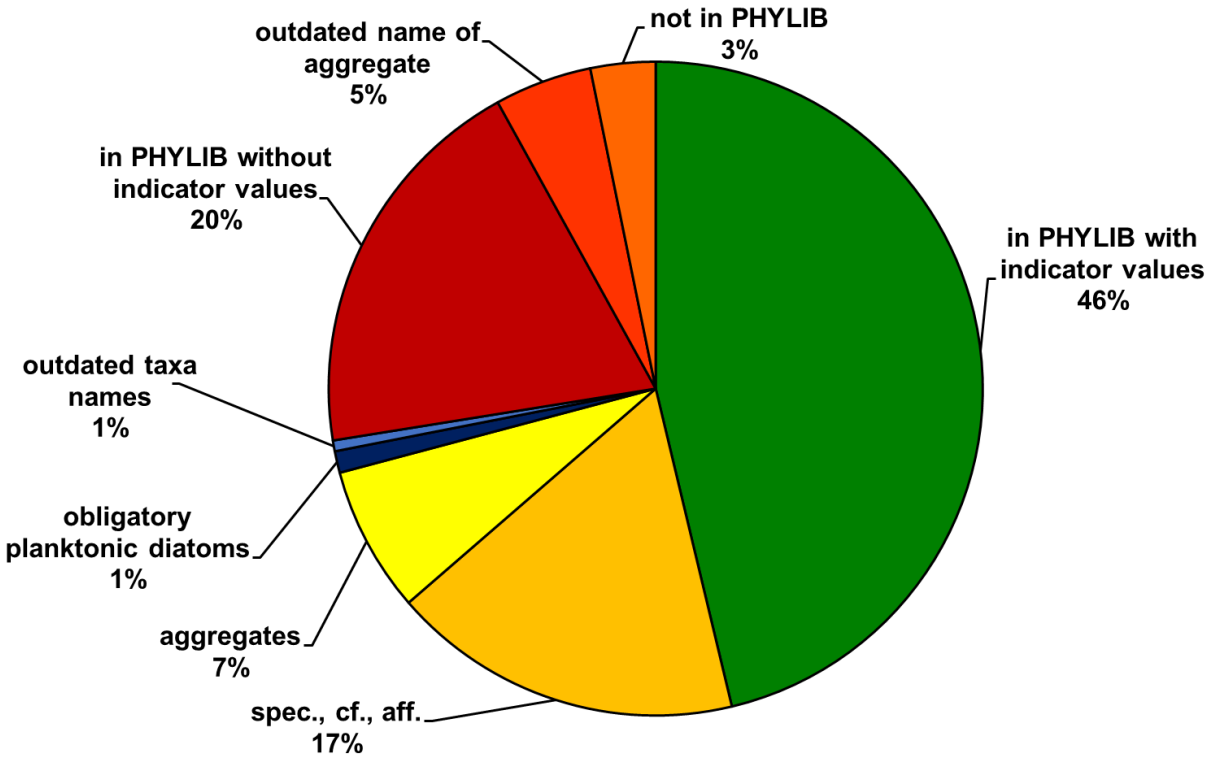


Figure 65. The proportion of **diatom taxa names that contributed (green) or did not contribute (remaining colours) to the assessment** of the ‘**Stream**’ **sample** when using the German PHYLIB-method based on the total number of recorded taxa names (n=374).

For the ecological assessment of lotic waters, the number of indicative taxa is irrelevant when using the German PHYLIB-method. Instead, the relative abundance of indicative taxa is relevant. For the auditors, indicative taxa in the PHYLIB-software contributed relative abundances of 75.4 %, 67.0 % and 79.6 % to the ‘Stream’ sample (Fig. 66-67). For the participants, indicative taxa contributed on average 71.7 % (n=40) to the assemblage (ranging from 13.4-92.6 %; Fig. 66-67).

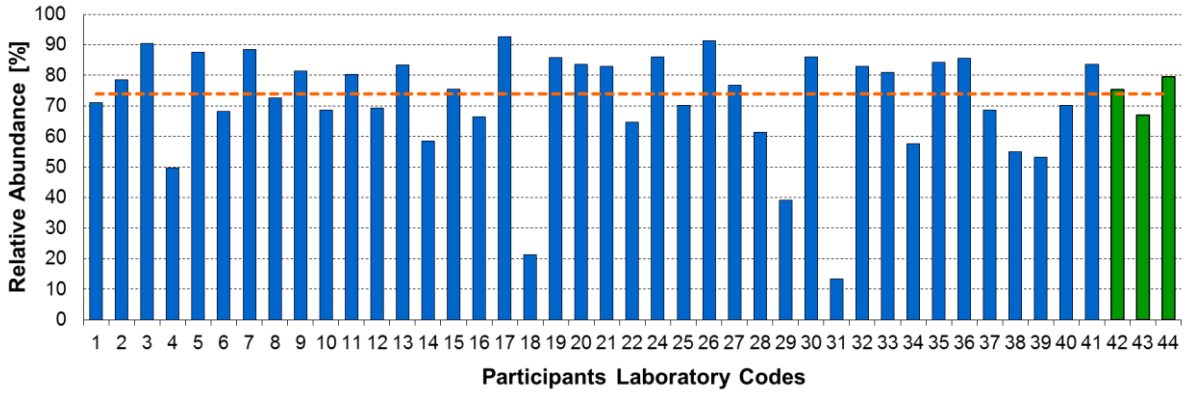


Figure 66. The **relative abundance of diatom taxa that contributed to the trophic state assessment** determined by each participant in the ‘**Stream**’ **sample** when using the German PHYLIB-method. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44. Red horizontal dashed line = average of auditors.

In the ‘Stream’ sample, taxa that did not contribute to the water quality assessment using PHYLIB (version 5.3, December 2015) contributed the following relative abundances (Fig. 67):

(1) Obligatory planktonic species contributed high relative abundances (>4-9 %) for four participants.

(2) Taxa listed in the PHYLIB software without indicator value contributed relative abundances of 6.0-8.8 % (auditors) and zero to 18.0 % (participants; average 6.2 %, n=40), respectively. Taxa responsible were mainly *Planothidium frequentissimum* var. *frequentissimum* (2.9 % to 3.9 % in the auditor counts), and also *Nitzschia palea* var. *tenuirostris* and *Fragilaria biceps*.

(3) Taxa not listed in the PHYLIB software hardly contributed to the relative abundances (<3.5 %).

(4) Taxa with insufficient level of taxonomic resolution. (A) Taxa entered as “spec.”, with “cf.“ or “aff.“ contributed a high relative abundance for three participants with 14.3 % to 48.0 %. (B) Taxa identified to an “aggregate“-species level contributed a relative abundance of zero to 6.0 % (auditors) and zero to 36.5 % (participants), respectively.

(5) Taxa referring to an outdated taxonomic concept occurred with relative abundances of 2 % and 4 % for only two participants.

(6) Taxa belonging to an “outdated aggregate“ contributed a relative abundance of 8.3-14.0 % to the assemblages of the auditors and on average 7.7 % (n=40) to the assemblages of the participants.

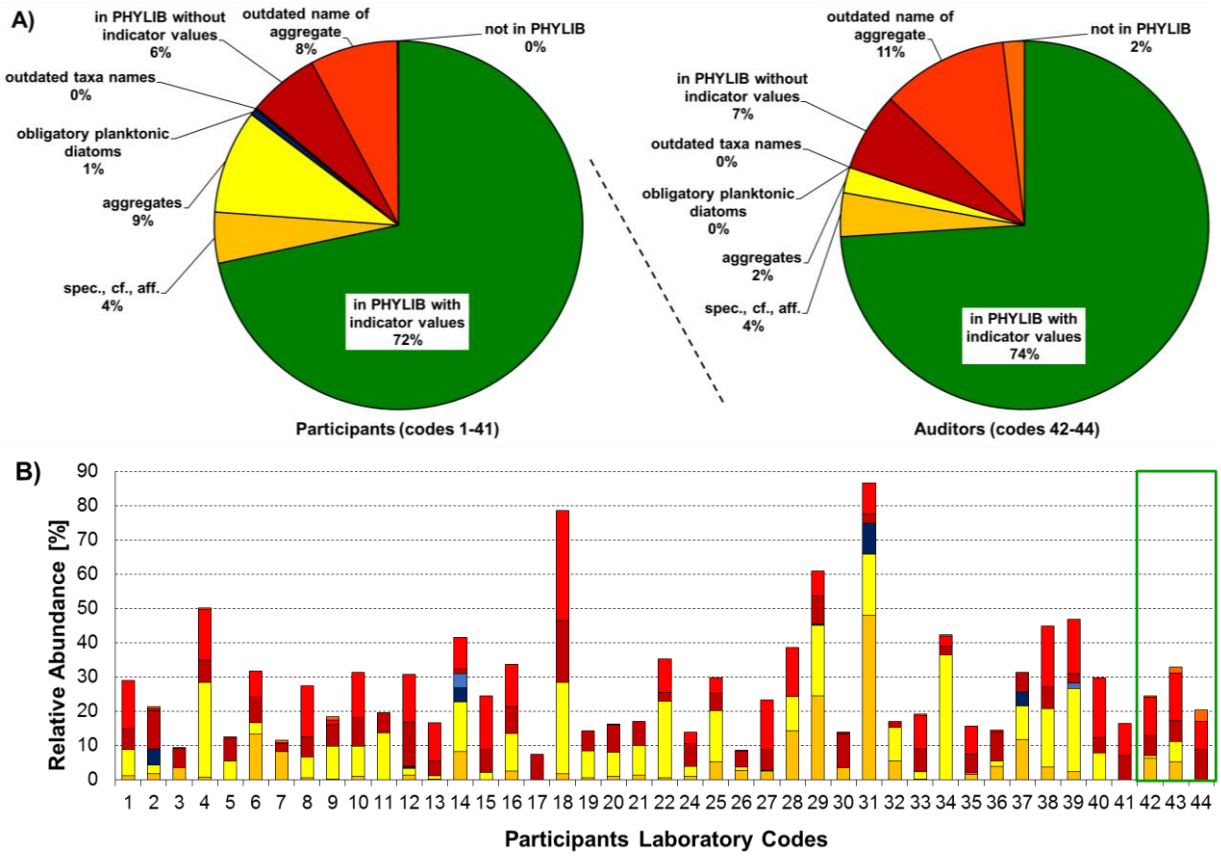


Figure 67. **The relative abundance** of diatom taxa that contributed (green) or did not contribute (remaining colours) to the assessment of the ‘**Stream**’ **sample** when using the German PHYLIB-method, A) based on the average contribution to the participant and auditor assemblages, respectively and B) as recorded by each participant.

The “outdated aggregate“ included mainly *Navicula cryptocephala* and *N. reichardtiana* (Fig. 68), who only contribute to the water quality assessment, if they are entered as *Navicula cryptocephala* var. *cryptocephala* and *N. reichardtiana* var. *reichardtiana* (DV-Nr. 6010 and 6221), respectively. Therefore, despite the correct identification, the allocation of *N. cryptocephala* and *N. reichardtiana* by the auditors and eleven participants prevented a contribution of their relative abundances to the assessment. This is because these two species are listed in the sense of aggregates (without further differentiation) in PHYLIB and the DV-register. Eight other participants only listed *Navicula cryptocephala* instead of *Navicula cryptocephala* var. *cryptocephala* (Fig. 68). This specific information was not included in the accompanying letter of this intercalibration exercise nor part of the German instruction protocols for lakes and lotic waters (Schaumburg et al. 2011c & 2012). Therefore, using “outdated aggregates“ only reflects, if a participant or auditor is familiar with the German PHYLIB-software that is used to assess the water quality.

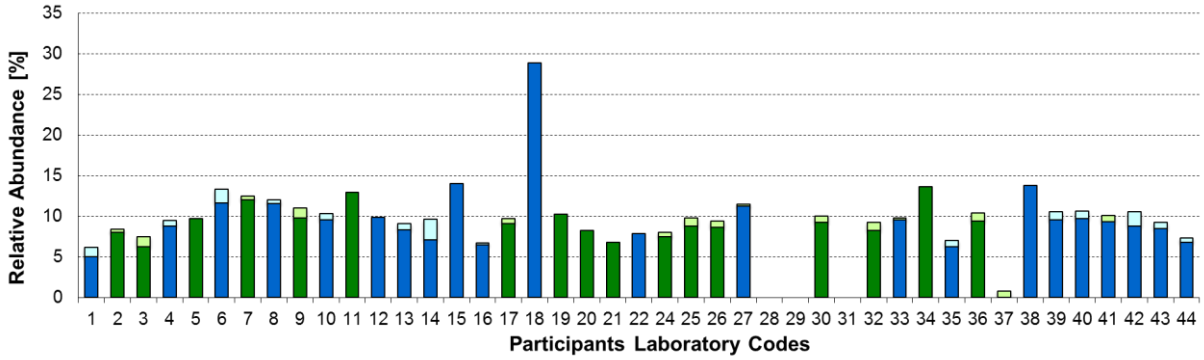


Figure 68. The relative abundance of ‘*Navicula cryptocephala* var. *cryptocephala’* (dark green) and ‘*N. reichardtiana* var. *reichardtiana’* (light green) that **contribute to the assessment (green)** when using the German PHYLIB-method for the ‘Stream’ sample. The relative abundance of ‘*Navicula cryptocephala’* (dark blue) and ‘*N. reichardtiana’* (light blue) belong to an “outdated aggregate“ and therefore **do not contribute to the assessment (blue)**. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44.

Overall a relatively high percentage of diatom taxa do not contribute to the assessment as identified by the auditors and participants (Fig. 67). Mainly, taxa are not indicative due to an insufficient level of taxonomic resolution or due to a DV-register and PHYLIB-software that lists some taxa in the sense of “outdated aggregates”. Concurrently, the nominate varieties (usually listed with indicator values) of these outdated aggregates are listed to differentiate them from the alleged “aggregates”. Consequently, a taxonomic correct allocation to the species name (e.g. *Navicula cryptocephala* or *N. reichardtiana* instead of *Navicula cryptocephala* var. *cryptocephala* or *N. reichardtiana* var. *reichardtiana*) prevents a contribution of their relative abundances to the assessment of the sample.

We recommend to harmonize the different diatom DV-no. for synonyms in the Mauch-register and adjust the PHYLIB-tool accordingly. Taxa from “outdated aggregates“ should be deleted from the DV-register or listed as inactive. Some indicative values from the training set may not be directly transferable from the species to the nominate variety, because the former and recent taxa names refer to different taxon concepts. For these occasions, a re-count of the training set is necessary.

Additionally, all changes in the data set should be documented in detail for each revised PHYLIB-software version. For example, no trophic indicator values for lotic systems were listed for *Planothidium frequentissimum* var. *frequentissimum* (DV-no. 16606) in the PHYLIB-software version 5.3 from December 2015. In contrast, the subsequent version from February 2016 (also termed version 5.3) lists a trophic value and weighting for lotic systems. Also, *Amphora minutissima* (DV-no. 36246) was listed with indicator values for lakes and lotic systems in the version from December 2015. In the version from February 2016, this taxon is entirely missing. Both changes are not provided in the technical documentation of the most recent version from February 2016. For the historical comparison of data sets, it is essential to account for both, the PHYLIB-version used (including the time of release) and the data the used version is based on.

In summary, a high percentage of indicative taxa is necessary for high accuracy and an unambiguous water quality assessment. The diatoms should be identified to the highest possible taxonomic level to enable an allocation to the training set (indicative taxa) and therefore an assessment. It is crucial to identify the taxa correctly according to the given identification literature. Incorrect identifications, that only seemingly increase the accuracy, increase the possibility of a wrong assessment. Diatom valves that can not unambiguously be allocated to a taxon with the available identification literature

should be labelled with “cf.”, “aff.” or “spec.” and documented accordingly (in writing and with pictures).

Using the German PHYLIB-method, **the ecological assessment** of samples from **lotic waters** with the Module Diatoms is calculated by combining the modules “Species Composition and Abundance” and “Trophic Index and Saprobic Index” to obtain the “$DI_{Fließgewässer}$” (Diatom $Index_{running\ waters}$) (Schaumburg et al. 2012, Schaumburg et al. 2006a). The ecological assessment of samples are deemed unreliable, if the percentage of ambiguously identified diatom objects (spec., spp., cf., aff.) exceeds 5 % (Schaumburg et al. 2006a). This “5 %-cf limit” was exceeded by two auditors and nine participants (Fig. 67). However, this exclusion criterion is not useful, as the percentage of indicative taxa that contribute to the assessment is more important. Following the lake assessment, we recommend to use a 60 % limit and replace the “5 %-cf limit”. For samples where the assessment is based on less than 60 % indicative taxa, the assessment should be seen as not reliable and should only be used in support of an expert assessment. For eight participants, the assessment is based on <60 % indicative taxa (Fig. 66). In contrast, the assessment is based on more than 60 % indicative taxa based on the results of five of the nine participants and both auditors that exceed the 5 %-cf limit (sum of spec., cf., aff.)(Fig. 66).

Additionally, an assessment is deemed unreliable, if the relative abundance of aerophilic diatoms exceeds 5 % (Schaumburg et al. 2012). This did not occur for any results of this intercalibration exercise for the ‘Stream’ sample.

The Module “Species Composition and Abundance” for the ‘Stream’ sample had transformed sums of reference species (**RAS**) of 0.10 to 0.13 based on the counting results of the auditors (Fig. 69 A), i.e. the auditors identified a relative abundance of 10 % to 13 % reference species. For the participants the RAS-values ranged from 0.005 to 0.60 (average: 0.16; n=40; Fig. 69 A). The Module “Trophic Index and Saprobic Index” had a transformed Trophic Index (**TI**) of 0.25 to 0.26 based on the results of the auditors (Fig. 69 B). If the results of the auditors were converted to comply with PHYLIB, then the TI-values would be reduced to range from 0.23 to 0.24 (Fig. 69 B), indicating a higher trophic state. For example, if *Navicula cryptocephala* (outdated aggregate in PHYLIB) is transferred to *Navicula cryptocephala var. cryptocephala* and *N. reichardtiana* to *N. reichardtiana var. reichardtiana*. The participants transformed TI-values ranged from 0.14 to 0.72 (average: 0.25). After transferring the *N. cryptocephala*- and *reichardtiana*-data, the TI-values indicated a higher trophic state (Fig. 69 B). After combining the modules of RAS and TI to obtain the diatom index “**$DI_{Fließgewässer}$**” the auditors had values between 0.18 to 0.20 (after taxa conversion: 0.17-0.19), which corresponded to the ecological status class (EC) 4 (unsatisfactory) (Fig. 69 C). The diatom index (DI) of the participants ranged from 0.07 to 0.66 (average: 0.20, n=40). Most of the participant results assessed the sample with EC 4 (unsatisfactory), others with EC 3 (moderate) and one with EC 1 (very good). The transfer of the *N. cryptocephala* and *N*. *reichardtiana*-data distinctly changed the assessment of the ‘Stream’ sample for the auditors and above mentioned participants (resulting in a slightly poorer assessment) (Fig. 69 C).

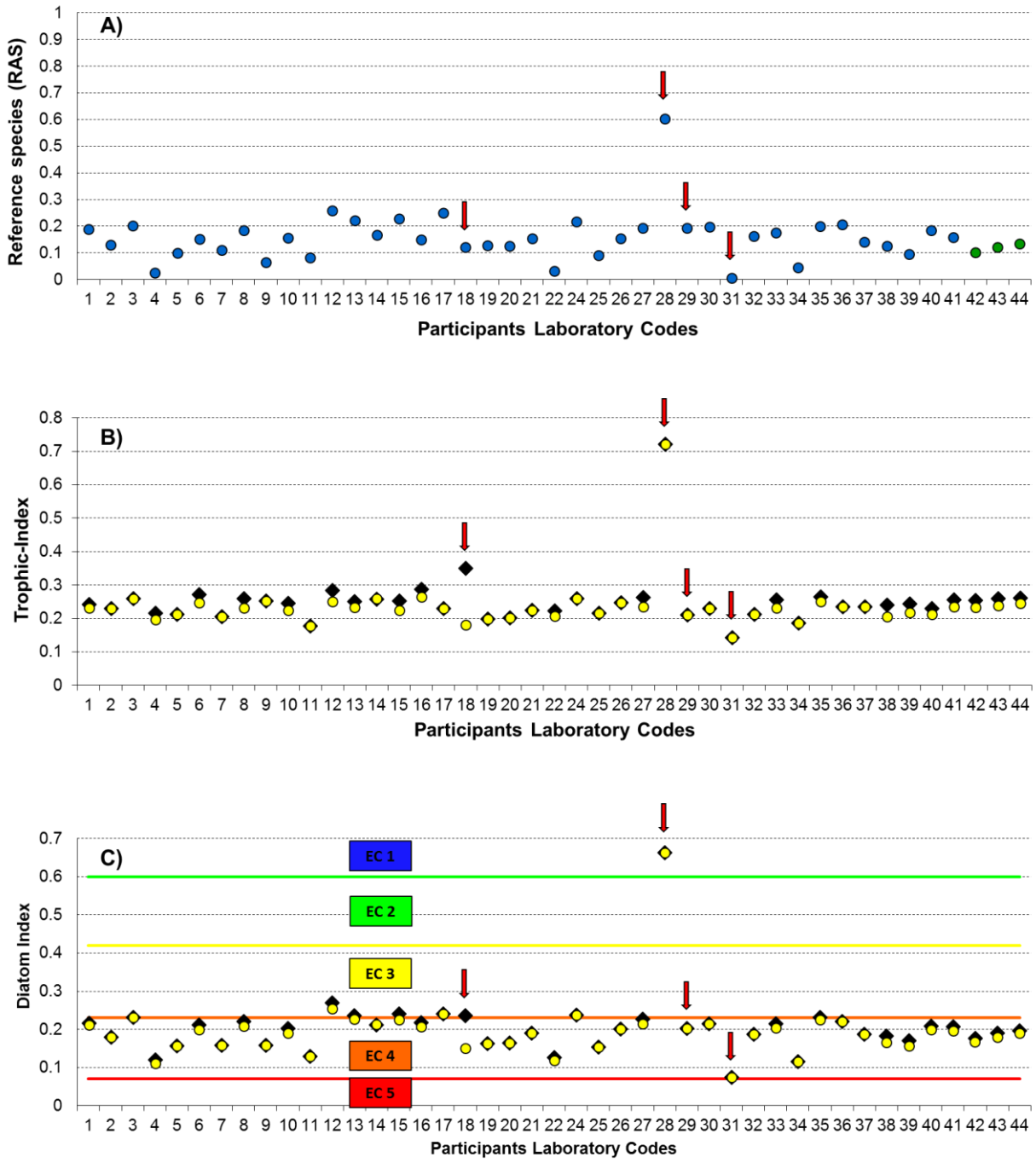


Figure 69. Results of the **ecological assessment using the German PHLYIB-tool** for the **‘Stream’ sample**: **A)** Module ”Species Composition and Abundance”, transformed sums of **reference species** (RAS) of the participants (laboratory codes 1-41) and auditors (laboratory codes 42-44); **B)** Module “Trophic Index and Saprobic Index”, shown as transformed **Trophic-Index**; Legend: solid diamonds: based on the entered counting results, yellow circles: relative abundances from *Navicula cryptocephala* and *N. reichardtiana* were corrected to *Navicula cryptocephala* var. *cryptocephala* and *N. reichardtiana* var. *reichardtiana*, **C) Diatom Index** “$DI_{Fließgewässer}$“; legend see B); coloured lines = index limits of ecological status classes (EC) for ‘running water-type’ D 12.1 with EC 1 = very good, EC 2 = good, EC 3 = moderate, EC 4 = unsatisfactory, EC 5 = poor EC; **red arrows**: participants that conducted the ‘Stream’ sample **unsuccessfully**. For three of those four participants (18, 29 and 31) the assessment was additionally based on <60 % of indicative taxa (compare with Fig. 66).

The assessments of the ‘Stream’ sample with the PHYLIB-tool were similar among auditors and most participants (Fig. 69). The assessments were based on similar counting results, i.e. low Bray-Curtis dissimilarity distances, with the exception of four participants (Chapter 4.1.2). The four participants had Bray-Curtis dissimilarity distances outside the confidence intervals of all three auditors and therefore, had counted this sample “unsuccessfully” (Fig. 69, red arrows). One participant (laboratory code (LC) 28) identified 50.7 % *Encyonopsis subminuta*. This species was not determined by the auditors. This misidentification led to a distinctly higher sum of reference species, better assessment of the trophic state and consequently a diatom index indicating a “very good” ecological state (Fig. 69). The assessment results of two participants (LC 18 and 29) ranged in the auditor assessments (ecological status class 4), despite differing counting results. One participant (LC 29) did not identify any of the most abundant auditor taxa and another (LC 18) identified *Navicula cryptocephala* var. *cryptocephala* with distinctly above average abundances (see Table 3 in Chapter 4.1.1). For both participants, the relative abundance of indicative taxa was relatively low (<40 %, Fig. 66). Most of their indicative species were misidentifications that “by chance” assessed the sample similar to the auditors.

The transfer of the originally entered *Navicula cryptocephala* and *N. reichardtiana* to *Navicula cryptocephala* var. *cryptocephala* and *N. reichardtiana* var. *reichardtiana*, distinctly increased the relative abundances of taxa that contributed to the assessment (Fig. 68). As both species are not reference species for the running water-type D 12.1, this transfer did not affect the Module “Species Composition and Abundance”, however, the transfer affected the TI-values and therefore the diatom indices (Fig. 69).

4.5.2 ‘Lake’ sample

In the ‘Lake’ sample the participants and auditors identified a total of 484 taxa, i.e. 484 different names for the encountered taxa. Different taxa names that were used for the same taxon were not pooled (see Chapter 4.5.1). The 484 taxa include 51 taxa that were only found by the auditors and participants during the search for rare taxa (after the count).

Of the 433 identified taxa names that were exclusively identified during the counts, only 42.0 % contributed to the water quality assessment with the German PHYLIB-method, as only those taxa (with these chosen taxa-names) have indicator values based on the training set of the PHYLIB-software (Fig. 70). In Figures 70-73 ‘indicator values’ or ‘indicative taxa’ only refer to the presence of a trophic value and weighting, not to the classification as reference or degradation species. Taxa names did not contribute to the water quality assessment (58 %) for the following reasons (Fig. 70):

(1) Obligatory planktonic species (0.7 % of the taxa names). These taxa had to be excluded from the count according to the instruction protocol (Schaumburg et al. 2011c).
(2) Taxa name listed in the PHYLIB software, but without indicator value (23.3 %).
(3) Taxa name not listed in the PHYLIB software or in the German DV-number register (DV-no.; Mauch et al. 2003, version 2011) (4.4 %).
(4) Insufficient level of taxonomic resolution. (A) Taxa names were entered as Bacillariophyceae, *Pennales*, on genus level (“spec.”), with “cf.“ or “aff.“ (21.7 %). (B) Taxa were identified to species level (or “species/varieties-aggregate” level). However, a higher taxonomic resolution would have been necessary for the assessment, i.e. the distinction of variety, subspecies or form (“aggregates“, 5.3 %).
(5) Outdated taxonomic concept (0.5 %). Taxa names could not be unambiguously allocated to a current taxon. And finally,
(6) Taxa name refers to an “outdated aggregate“ (2.1 %; explanations see Chapter 4.5.1).

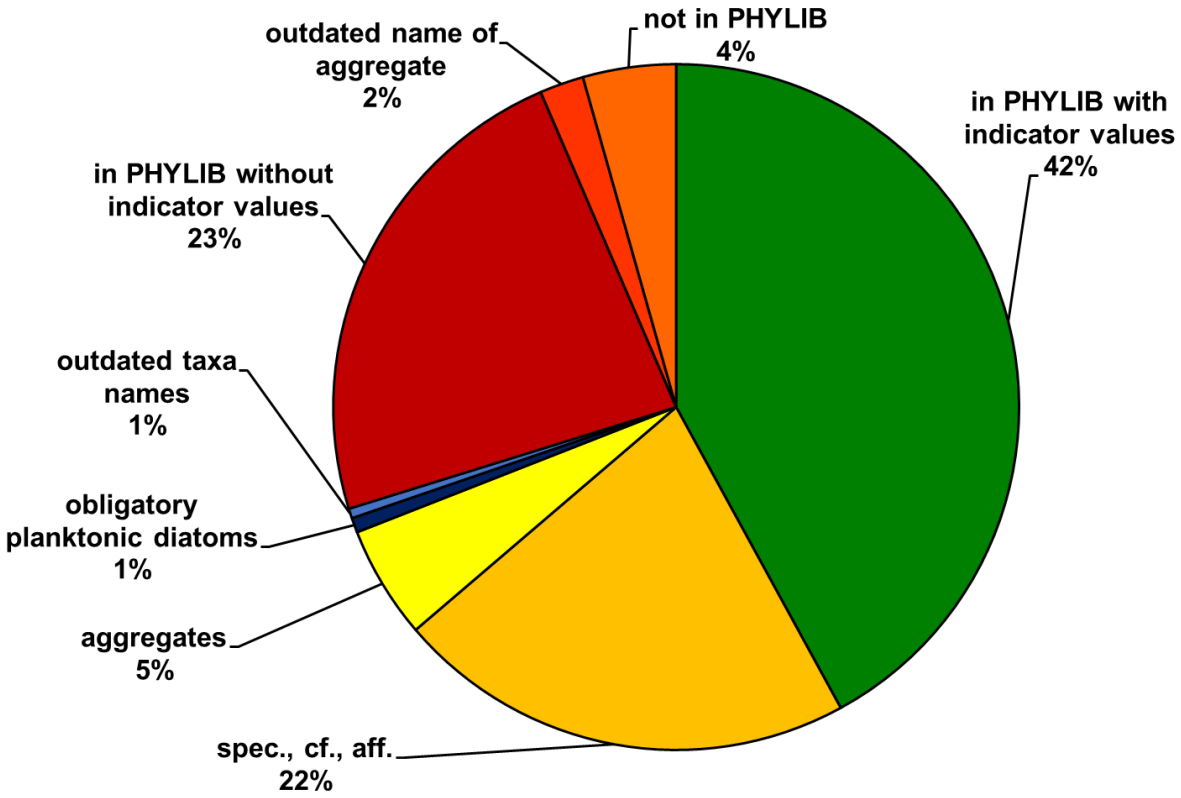


Figure 70. The proportion of **diatom taxa names that contributed (green) or did not contribute (remaining colours) to the assessment** of the ‘**Lake**’ **sample** when using the German PHYLIB-method based on the total number of recorded taxa names (n=433).

For the ecological assessment of a lake sample with the German PHYLIB-method, both are relevant, the number of indicative reference species and the sum of the relative abundance of taxa with a trophic indicator value. For a reliable assessment at least 8-12 taxa (depending on the lake-type) need to be classified as either reference or degradation species. Also, at least 60 % of the counted diatom objects need to belong to a taxon with a trophic value (with weighting) in the PHYLIB-software. For the auditors, indicative taxa in the PHYLIB-software contributed relative abundances of 50.0 %, 51.0 % and 63.4 % to the ‘Lake’ sample (Fig. 71-72). For the participants, indicative taxa contributed on average 55.1 % (n=40) to the assemblage (ranging from 16.6 % to 71.7 %; Fig. 71-72).

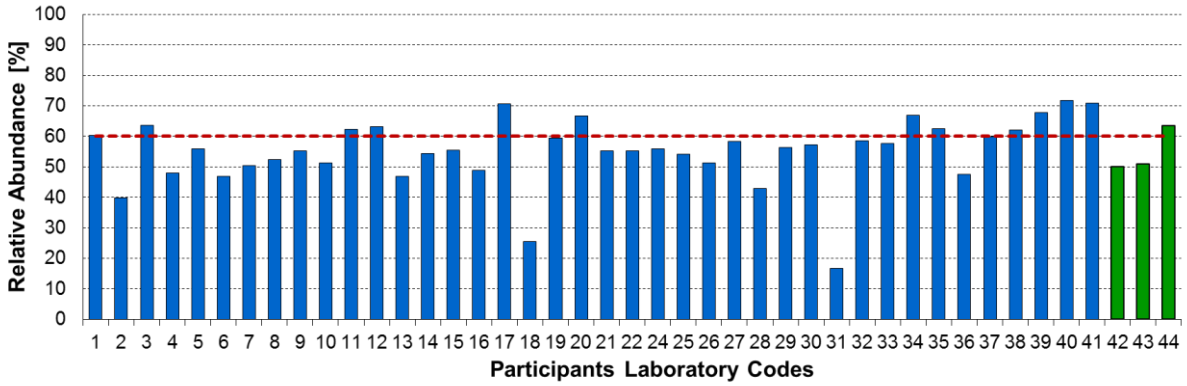


Figure 71. The **relative abundance of diatom taxa that contributed to the trophic state assessment** determined by each participant in the ‘**Lake**’ **sample** when using the German PHYLIB-method. Participants: laboratory codes 1-41, auditors: laboratory codes 42-44. The dashed horizontal line indicates the minimum of 60 % relative abundances that the sum of indicative taxa (taxa with trophic value) have to contribute for a reliable assessment following the PHYLIB-tool.

In the ‘Lake’ sample, taxa that did not contribute to the water quality assessment using PHYLIB (version 5.3, December 2015) contributed the following relative abundances (Fig. 72):

(1) Obligatory planktonic species hardly contributed to the relative abundances (<0.4 %, n=43).

(2) Taxa listed in the PHYLIB software without indicator value contributed relative abundances of on average 28.1 % (participants and auditors, n=43). Taxa responsible were mainly *Achnanthidium minutissimum* var. *minutissimum* (in the auditor samples with 8.8 % to 15.4 %) and *Cymbella vulgata* (in the auditor samples with 8.6 % to 13.0 %).

(3) Taxa not listed in the PHYLIB software hardly contributed to the relative abundances (average 0.8 %; n=43).

(4) Taxa with insufficient level of taxonomic resolution. (A) Taxa entered as “Bacillariophyceae”, “*Pennales*”, “spec.”, with “cf.“ or “aff.“ were identified with abundances of on average 5.0 % (n=43); (B) Taxa identified to an “aggregate“-species level contributed relative abundances of on average 5.8 % (n=43).

(5) Taxa referring to an outdated taxonomic concept hardly contributed to the relative abundances (average: 0.02 %; n=43).

(6) Taxa belonging to an “outdated aggregate“ (definition see Chapter 4.5.1) contributed a relative abundance of on average 5.1 % (n=43) to the assemblage. This pertained to *Fragilaria* (*Pseudostaurosira*) *brevistriata*. This taxon only contributes to the assessment, if listed as *Fragilaria brevistriata* var. *brevistriata* (DV-no. 6388) or *Staurosira brevistriata* (DV-no. 16616, listed only by the participant with Laboratory Code 4), but not, if listed as *Fragilaria brevistriata* (DV-no. 36079)(Fig. 73; see detailed explanations in Chapter 4.4.4).

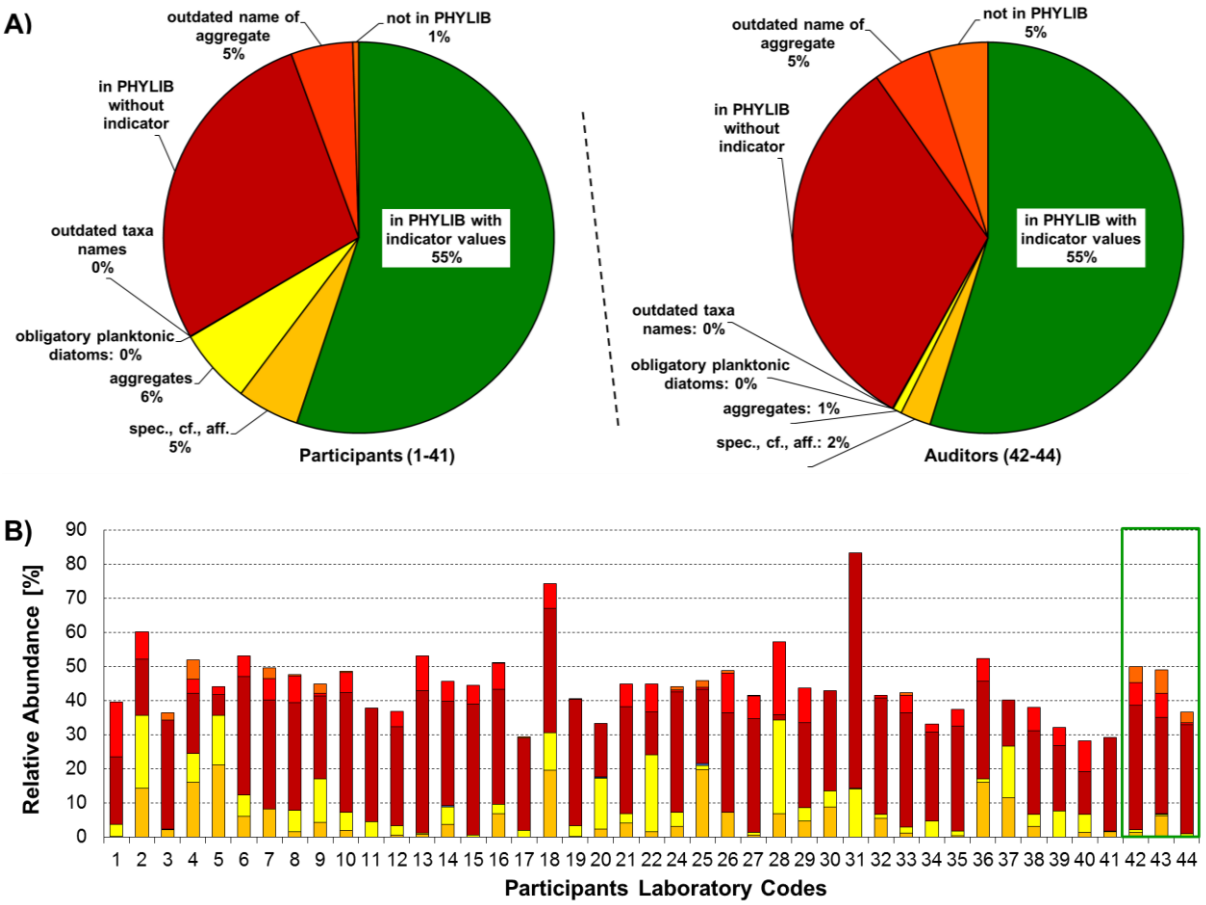


Figure 72. **The relative abundance** of diatom taxa that contributed (green) or did not contribute (remaining colours) to the assessment of the **‘Lake’ sample** when using the German PHYLIB-method, A) based on the average contribution to the participant and auditor assemblages, respectively and B) as recorded by each participant.

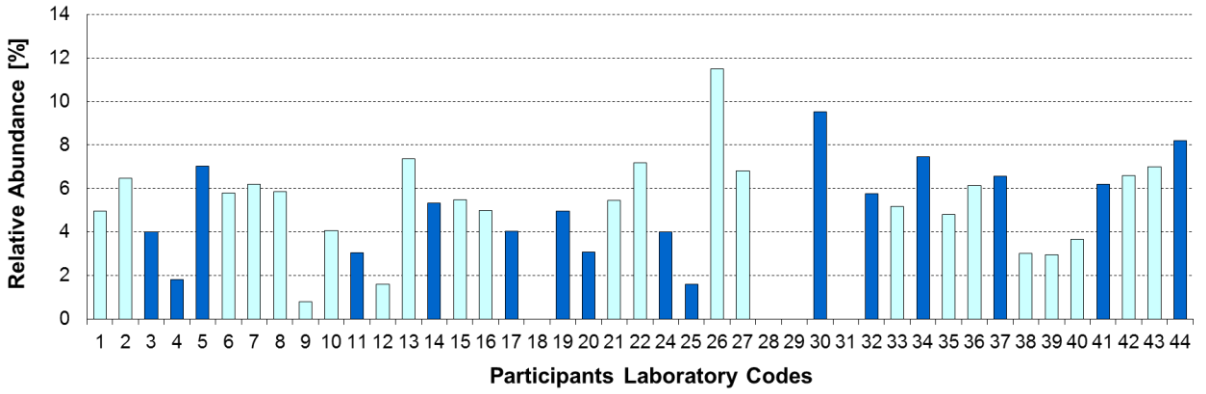


Figure 73. The relative abundance of *Fragilaria brevistriata* var. *brevistriata and Staurosira brevistriata* that **contribute to the assessment (dark blue)** when using the German PHYLIB-method, and abundances [%] of *Fragilaria brevistriata*, that **do not contribute to the assessment** (**light blue**), as identified by the participants (codes 1-41) and auditors (codes 42-44) for the **‘Lake’ sample**.

Using the German PHYLIB-method, **the ecological assessment** of samples from **lakes** with the Module Diatoms is calculated by combining the modules “Trophic-Index (TI)“ and “Quotient of Reference Species (RAQ)“ to obtain the Diatom-Index for lakes (DI_{Seen})(Schaumburg et al. 2011c, Schaumburg et al. 2007a). The ecological assessment of samples are deemed unreliable, if the percentage of ambiguously identified diatom objects (spec., spp., cf., aff.) exceeds 5 % (Schaumburg et al. 2007a). In the ‘Lake’ sample this “5 %-cf limit“ was exceeded by one auditor (slightly) and 14 participants (Fig. 72 and Table 6 in Chapter 4.1.2). Additionally, an assessment is deemed unreliable, if the relative abundance of aerophilic diatoms exceeds 5 % (Schaumburg et al. 2011c). This did not occur for any results of this intercalibration exercise for the ‘Lake’ sample.

For a reliable assessment of the Module “**Quotient of Reference Species (RAQ)**“ at least 12 indicative species are required to be present in the assemblage according to Lake-Type D 10.1, i.e. species that are classified as either reference or degradation species (Schaumburg et al. 2011c). Five participants (laboratory codes 18, 28, 29, 31 and 39) identified less than 12 indicative species and consequently their assessment is unreliable according to the PHYLIB-tool. Four of these participants also did not search for rare taxa after the count (Table 6 in Chapter 4.1.2), which is required for lake samples according to the PHYLIB-method (Schaumburg et al. 2011c). This emphasizes the necessity to strictly follow the instruction specified of each method, to generate a reliable assessment.

For a reliable assessment of the Module “**Trophic-Index (TI)**“ indicative taxa need to contribute a minimum of 60 % (relative abundance) to the assemblage, i.e. taxa with a trophic value (and weighting) in PHYLIB (Schaumburg et al. 2011c). With 63.4 % the sum of TI-indicative taxa was only slightly above the 60 %-limit in one of the three auditor samples (Fig. 71). Only 12 of the 40 participants (i.e. 30 %) identified at least 60 % TI-indicative taxa. The Module “Trophic-Index (TI)“ of all remaining participants and two auditors is unreliable according to PHYLIB, as the assessment is based on an insufficient percentage of the data. This is due to the relatively high percentage of taxa in the ‘Lake’ sample that are listed in PHYLIB but have no trophic value (Fig. 72), i.e. taxa with no indicator data in the PHYLIB training set. After combining the modules “Trophic-Index (TI)“ and “Quotient of Reference Species (RAQ)“ to obtain the overall assessment with diatoms (**Diatom-Index for lakes, DI_{Seen}**) for the ‘Lake’ sample, only one auditor and eleven participant assessments (i.e. 27.5 %, n= 40) are determined as reliable (e.g. Fig. 71).

The transfer of the originally listed *Fragilaria brevistriata* to *Fragilaria brevistriata* var. *brevistriata* increased the relative abundances of taxa with trophic values (indicative taxa) distinctly (Fig. 73), leading to a reliable assessment of the TI-module for 18 participants (corresponding to 45 % of the 40 participants). This also increased the reliable overall assessment of DI_{Seen} to 40 % (16 of the 40 participants) and also affect the values of the diatom indices (Fig. 74).

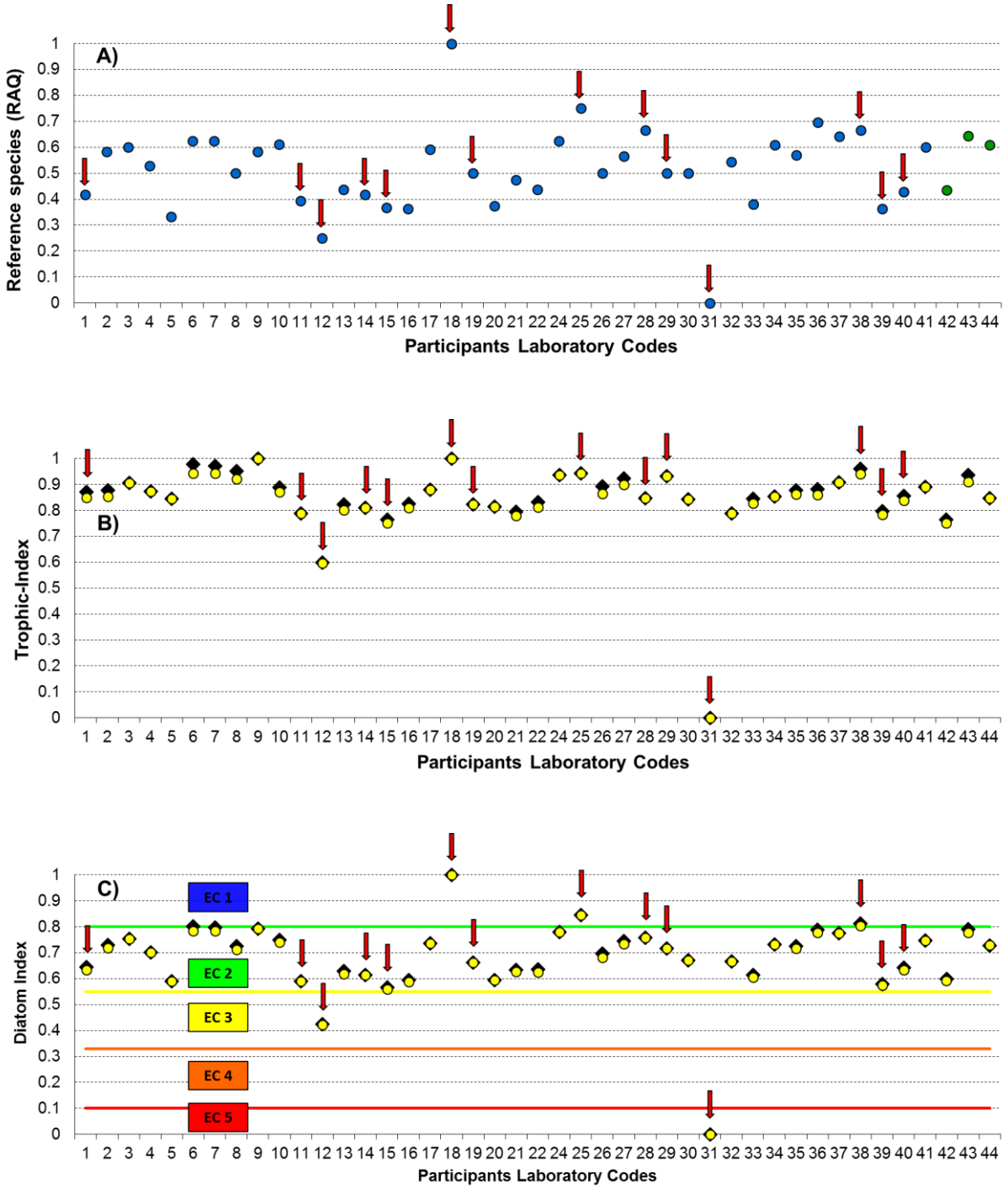


Figure 74. Results of the **ecological assessment using the German PHLYIB-tool** for the **‘Lake’ sample**: **A)** Module “Quotient of **Reference Species** (RAQ)“, shown are transformed RAQs of the participants (laboratory codes 1-41) and auditors (laboratory codes 42-44); **B)** Module “**Trophic-Index** (TI)“, shown as transformed TIs; Legend: solid diamonds: based on the entered counting results, yellow circles: relative abundances from *Fragilaria brevistriata* were corrected to *Fragilaria brevistriata* var. *brevistriata*; **C) Diatom-Index** for lakes (DI_{Seen}); legend see B), coloured lines = index limits of ecological status classes (EC) for Lake Type D 10.1 with EC 1=very good, EC 2 = good, EC 3 = moderate, EC 4 = unsatisfactory, EC 5 = poor EC; **red arrows** indicate participants that analysed the ‘Lake’ sample taxonomically **unsuccessfully**.

The Module “Quotient of Reference Species (**RAQ**)” for the ‘Lake’ sample had transformed RAQs of 0.44 to 0.64 based on the counting results of the auditors (Fig. 74 A). For the participants, the transformed RAQs ranged from 0.25-0.75 and two exceptions (with ‘zero’ and ‘one’, respectively) with an average of 0.52 (n=40; Fig. 74 A). The Module “Trophic-Index (**TI**)“ had transformed TIs of 0.76 to 0.94 based on the results of the auditors. The participants transformed TI-values ranged from 0.75 to 0.94 and two exceptions (with ‘zero’ and ‘one’, respectively) with an average of 0.85 (n=40; Fig. 74 B). If the counting results of the auditors were converted to comply with PHYLIB, i.e. if *Fragilaria brevistriata* (outdated aggregate in PHYLIB) was transferred to *Fragilaria brevistriata* var. *brevistriata*, then the auditor TI-values would be reduced to range from 0.75 to 0.91 (Fig. 74 B), indicating a higher trophic state. Similarly, transferring the *F. brevistriata* - data of the participants led to decreased TI-values (average 0.84, n=40) which also indicated a higher trophic state (Fig. 74 B).

The combination of TI and RAQ to calculate the Diatom-Index for lakes (**DI_{Seen}**) resulted in DIs of 0.60 to 0.79 for the auditors (after taxonomic correction to 0.59-0.78), which corresponded to the ecological status class (EC) 2 (good)(Fig. 74 C). The participants DIs ranged from 0.42 to 0.85 and two exceptions (with ‘zero’ and ‘one’, respectively) with an average of 0.681 (after taxonomic correction: 0.677). Therefore, most participant results also indicated EC 2 (good). Some participant results indicated EC 1 (very good) or EC 3 (moderate) and one indicated EC 5 (poor)(Fig. 74 C). The conversion of the *F. brevistriata*-data distinctly changed the assessment of the ‘Lake’ sample for the auditors and some participants, which resulted in a slightly poorer assessment (Fig.74 C).

According to the auditors, this ‘Lake’ sample was assessed as EC 2 (good). Of 40 participants 35 found the same result and five participants generated a different assessment (Fig. 74 C). These five participants also counted the ‘Lake’ sample unsuccessfully. The results of the other nine participants that counted the ‘Lake’ sample unsuccessfully had, by chance, also an assessment of EC 2 (good), despite partly considerable misidentifications (Fig.74 C).

In this intercalibration exercise, the **variability of the assessment** of the water quality with the PHYLIB-tool was considerably less for the ‘Stream’ sample compared to the ‘Lake’ sample. In contrast to the ‘Stream’ sample (Fig. 69), the values for the modules TI, RAQ and consequently also DI_{Seen} differed more strongly for the ‘Lake’ sample among auditors and participants (Fig. 74). Similarly, the counting results of the auditors differed more strongly to each other in the ‘Lake’ sample compared to the ‘Stream’ sample (Table 4 and 6 in Chapter 4.1). Overall, this higher variability reflects the higher proportion of taxa that were difficult to identify in the ‘Lake’ sample compared to the ‘Stream’ sample (Chapter 4.1).

Summary of the effects of counting result variances on the ecological assessment with PHYLIB

The 40 participants and three auditors listed 374 different taxa names for the 'Stream' sample and 433 names for the 'Lake' sample (Table 24). A large percentage of taxa did not contribute to the water quality assessment for varying reasons (Fig. 65, 67, 70 and 72). When considering both samples, the largest portion of **non-indicative taxa** were identified to an insufficient level of taxonomic resolution (Table 24). This emphasizes the importance to identify taxa to the required level of taxonomic resolution and with as much precision as possible. The second largest portion of non-indicative taxa were taxa that were listed in the PHYLIB-software, but had no trophic values (Table 24), i.e. that had no trophic state data in the PHYLIB training set. In the lake sample these taxa contributed on average 28.1 % to the assemblages (n=43). Consequently they were responsible to a large extent for less than 60 % indicative taxa (i.e. assessments of the water quality that were deemed unreliable) that occurred in two auditor samples and in 70 % of the participant samples (28 participant results).

Table 24. Summary of effects of counting result variances on the ecological assessment with PHYLIB for the 'Stream' and 'Lake' sample.

	Stream	Lake	Figure
# of taxa names identified	374	433	-
Taxa with insufficient level of taxonomic resolution (% of taxa names)	24.6 %	27.0 %	65 & 70
Taxa with insufficient level of taxonomic resolution (average relative abundances; n=43)	13.1 %	10.8 %	67 & 72
Taxa in PHYLIB without indicator values (% of taxa names)	19.5 %	23.3 %	65 & 70
Taxa in PHYLIB without indicator values (average relative abundances; n=43)	6.2 %	28.1 %	67 & 72
Taxa with indicator values (average relative abundances; n=43)	71.8 %	55.1 %	66 & 71
Inferred ecological status class (auditors)	4	2	69 & 74

Taxa names that belonged to an "outdated aggregate" in PHYLIB (explanations see Chapter 4.5.1) also contributed greatly to the relative abundance of non-indicative taxa (Fig. 67 & 72). In contrast, only 3.2 % (Stream sample) and 4.4 % (Lake sample) of the listed taxa names had no German data processing number (DV-no ; Mauch et al., 2003, version 2011) and were therefore not in PHYLIB. Despite a minimal affect on the abundances of indicative taxa, we recommend to update and supplement the DV-no-registry and correspondingly the PHYLIB software.

The counting results of the auditors assessed the **'Stream'** sample with an **ecological status class** (EC) of EC 4 (unsatisfactory). Most results of the participants generated the same assessment, including all participants that counted the sample successfully, i.e. Bray-Curtis dissimilarity distances were within the confidence intervals of all auditors.

The counting results of the auditors assessed the **'Lake'** sample with EC 2 (good). The results of five participants generated a different assessment. These participants had counted the 'Lake' sample unsuccessfully. Therefore, we conclude that the exact diatom identification is an essential prerequisite for a correct ecological assessment.

5. Recommendations

5.1 Recommendations for diatom counts in applied areas

Diatom analysis is a highly sophisticated task that requires a high level of experience and certain technical equipment. The microscope should have a minimum of 1,000x magnification (Chapter 4.2). Also desirable is a microscope that uses differential interference contrast or phase contrast and a 100x objective with an aperture of at least 1.3. The microscope should routinely be tested for performance, cleaned and calibrated. For example, the performance can be tested using slides containing *Amphipleura pellucida*. Resolution should be sufficiently high so that the striae are visible.

As already demonstrated during the first German intercalibration exercise for benthic diatoms, it is essential that all characteristics visible with the light microscope are taken into account for the identification of diatoms (Dreßler et al. 2014). This includes all characteristics of each taxon, from given size dimensions, to the length to width ratio, the striae and fibulae density, to valve shape and any special features of the valve (e.g. stigma, shape of areolae, striae orientation).

We recommend to always use the most current identification literature given in the applied method. For the German method in terms of identifying the ecological state of water bodies (PHYLIB-method) we recommend to at least use the literature given in the PHYLIB-instructions, i.e. in Schaumburg et al. (2011c, 2012). The given standard identification literature (Hofmann et al. 2011 or 2013) covers most of the diatoms relevant for water quality monitoring. However, the volume does not include all diatoms present in an area (Hofmann et al. 2013). This holds particularly true, but not exclusively (!), for taxa from the following genera: *Cymbella*, *Cymbopleura*, *Encyonema*, *Encyonopsis*, *Gomphonema* and *Pinnularia*. Therefore, it is necessary to use the following supplementary volumes (Schaumburg et al. 2011c, 2012): Krammer & Lange-Bertalot (1986-2004), Lange-Bertalot (1993, 2001), Lange-Bertalot & Moser (1994), Lange-Bertalot & Metzeltin (1996), Krammer (1997a, 1997b, 2000, 2002, 2003), Reichardt (1999), Witkowski et al. (2000) and Levkov (2009). We recommend adding Lange-Bertalot et al. (2011) and Levkov et al. (2016) to the list of supplementary literature.

Information about the ecology, distribution and occurrence of diatoms provided in the identification literature should preferably not be used as aid to identify a species (Dreßler et al. 2014).

We recommend to document diatom taxa that are difficult to identify, i.e. taxa where a differentiation with certainty is difficult. Adequate pictures and a brief description of the taxonomic problem should be given together with the identification literature used. We suggest to label diatoms determined with uncertainty with ”sp.”, ”aff.” or ”cf.”. Together with the precise documentation, this potentially enables a later allocation to an unambiguous taxon.

The German DV-number register (Mauch et al. 2003, version 2011) still lists superfluous and outdated taxa names concurrent with the current names that incorporate the current taxonomic concepts. In practice, it needs to be checked, which name and DV-number is contained in the PHYLIB-software (German software to identify ecological state of waters from diatom assemblages) and has indicator values (see comments in Chapter 4.5.1). These taxa are listed in the Appendix (Table A1).

We recommend for personnel that count and identify diatoms in applied areas (Water Framework Directive, bio-monitoring) to regularly participate in intercalibration exercises or taxonomic workshops. This should ensure both a permanently high quality standard and the comparability of diatom counts among diatomists. In the future, the participation shall be a prerequisite for successful public contract bids. Alternatively, we recommend that the authorities that assign diatom contracts conduct quality control via independent third parties.

5.2 Recommendations for the German assessment method

For ensuring quality and accuracy of the assessment results using the German PHYLIB-method, the highest possible percentage of indicative taxa is essential, i.e. a high number of taxa that contribute to the ecological assessment. The PHYLIB-method is based on a training set that was generated using the taxonomic concept of 2003 and earlier. Since then, the taxonomy was adjusted according to the continuously renewed diatom taxonomy several times (Mauch et al. 2003, version 2011). Still, a substantial percentage of taxa are not part of the PHYLIB-method due to the use of the most current taxonomy (Chapter 4.5). Accordingly, we recommend to:

- harmonize the various German DV-numbers for diatom synonyms in the Mauch-register and adjust the PHYLIB-software accordingly. ”Outdated taxa-aggregates (lumping groups)” (for more explanations see Chapter 4.5.1) should be deleted from the Mauch-register or marked as inactive. Also, the continuous and prompt updates and supplements of the DV-numbers should be continued.

- incorporate the advanced and now established taxonomic knowledge level with the accordingly updated taxa names (e.g. *Gomphonema parvulum*, *Pseudostaurosira brevistriata*, see also Chapters 4.3.2 and 4.4.4). Additionally, the data incorporated in the PHYLIB-software and the DV-no.-register (Mauch et al. 2003, version 2011) should be checked for consistency. For example, there are three different DV-no. listed for *Fragilaria brevistriata* (*F. brevistriata*: 36079, *F. brevistriata* var. *brevistriata*: 6388 and *Staurosira brevistriata*: 16616) and no DV-number for *Pseudostraurosira brevistriata* in the Mauch-register or PHYLIB-software (Table 22). In the PHYLIB-software, only numbers 6388 and 16616 have allocated indicator values. Therefore, we additionally recommend to only list one unambiguous taxon in the PHYLIB-software that has indicator values. Ideally, the synonyms then generate an error message or are automatically transferred to the unambiguous taxon name (see Chapter 4.5).

- recount and potentially enlarge (rare taxa) the PHYLIB-training set. The goal is a distinct increase of the number of indicative species whilst taking the current taxonomic knowledge into account to fully use the entire potential of benthic diatoms as robust bio-indicators (Dreßler et al. 2014).

Furthermore, we support the recommendations given in the final report of the first German intercalibration exercise (Dreßler et al. 2014), to count diatom valves instead of diatom objects (frustules or valves). The results of the second intercalibration exercise complement former recommendations, as the variability of the counting results were probably partly due to some participants that counted valves and not objects. During the workshop we learned that an international comparison of counting results is not possible when counting objects, as all other European countries count valves. Therefore, counting valves in Germany would enhance the comparability of results across international borders for monitoring and management.

A general separation of (facultative) benthic and obligatory planktonic taxa is often difficult but implemented in the German PHYLIB-method. Several European countries (e.g. Sweden) have developed standard methods that incorporate all species encountered in a benthic sample. Thus, if the German training set is recounted (see below) all species should be counted and the new training set should be re-investigated to identify, if the new model is improved when all species are considered. If the exclusion of planktonic taxa is to be continued, we recommend expanding the list of obligatory planktonic taxa according to the standard identification literature (Appendix, Table A2). See also Chapter 7 in Dreßler et al. (2014).

Therefore, we strongly recommend a recount of the PHYLIB-training set to further increase the strength and accuracy of the model. As follows:

- enabling the integration of the current taxonomic knowledge status, and thus to distinctly increase the number and percentage of indicative species,
- counting diatom-valves and NOT diatom objects,
- revisiting the separation of planktonic and benthic taxa and including all diatom taxa for the assessment of a sample, if that maximizes the strength of the model, and
- using the newest, revised edition of Hofmann et al. (2011 or 2013), i.e. Lange-Bertalot et al. (2017), as the new standard identification literature for the future and for the recount of the training set.

Additionally, we recommend to create a publicly available database that presents the current (and future recounted) PHYLIB-training set. The database should (1) present the training set including a list of all taxa with both, their images from the training set-samples and their indicator values, (2) list all identified water parameters of the training set-samples, (3) contain the intercalibration exercise reports, and (4) document updates of the latest taxonomic versions. Therefore, this database would help harmonize information of the most recent German DV-number register (Mauch et al. 2003, version 2011), the PHYLIB-instruction protocols (Schaumburg et al. 2011c, 2012) and the standard identification literature (Hofmann et al. 2013 or suggested Lange-Bertalot et al. 2017). This database would also increase the accuracy of users of the PHYLIB-method, facilitate future taxonomic adjustments and help identify problematic areas that need further work. These areas could be the focus of the next intercalibration exercise. We also recommend to provide access to the training set samples and slides for taxonomic examinations. All changes to new PHYLIB-software versions should be clearly documented, providing detailed information about differences to former versions. Overall, this database would concentrate all information and data relevant to the German PHYLIB-method that assesses the water quality in German surface waters in one location.

5.3 Recommendations for taxonomists

In addition to the recommendations given in Dreßler et al. (2014), we suggest the following complementation in Hofmann et al. (2013):

- An introductory chapter should explain how to measure striae density (according to Chapter 6 in Dreßler et al. 2014).
- We recommend to refrain from stating that ”a further differentiation of a taxon is not necessary, because the German assessment method (PHYLIB) does not require a differentiation”. Instead, we recommend to always suggest to differentiate as much as taxonomically possible. This enables a later allocation of the taxa, if they are added to PHYLIB. Also the assessment results are less likely to be affected, e.g. critical relative abundances of reference species may generate different results depending on the level of differentiation (see Chapter 4.3.1).
- *Fragilaria brevistriata* should be named *Pseudostaurosira brevistriata*, as internationally established now, due to the striae structure. In Hofmann et al. (2013) the allocated name *Staurosira brevistriata* is not correct. Additionally, we recommend the allocation of *Fragilaria pinnata* and *F. lapponica* to *Staurosirella*.

- Several common species should be added to the standard book, for example:
  - *Navicula submuralis* (German DV-no. 16373), listed in the German PHYLIB-software with a trophic value for lotic systems;
  - *Pseudostaurosira elliptica*, a relatively newly described species that is similar to *Pseudostaurosira brevistriata*;
  - *Pseudostaurosira trainorii*, with currently no indicator values (yet) in the PHYLIB-software, but described and depicted in Krammer & Lange-Bertalot 2000 (Plate 130), as *Fragilaria elliptica* SCHUMANN (DV-Nr. 6400).
  - We also recommend to potentially include additional common *Encyonopsis*-species because Krammer (1997b) has been out of print for years and will not be available in the near future.

6. Summary

The second German intercalibration exercise for benthic diatoms took place in 2014 and 2015. The purpose of the exercise was to: 1) improve the accuracy of the German PHYLIB-method that assesses the water quality of lakes and lotic systems (Schaumburg et al. 2011c, 2012), 2) to enable a harmonization of handling taxonomic problems and to 3) conduct a basic quality control of diatom counting results. The intercalibration exercise was open to independent, government-funded and scientific personnel that conduct water quality assessments and monitoring of water bodies using benthic diatoms.

The 40 participants of the intercalibration exercise were from 15 countries (Belgium, Estonia, France, Great Britain, Germany, Hungary, Ireland, Italy, the Netherlands, Peru, Portugal, Serbia, Slovenia, Spain and Sweden). Additionally, three internationally renowned diatom specialists participated as auditors. The 40 participants and three auditors had to count and identify diatoms from two samples according to the German instruction protocols (Schaumburg et al. 2011c, 2012). These were one lake sample (Lake **Lychensee**, Diatom-Lake-Type 10.1, carbonate-rich, dimictic) and one stream sample (Stream **Saaler Bach**, Diatom-Running Water-Type 12.1, carbonate-rich), both from the North German Lowlands.

For an evaluation of the participant performances their counting results were statistically assessed and their deviations from following the instruction protocols were examined based on pre-determined parameters. The statistical analysis included two independent methods: the calculation of Bray-Curtis-dissimilarity distances and the analysis and graphical depiction of the similarity of the counting results using a Detrended Correspondence Analysis (DCA).

Most participants followed the appropriate (i.e. lake or stream) instruction protocols. Of 40 participants, 30 had ”no deviations” from the instruction protocols, five participants showed ”minor deviations” and another five participants had ”substantial” deviations. The ‘Stream’ sample was successfully counted by 36 participants. The results of four participants were outside the Bray-Curtis-distances plus standard deviations to all three auditors. This result was confirmed by the DCA. The ‘Lake’ sample was counted successfully by 26 of 40 participants.

In July 2015, we conducted a two-day long workshop at the Technische Universität Braunschweig, Germany, with the participation of two auditors. We presented the results of the intercalibration exercise, discussed taxonomically difficult diatom-groups, determined characteristics of these diatoms to facilitate identification together with the workshop participants and composed recommendations to improve the German PHYLIB-method for assessing the ecological state of water bodies.

The analysis of the counting results of both intercalibration exercise samples and the discussion during the workshop identified difficulties of the participants to identify the following taxa and taxa groups. Stream sample: (1) *Cocconeis placentula*-aggregate, (2) *Gomphonema parvulum*-aggregate, (3) *Navicula cryptocephala*, (4) *Nitzschia paleacea*, (5) *Nitzschia palea*-aggregate, (6) *Planothidium lanceolatum* and *P. frequentissimum*; Lake sample: (7) *Achnanthidium minutissimum*-aggregate, (8) *Cymbella vulgata* and other *Cymbella*-species, (9) *Encyonopsis minuta* and *E. subminuta*, (10) *Fragilaria (Pseudostaurosira) brevistriata* and (11) *Navicula cryptotenella* and *N. cryptotenelloides*.

Following the results of the first German intercalibration exercise (Dreßler et al. 2014) this exercise similarly identified the following reasons for misidentifications: (1) Not all characteristics visible in the light microscope were used to identify diatoms, e.g. size dimensions, length to width ratio, striae and fibulae density, valve shape and other special features of the valve (e.g. stigma, shape of areolae,

striae orientation). (2) Species description and documentation are sometimes ambiguous in the given identification literature, e.g. for the *Cocconeis placentula*-aggregate. (3) Some participants exclusively used Hofmann et al. (2011 or 2013) as identification literature, which is the standard identification literature. However, it only covers most but not all diatom species relevant for water quality monitoring. For example, additional identification literature was necessary to unambiguously identify *Cymbella vulgata* and other *Cymbella*-species as well as *Encyonopsis minuta* and *E. subminuta* in the lake sample. (4) Taxa were listed as unambiguously identified, despite at least one characteristic that differed distinctly from the descriptions in the identification literature, i.e. often participants refrained from labelling such taxa as determined with uncertainty (”cf. ”, ”aff. ”) and if necessary to document them for later unambiguous identification.

The counting results of most participants indicate the same ecological status of the water bodies when using the German PHYLIB-method, as the assessment based on the counting results of the auditors. The assessment was in agreement for all participants that counted the sample successfully. This emphasizes how robust and well the PHYLIB-method works, whereby the precise identification of diatoms is an essential pre-requisite for a correct ecological assessment.

To ensure quality and accuracy of the assessment results using the PHYLIB-method, the percentage of indicative taxa needs to be as high as possible, i.e. the number of taxa that contribute to the ecological assessment. Therefore, the identifications need to be as exact as possible and also at the postulated level. Also, taxa from ”outdated aggregates (lumping groups)” need to be deleted or listed as inactive in the German DV-register that identifies each taxon. We strongly recommend to recount the PHYLIB-training set based on the current taxonomic knowledge level with the goal to increase the number of indicative species and consequently to further increase the strength and accuracy of the model for water quality assessment.

Following up the first German intercalibration exercise, the second intercalibration exercise for benthic diatoms in Germany demonstrated that the quality of counting results critically affects the assessment results when using the German PHYLIB-method. The analysis of the counting results identified taxonomic problems for which proposed solutions were developed during the workshop and in this report. The harmonization of taxonomic concepts facilitates a reduction of the variability of counting results and thus improves the accuracy and ultimately the quality of the water quality assessment. Additionally, we formulated recommendations for the diatom analysis when using the German PHYLIB-method, for additional recommended identification literature and for improving the PHYLIB-method itself based on the results of this intercalibration exercise. Overall, a periodic conduction of intercalibration exercises with a similar approach remains extremely important in future years.

7. Acknowledgements

We are especially thankful to the auditors Dr. Krisztina Buczko (Hungarian Natural History Museum, Hungary, Budapest), Prof. Dr. Bart Van De Vijver (Botanic Garden Meise, Belgium) and Dr. Luc Ector (Luxembourg Institute of Science and Technology (LIST), Luxemburg) and all participants of the intercalibration exercise and workshop. Thanks to Cornelia Fischer for her help during the conduction of the workshop at the Technical University Braunschweig, Germany. We would also like to thank Francine Forrest (LimnoLogic Solutions Ltd) for her editorial and technical review of the English version. Finally, thank you to Andreas Meybohm of the State Reservoir Administration of Saxony (LTV), Germany, for the administrative operation of the intercalibration exercise and for providing and maintaining the webpage www.planktonforum.eu.

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Appendix

Table A1. List of taxa as they need to be entered for an assessment with the German PHYLIB-software, partly in deviation to the current taxonomic concepts.

Taxon	**DV-number**
Chamaepinnularia soehrensis var. *soehrensis*	26628
Cymbella cymbiformis var. *cymbiformis*	6979
Cymbella helvetica var. *helvetica*	6184
Cymbopleura hybrida var. *hybrida*	26182
Cymatopleura elliptica var. *elliptica*	6057
Cymatopleura solea var. *solea*	6031
Encyonopsis cesatii var. *cesatii*	26147
Eunotia bilunaris var. *bilunaris*	6213
Eunotia exigua var. *exigua*	6975
Eunotia fallax var. *fallax*	6359
Eunotia monodon var. *monodon*	6885
Eunotia paludosa var. *paludosa*	6373
Eunotia parallela var. *parallela*	6765
Eunotia praerupta var. *praerupta*	6851
Eunotia rhynchocephala var. *rhynchocephala*	16230
Eunotia serra var. *serra*	6850
Fragilaria brevistriata var. *brevistriata*	6388
Fragilaria famelica var. *famelica*	6915
Fragilaia pinnata var. *pinnata*	6078
Fragilaria virescens var. *virescens*	6169
Frustulia rhomboides var. *rhomboides*	6187
Gomphonema parvulum var. *parvulum* f. *parvulum*	6158
Gyrosigma acuminatum var. *acuminatum*	6036
Luticola mutica var. *mutica*	26577
Mastogloia elliptica var. *elliptica*	16281
Mastogloia smithii var. *smithii*	6444
Navicula cryptocephala var. *cryptocephala*	6010
Navicula kotschyi var. *kotschyi*	6508
Navicula menisculus var. *menisculus*	6094
Navicula radiosa var. *radiosa*	6103
Navicula reichardtiana var. *reichardtiana*	6221
Navicula viridula var. *viridula*	6037
Neidium affine var. *affine*	6820
Nitzschia capitellata var. *capitellata*	6964
Nitzschia fonticola var. *fonticola*	6025
Nitzschia liebetruthii var. *liebetruthii*	16423
Nitzschia linearis var. *linearis*	6024
Nitzschia recta var. *recta*	6029
Nitzschia tryblionella var. *tryblionella*	6119
Pinnularia appendiculata var. *appendiculata*	6623
Placoneis pseudanglica var. *pseudanglica*	16603
Planothidium frequentissimum var. *frequentissimum*	16606
Rhopalodia gibba var. *gibba*	6677
Sellaphora laevissima var. *laevissima*	16612
Sellaphora pupula var. *pupula*	16614
Stauroneis anceps var. *anceps*	6129
Stauroneis smithii var. *smithii*	6131
Tabellaria flocculosa var. *flocculosa*	6091

Table A2. Recommendation for a revised plankton exclusion list for A) *Centrales*, B) *Pennales*, particularly freshwater taxa and C) *Pennales*, particularly brackish- and salt water taxa; H = according to Hofmann et al. 2011 or 2013, HU = according to Lange-Bertalot & Ulrich (2014), KLB = Krammer & Lange-Bertalot (1986–2004), MN = Mischke & Nixdorf 2008, S = according to Schaumburg et al. (2011c, 2012), see also Chapter 7 (Recommendations) in Dreßler et al. (2014).

Taxon	DV-number	Source
A) *Centrales*, all, except:		
Ellerbeckia arenaria	6211	KLB
Melosira varians	6005	S
Pleurosira laevis	16487	KLB
B) *Pennales*, particularly in freshwater:		
Asterionella	6142	S
Asterionella formosa	6050	S
Asterionella formosa var. *acaroides*	6863	S
Diatoma tenuis	6210	H, MN
Fragilaria berolinensis	6235	KLB
Fragilaria crotonensis	6075	S
Fragilaria longifusiformis	26383	Siver et al. 2006
Fragilaria reicheltii	6215	S
Fragilaria saxoplanctonica	keine	HU
Fragilaria tenera var. *lemanensis*	keine	HU
Fragilaria ulna angustissima-aggregates	6410	S
Nitzschia acicularis	6023	S
Nitzschia acicularis- aggregates	16856	S
Nitzschia acicularis var. *closterioides*	16600	S
Nitzschia draveillensis	6588	H
Nitzschia fruticosa	6806	S
Nitzschia graciliformis	6594	H
Nitzschia reversa	16445	KLB
Surirella splendida	6695	S
Tabellaria fenestrata	6074	S
C) *Pennales*, particularly in brackish- and salt water:		
Asterionellopsis	16820	S
Asterionellopsis glacialis	16797	S
Asterionellopsis kariana	16819	S
Cylindrotheca closterium	26929	S
Delphineis surirella	16831	S
Nitzschia behrei	16394	S
Nitzschia closterium	16398	S
Pseudo-nitzschia	16847	S
Rhaphoneis	16659	S
Rhaphoneis amphiceros	16812	S
Thalassionema nitzschioides	16849	S