Standardisation of river classifications:

Framework method for calibrating different biological survey results against ecological quality classifications to be developed for the Water Framework Directive

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Results of the La Bresse diatom sampling and analysis workshop

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Results of the La Bresse Diatom sampling and analysis workshop

Final Draft

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1 Introduction

1.1 The workshop

The STAR workshop in La Bresse, France, was organised by Metz University between 28 April and 3 May, 2002. During this workshop the sampling of various aquatic organism groups was discussed. One of these groups is benthic algae, in particular benthic diatoms. During the workshop a sampling and analysis protocol for benthic diatoms was proposed (Van der Molen & Verdonschot, 2002). Two sampling sites were visited to give participants hands-on experience in diatom sampling. This simultaneous sampling effort was used to perform a comparison between the results generated by participating partners in STAR and to get an indication of the overall variation introduced by the different steps in the sampling and analysis process.

The researchers that collected the samples and performed the laboratory analyses, had a different level of experience in diatom research, ranging from almost none to several years of professional experience. Experience is, however, only one of the sources of variation. It needs to be stressed that this workshop was not intended as a 'ring test' as the samples were not re-analysed by different laboratories.

Sources of variation
In the process of diatom sampling and analysis, variation can be introduced during sample collection, treatment, microscope preparation and identification and counting of diatom valves.

Sample collection
Samples are collected from various parts of the stream. A possible patchy distribution of diatoms can introduce differences between replicate samples collected from one sampling site.

Sample treatment in the laboratory and microscope slide preparation
Diatom field samples are chemically treated in the laboratory before microscopic examination. The organic components in the sample (such as chlorophyll) are destroyed in this procedure leaving the silicate cells walls of the diatoms intact. The cleaned suspension is evaporated onto a coverslip and mounted onto a microscope slide. As only a small portion of the original sample is used for slide preparation, this adds to the error.

Microscope slide analysis

The analysis of the slides is done by identifying and counting diatom valves until 300 valves have been counted. In most cases more than 300 valves are present on the slide. Counting a subsection of the slide introduces a subsampling error.

Total error
The variation that is measured between replicate samples collected by one partner is the result of the combined errors that are introduced in the steps of sample collection, treatment and analysis. The total variation that is observed when comparing samples between partners comprises this combined error plus the error as a result of different identifications and errors introduced by possible different interpretation of the sampling and analysis protocol.

La Bresse workshop versus the audit
During the course of the STAR project, an audit will be performed for quality control of benthic diatom samples. The methods of performing the audit are introduced in this report by applying these methods on the results of the La Bresse workshop. The outcome of these analyses however, is of a different order than those that will be generated as part of the audit.

This report focuses on the differences between replicate samples, microhabitats and partners. This variation is studied based on two sampling sites where samples were collected from three different microhabitats. This setup differs from the design of the audit. During the audit each microscope slide is analysed twice: once by the principal analyst of the STAR partner that collected the sample and once by the auditor. This means that the variation between the results of the principal analyst and the auditor are only the result of differences in identification and counting (see Figure 1 for an illustration of the different levels at which variation between analyses is investigated during the workshop and audit).

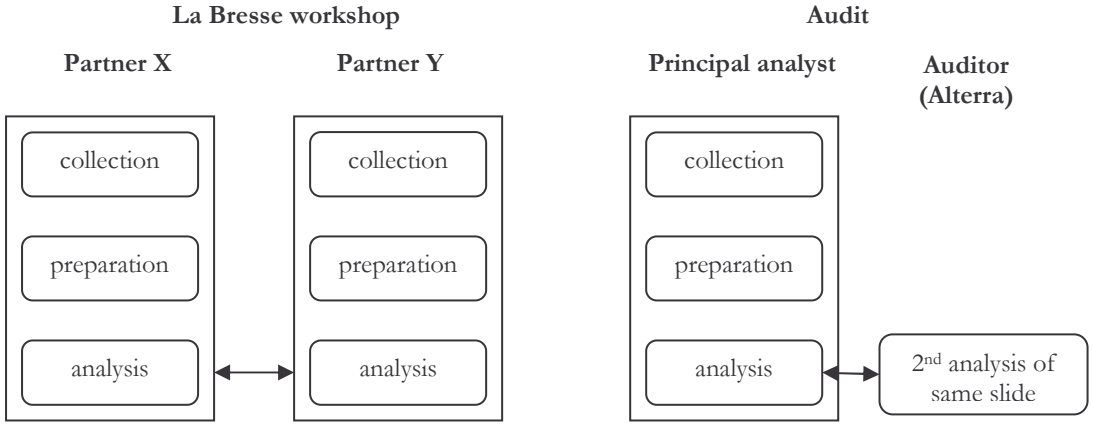

Figure 1Diagram illustrating the different levels at which results are being compared between partners during the La Bresse workshop and during the audit. Each square represents a stage in the analysis at which error is introduced. In the La Bresse workshop the overall variation based on collection, preparation and analysis is assessed. During the audit comparison is only made on the analysis level (taxon identification and counting).

The outcome of the La Bresse workshop gives valuable information on the variation that can occur between replicates, habitats and different operators (partners) performing the analyses. The audit will provide information on the variation at the level of diatom identification and counting only.

2 Materials and Methods

Sample collection and analysis

Samples were collected from the Plaine River, France, at two locations (PL0 and PL5) on 2 and 3 May 2002. Samples were collected from three different habitat types: Stones (H), Macrophytes (M) and Sediments (S) following the STAR sampling protocol (Van der Molen & Verdonschot, 2002). Each partner sampled two habitats in triplicate on both sampling sites. The participants that collected the samples also made sure that these samples were analysed (i.e. identified and counted) by their respective laboratories.

The proposed diatom nomenclature used for identification was according to the taxonlist used in the OMNIDIA programme (Lecointe *et al.*, 2003). This list is widely used throughout Europe and is kept up to date to allow for taxonomic revisions and new autecological information for individual taxa. The taxonlist generally uses the nomenclature following Krammer and Lange-Bertalot (1986-1998) and includes information on synonymous taxa.

At least 300 valves were identified and counted in each slide following the procedures suggested in the STAR sampling protocol (Van der Molen & Verdonschot, 2002). The results were sent to Alterra in Wageningen, the Netherlands.

Compilation and analysis of the results

The overall list of taxa found by the participants was harmonised by converting synonymous taxa to one valid name, based on information contained in the OMNIDIA programme (Lecointe *et al.*, 2003).

For each sample the relative abundance of taxa (number of valves/total number of valves counted), shannon diversity and evenness (Zar, 1996) were calculated. The Shannon diversity was calculated following:

$$H' = -\sum_{i=1}^{k} p_i \log p_i .$$

where p_i is the relative abundance and k the number of taxa in a sample.

Evenness:

$$J' = \frac{H'}{H'_{\max}} \text{ where } H'_{\max} = \log k .$$

Furthermore, the similarity between samples was calculated with the Bray-Curtis similarity index:

$$D_{ij} = 1 - \frac{\sum_{k=1}^{n} \left| x_{ik} - x_{jk} \right|}{\sum_{k=1}^{n} (x_{ik} + x_{jk})}$$

where D_{ij} is the similarity between samples i and j and x is the abundance of the k^{th} taxon in sample i and j. The Bray-Curtis index was calculated using MVSP (Kovach Computing Services, 2002). The Bray-Curtis similarity between partners was plotted in a box and whisker plot for each habitat and each site (see results). Box and whisker plots provide a

graphic means of summarizing a variable in raw data. It illustrates the spread of values about the median. Visually each variable is represented by a box with a waisted notch about the median and vertical lines ("whiskers") extending from the top and bottom. The notches delimit the quartiles of data. The whiskers delimit the 5th and 95th percentiles. The entire box delimits the 10th and 90th percentiles (Kovach Computing Services, 2002).

The OMNIDIA programme (Lecointe *et al.*, 2003) was used to compute 13 different diatom indices that are regularly used to asses several aspects of water quality, mainly in flowing waters (Table 1). The index results were standardised to a scale between 1 and 20 to allow for easy comparison. The meaning of index values as such (quality assessment) was not subject to interpretation in this report. The objective was to analyse the degree of variation of the indices calculated for samples originating from the same site and habitat but collected, processed and analysed by different partners.

Table 1 Diatom indices available in the OMNIDIA programme that were used to compute index values for all diatom samples from sites PL0 and PL5.

Abbreviation	*Full name*	*Year*	*Reference*
IPS	Specific Pollution Sensitivity Index	1987	(Coste, 1987)
SLAD	Sládecek's pollution index	1986	(Sladecek, 1986)
DESCY	Descy's pollution index	1979	(Descy, 1979)
LMI	Leclercq & Maquet's pollution index	1987	(Lecointe *et al.*, 2003)
SHE	Steinberg & Schiefele trophic index	1988	(Steinberg & Schiefele, 1988)
WAT	Watanabe et al pollution index	1990	(Lecointe *et al.*, 2003)
TDI	Trophic Diatom index	1995	(Kelly & Whitton, 1995)
EPI_D	Pollution index based on diatoms	1996	(Dell'Uomo, 1996)
ROTT	Trophic index	1999	(Rott *et al.*, 1999)
IDG	Generic Diatom Index	1991	(Lecointe *et al.*, 2003)
CEE	Commission for Economical Community index	1991	(Descy & Coste, 1991)
IBD	Biological Diatom Index	1991	(Prygiel & Coste, 1999)
IDAP	Indice Diatomique Artois Picardie	2002	(Lecointe *et al.*, 2003)

Diatom indices were compared between partners and between replicates in an analysis of variance. Variance components were estimated by means of restricted maximum likelihood (Patterson & Thompson, 1971). The hypothesis that there were no differences in variance in index values regardless of the habitat, was tested with a chi-squared test. Analyses were performed in GenStat 6.1 (VSN International Ltd, 2002).

Comparison with a standard sample

For the purpose of introducing the methods that will be used in the audit of diatom samples during the course of the STAR project, a comparison was made between a 'standard sample' collected and analysed by Alterra (the audit sample of the workshop) and those collected by the other participants (primary samples). Results from partners and auditor were compared in two ways. The similarity between primary (partner) and audit (Alterra) samples was evaluated with the Bray-Curtis similarity measure. Also, after calculating diatom index values for all samples, a comparison was made between primary and audit index results. The latter method is indirect as it involves an interpretation of the composition of a sample by weighting known indicator taxa, whereas the Bray-Curtis similarity measure was calculated from original data (taxon lists).

3 Results

3.1 General composition of the data matrix

Siatom taxon lists and the relative abundance of all taxa in a count of 300 valves were received from 10 STAR partners (Table 2). The results delivered by partner 7 contained abundance classes (abundant, common and rare) and could therefore not be used in the numerical analyses. In total results on 116 samples were received divided over two sampling sites (PL0 and PL5) and three habitats: Stone (H), macrophyte (M) and sand/sediment (S) (Table 2).

*Table 2 Institutes participating in the La Bresse diatom sampling workshop and number of samples analysed per habitat (Stone (H), macrophyte (M) and sand/sediment (S)) and per sample site (PL0 and PL5) by each partner. * = data was not in numerical form.*

Partner	*Institute*	*PL0H*	*PL0M*	*PL0S*	*PL5H*	*PL5M*	*PL5S*	*Total*
1	Centre for Ecology and Hydrology, UK	3		3	3			9
2/15	University of Essen/Research Institute Senkenberg, Germany		3	2		3	3	11
3	University of Agricultural Sciences, Vienna, Austria	3		3	3		3	12
5	Swedish University of Agricultural Sciences, Sweden	3	3		3	3		12
6	Masaryk University, Brno, Czech Republic	3		3	3		3	12
7	National Centre for Marine Research, Greece	*		*				*
8	Istituto di Recerca sulle Acque (IRSA-CNR), Italy	3		3	3		3	12
9	University of Evora, Portugal	3	3		3	3		12
10	National Environmental Research Institute, Denmark		3	3		3	3	12
13	Province of Bolzano (LABBIO), Italy	3	3		3	3	0	12
14	University of Metz, France		3	3		3	3	12
	Total	21	18	20	21	18	18	116

From the results received from the STAR partners a data matrix was compiled. After correction for synonymous taxa the resulting taxon list contained 307 taxa. The average number of taxa in samples from sites PL0 and PL5, the shannon diversity and evenness are listed in Table 3. In general, samples collected from stone substrata (H) had a lower number of taxa than the macrophyte (M) and sand (S) samples. This can mean two things: Stone habitats generally contain less taxa or the method of sampling stones does not collect all taxa present. The latter is unlikely as the method for collecting diatoms from macrophytes is less severe in removing cells from the host substrate. Shannon diversity and evenness were similar in all habitats and sites, indicating that all substrata showed a similar relation between abundant, common and rare taxa.

Table 3 Average (standard deviation) of numbver of diatom taxa, shannon diversity and evenness of samples collected from the Plaine river at sites PL0 and PL5 from habitats stone (H), macrophyte (M) and sand/sediment (S).

	PL0H	*PL0M*	*PL0S*	*PL5H*	*PL5M*	*PL5S*
Number of taxa	22.8 (4.8)	33.0 (13.3)	33.7 (16.5)	27.0 (7.6)	41.9 (16.9)	39.5 (18.7)
Shannon diversity	1.0 (0.1)	1.1 (0.1)	1.0 (0.2)	1.1 (0.1)	1.1 (0.2)	1.0 (0.3)
Eveness	0.8 (0.1)	0.8 (0.0)	0.7 (0.1)	0.7 (0.1)	0.7 (0.1)	0.7 (0.1)

3.2 Similarity

Similarity between the replicates

For the purpose of comparing replicate samples, the Bray-Curtis similarity between replicate samples was determined for each STAR partner at both sampling sites and for all three habitats. Variation between replicate samples can be seen as the background variation that is inherent when sampling a site. Furthermore, the variation also includes the error introduced during processing and analysing samples in the laboratory. The results showed that similarity between replicates was generally high (between 60 and 80%), indicating that replicate samples had been collected and analysed consistently by each partner.

Similarity between partners

In order to compare samples between partners, similarity between average samples, based on all three replicates, was determined. Comparisons were made within sites and habitats only (Figure 2). The similarity between stone samples was about 40 % at both sites. At site PL0 the similarity was generally higher for macrophyte and sand samples (around 60%). This was not the case at site PL5 were similarities between partners were around 40% for all habitats.

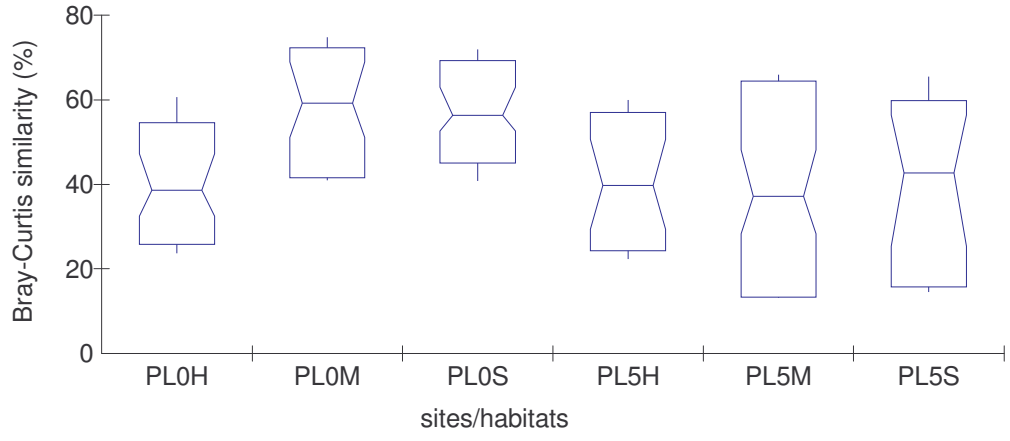

Figure 2 Spread about the median of the Bray-Curtis similarity values between samples from different partners at sites PL0 and PL5 from habitats stone (H), macrophyte (M) and sand/sediment (S).

3.3 Diatom indices

The diatom indices computed for all samples were standardised to a scale between 1 (bad conditions) and 20 (very good conditions). Although the scales are standardised, class

boundaries (between good and bad conditions) are different for each index system hence the differences between the mean value for each index. Also, each system uses its own set of taxa for which indicator values are known. It is therefore not relevant to compare the absolute values of the indices. It is interesting though to compare the variation within each index system between replicates, habitats and partners. This is done in the following paragraph.

Table 4 Mean index values (standard deviation) of samples at sites PL0 and PL5 from habitats H (stone), M (macrophyte) and S (sand). For explanation of the codes used for the index systems see Table 1.

	PL0H	*PL0M*	*PL0S*	*PL5H*	*PL5M*	*PL5S*
IPS	17.2	16.0	15.6	16.7	15.0	15.0
	(1.3)	(0.7)	(0.9)	(0.9)	(1.1)	(1.7)
SLAD	13.5	13.7	11.5	13.3	12.6	12.1
	(1.4)	(0.8)	(1.1)	(1.6)	(0.6)	(2.8)
DESCY	17.1	16.3	16.8	14.8	14.2	14.4
	(1.2)	(0.9)	(1.1)	(1.1)	(1.1)	(1.1)
L&M	11.7	12.4	10.3	11.3	11.0	9.5
	(1.4)	(0.6)	(0.8)	(1.5)	(1.1)	(0.7)
SHE	13.2	12.5	10.9	13.9	14.1	12.6
	(2.0)	(1.1)	(1.0)	(1.1)	(1.7)	(2.0)
WAT	16.5	15.8	13.8	16.4	14.7	13.5
	(2.0)	(1.4)	(1.1)	(1.2)	(1.4)	(1.1)
TDI	13.5	12.0	14.8	12.9	11.6	12.8
	(1.3)	(0.9)	(1.9)	(1.4)	(3.0)	(2.6)
EPI-D	9.8	10.6	8.5	10.9	9.6	9.3
	(2.3)	(1.4)	(1.0)	(2.1)	(1.0)	(3.7)
ROTT	13.8	13.2	12.6	14.9	14.7	14.4
	(1.4)	(0.9)	(0.8)	(1.5)	(1.7)	(2.1)
IDG	15.1	14.5	13.8	15.6	14.5	14.0
	(0.8)	(0.5)	(0.6)	(0.6)	(0.7)	(0.6)
CEE	15.0	13.6	13.2	14.6	13.3	12.7
	(1.7)	(0.8)	(1.1)	(1.2)	(0.8)	(0.5)
IBD	17.4	17.0	16.3	16.6	14.2	14.9
	(2.1)	(0.6)	(1.1)	(1.1)	(2.5)	(2.3)
IDAP	11.9	11.0	11.2	11.7	11.7	11.7
	(1.1)	(0.6)	(0.4)	(1.0)	(0.9)	(1.1)

The variation between index values was assessed between partners and replicates for each index system. The variation between partners gives an indication about the overall differences in sampling, processing, diatom identification and counting. The variation between replicates indicates the consistency in sampling and analyses of replicate diatom samples.

The estimated variance components between partners (Figure 3) showed that the variation between index results differs depending on the habitat that was used to compute the index. The IPS results for example showed relative little variation based on samples collected at PL0 Sand (• in Figure 3) and more variation when samples from PL5 Sand were used for index computation (+ in Figure 3). The lowest variation was achieved for all habitats when using the IDG index system. This system uses autecological information on genus level. Since the variation of this index was low, it appeared that most partners had, in most cases, identified the diatom taxa to the same genera and with comparable relative abundance. The variation of the other index values was much higher, suggesting that the discrepancies arose mostly at species level.

The estimated variance components between replicates (Figure 4) were generally lower than between partners (Figure 3). This indicates that the participants had sampled and

analysed the replicate samples in a consistent way and that differences between partners were more important than between replicates.

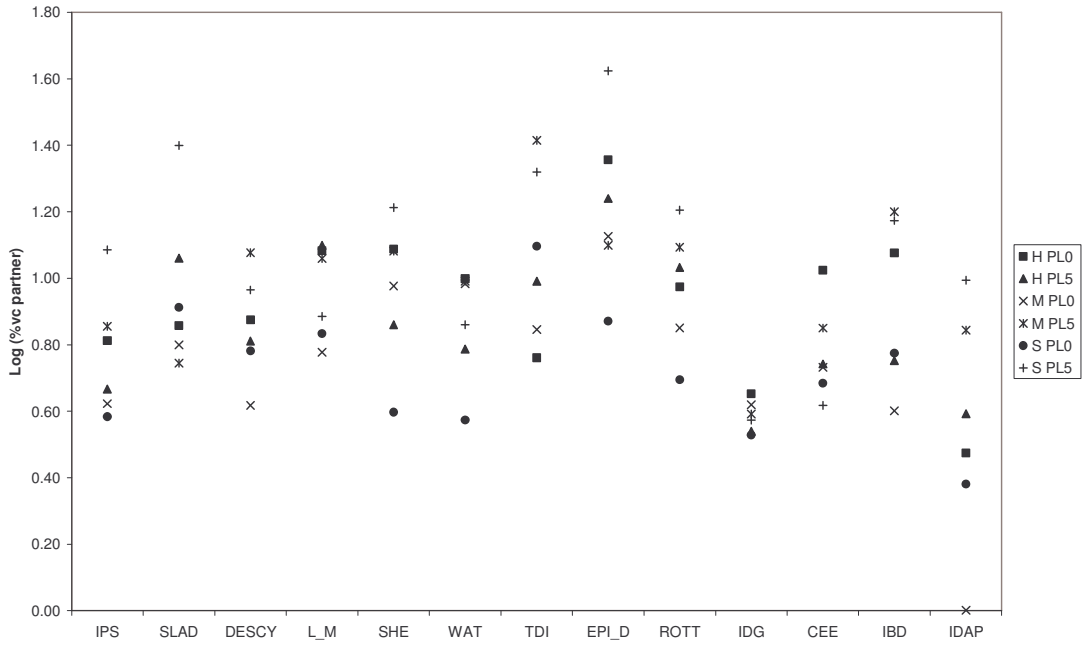

Figure 3 Estimated variance components between partners based on index values of Stone (H), Macrophyte (M) and Sand (S) samples from sites PL0 and PL5 in the Plaine River. For explanation of the codes used for the index systems see Table 1.

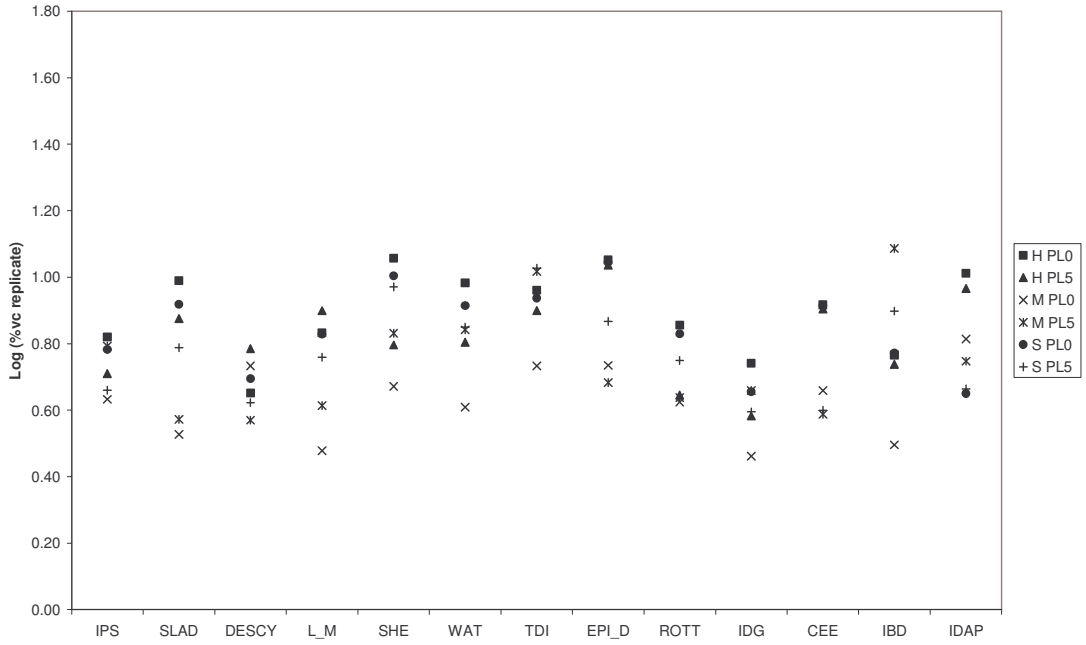

Figure 4 Estimated variance components between replicate samples based on index values of Stone (H), Macrophyte (M) and Sand (S) samples from sites PL0 and PL5 in the Plaine River. For explanation of the codes used for the index systems see Table 1.

A chi-squared test was done to determine if the variation in index values was consistently larger or smaller depending on the sampled habitat (stone, macrophyte or sand, Table 5).

Test results showed that for the indices IPS, DESCY, TDI, ROTT, IDG and IBD the variation was similar regardless of the habitat of sampling. For the other indices there are indications that habitat type affected the variation in index results.

Table 5 Summary of chi-squared test of significance between variation of index values based on different habitats. df = degrees of freedom. p = significance with which the null hypotheses (no differences between variation) is rejected.

index	X^2	*df*	*p*
IPS	2.3	2	0.31
SLAD	27.0	2	0.00
DESCY	0.9	2	0.64
L_M	18.0	2	0.00
SHE	6.3	2	0.04
WAT	7.2	2	0.03
TDI	2.4	2	0.30
EPI_D	18.8	2	0.00
ROTT	4.9	2	0.09
IDG	3.1	2	0.21
CEE	18.1	2	0.00
IBD	3.6	2	0.17
IDAP	23.5	2	0.00

3.4 Comparing sampling results with a standard

Bray-Curtis similarity

At both sampling sites 'standard samples' were collected from all three habitats and analysed by Alterra. These were considered as the audit samples. The similarity between the audit and the primary samples (Figure 5) was generally between 30 and 50% and differed depending on the sampling site and habitat. Variation in similarity was highest for the samples collected from sand habitats and lowest for samples collected from macrophytes.

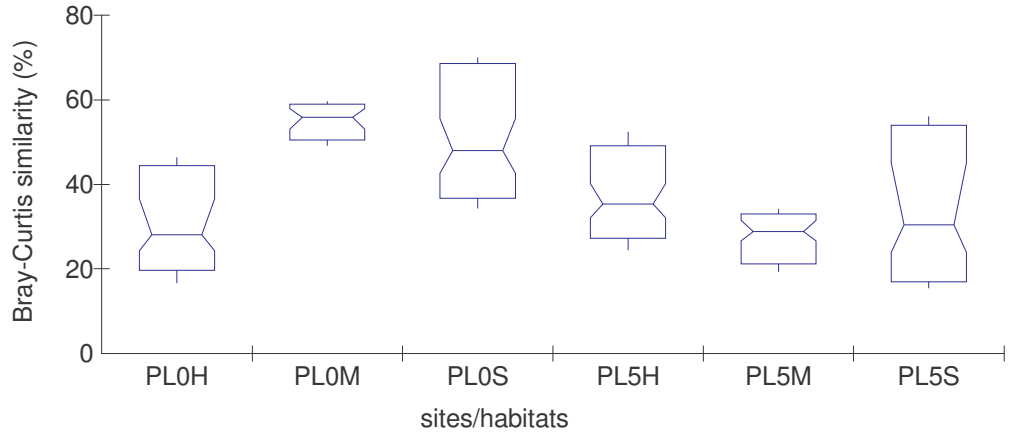

Figure 5 Bray-Curtis similarity between audit and primary samples at sites PL0 and PL5 from habitats H (stone), M (macrophyte) and S (sand).

Correlation of diatom indices between audit and primary samples

The correlation between the index values computed from primary and audit counts is shown for a selection of the indices (Figure 6). The IPS, TDI and IBD are among the

most widely used index systems. The IDG and EPI-D were chosen because these showed a respectively low and a high variation between partners (see Figure 3).

Figure 6 shows that the correlation between audit and primary samples is weak for most indices. The IDG index performs relatively well, but this index is based on genus level and therefore is not likely to provide enough separation between ecological classes to be a useful index. The IBD, IPS and TDI show a large spread and no significant correlation. This indicates that the low similarity between audit and primary samples (Figure 5) result in a large variation of index values. How much variation is acceptable depends on the index system that will be used for river classification. As yet there are no guidelines as to the magnitude of variation being acceptable.

3.5 Possible misidentified taxa

The overall list of taxa that was identified by the partners (see Appendix) was checked for possible misidentified taxa, using the following criteria:

- Select the taxa with a relative abundance >= 2% and are found in at least two samples.
- Select the taxa that have a similar count in samples from the same habitat and site but are named differently.
- Check these taxa in combination with knowledge on the appearance of these taxa whether a misidentification could be the cause of this similar count.

These taxa were discussed during a diatom identification workshop held on 22 and 23 May 2003 in Wageningen, The Netherlands. During this workshop, participants were able to clarify some identification problems. It became clear that in future feedback will remain important to deal with problematic taxa. Quality control of identifications can be done by distributing diatom slides between laboratories (ring tests). Presenting the results of these ring tests and providing feedback on the identifications will further raise the level accuracy of diatom identification.

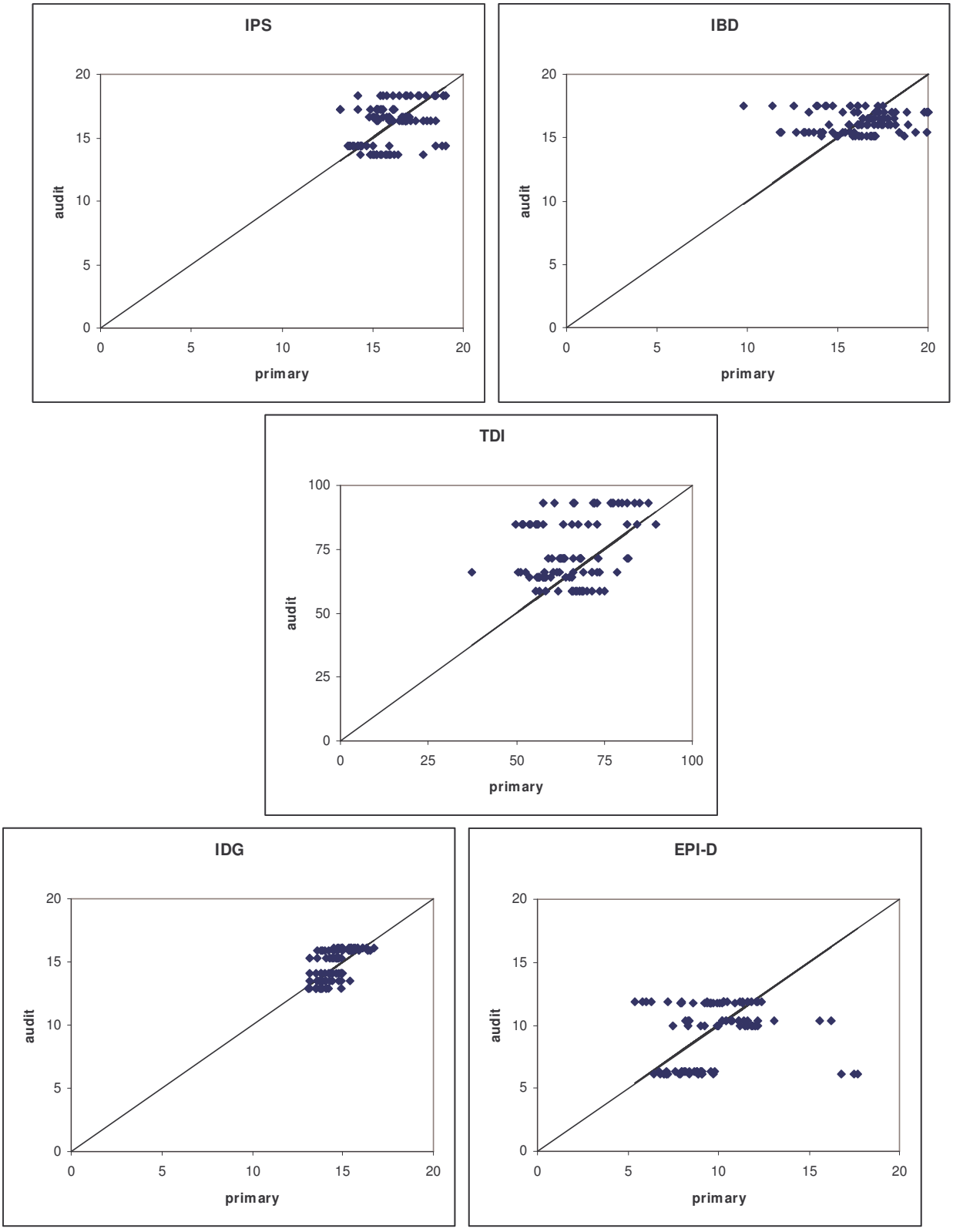

Figure 6 Correlation of diatom indices between primary and audit analyses for 110 samples from sites PL0 and PL5 from the La Plaine River. In the theoretical case that both primary and audit values are the same, the points would be plotted on the 1:1 line shown in each figure.

4 Conclusions & Discussion

Comparing replicates and partners

The variation in index values between replicates was smaller than between partners. This indicates that each partner collected, identified and counted the samples consistently (be it good or bad) but that partners did not produce comparable end results. The total variation that is observed between partners is made up of the combined errors that are introduced in the steps of sample collection, treatment and analysis plus the variation as a result of different identifications and possible different interpretation of the sampling and analysis protocol. The design of this project did not allow for estimation of the contribution to the variation of each of these sources.

The list of possible misidentified taxa consists of taxa that are often confused by diatom analysts. It is therefore likely that misidentification of taxa contributed to a large extent to the difference between the index values. Another study by Prygiel et al. (2002) confirms this theory. Kelly (1997) also notes that errors introduced by slide preparation are not likely to have a significant effect on the difference between replicate samples.

When index values were computed for IPS, DESCY, TDI, ROTT, IDG or IBD the variation in the outcome was similar regardless of the habitat. This indicates that there is no preferred habitat for applying these indices, although indices based on different habitats can not be compared without scrutiny. It does indicate however that when a habitat is sampled consistently in a river system or over time, these indices can be used to study temporal or spatial trends in the ecological status based on benthic diatom communities.

Comparison with standard samples

Samples from partners and auditor were compared by means of a similarity analysis (Bray-Curtis) and by comparing diatom index results. The first method showed that the highest similarity was around 60 % for macrophyte samples on one of the sites, but other sites and habitats showed much lower similarity (around 30%). As the similarity between replicate samples was around 70%, this can be seen as the maximum achievable similarity values between primary and audit samples using the Bray Curtis method.

The index results based on primary and audit samples did not show a significant correlation. This means that taxa with known indicator values are an important factor in determining the differences. It can be expected that this relationship improves after misidentifications are clarified and special emphasis should be place on the identification of indicative taxa.

During the audit of STAR samples, both methods of comparing primary and audit samples will be used. The variation that will be found during the audit, however, is of a different nature than what is shown on the basis of the La Bresse samples, because the audit only considers variance introduced by identification and counting. This report indicates the extent of the total variation that can be expected when different partners collect, process and analyse benthic diatom samples. It is expected that the variation between primary and audit samples during the audit (with less sources of error) will be lower than what is found in this report.

Is the observed variation acceptable?

When different analysts identify and count the same sample, there will always be variation in the results. A standard sampling protocol, a protocol for making slides and microscope procedures and a standard taxon list and experience of the operators using these standards will decrease the amount of variation. However, some variation will always remain. The degree of variation that is acceptable depends on the kind of metrics that are generated. There are no guidelines yet for an acceptable amount of variation. Diatoms (and other group of organisms) are used as indicators of the ecological classes that are defined by the WFD. It still needs to be determined in which way raw end results are going to be processed to determine the ecological classes (which metrics will be used). In fact, these metrics could be different for each country. These metrics determine however, the amount of variation that is acceptable without loosing the capacity to distinguish between ecological classes. Based on the metrics that were used in this exercise (the diatom indices) it can be stated that the variation between the partners was generally too large to be able to classify the sampled sites in a consistent manner. The variation can only be lowered by standardising methods (such as the protocols used in STAR or proposed by CEN) and, to minimize the error in identification, periodic ring tests followed by coherent and frequent feedback.

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Appendix

List of diatom taxa found in the samples collected from sites PL0 and PL5 in the Plaine River, France. Only those taxa recorded at least twice and with an abundance >= 2% are listed. The four letter code is used by the OMNIDIA programme (Lecointe *et al.*, 2003). The taxonomic comments were made during the diatom workshop in May 2003.

Code	*Name*	*Number of occurences*	*Mean*	*std*	*Comment*
ADET	*Achnanthes detha*	2	1.3	0.0	
ACHN	*ACHNANTHES* SPEC	18	0.7	0.4	
ADBI	*Achnanthidium biasolettianum*	22	2.3	2.9	possible mix up with ADSU
ADMI	*Achnanthidium minutissimum*	99	7.5	9.1	
ADSU	*Achnanthidium subatomus*	23	2.4	5.3	possible mix up with ADBI
AFOR	*Asterionella formosa*	36	0.9	1.0	
AUGR	*Aulacoseira granulata*	3	0.6	0.5	
CPLA	*Cocconeis placentula* var. *placentula*	40	0.6	0.6	
CCST	*CYCLOSTEPHANOS* SPEC	6	1.1	0.7	
CYCL	*CYCLOTELLA* SPEC	15	0.9	1.0	
CSPW	*Cyclotella stelligera* var. *pseudostelligera*	6	0.6	0.3	
DEHR	*Diatoma ehrenbergii*	3	15.9	7.0	
DHIE	*Diatoma hyemalis* var. *hyemalis*	6	41.0	15.3	
DMES	*Diatoma mesodon*	74	5.6	4.8	
DPRO	*Diatoma problematica*	19	20.4	11.0	w= 5-7, possible mix up with DITE
DITE	*Diatoma tenuis*	41	12.4	13.3	w = 3-5, long, possible mix up with DPRO
ECAE	*Encyonema caespitosum*	12	1.9	1.1	
EELG	*Encyonema elginense*	2	1.5	0.9	
ENMI	*Encyonema minutum*	108	7.4	5.7	small, dense striae. Possible mix up with ESLE
ESLE	*Encyonema silesiacum*	74	4.4	5.2	larger. Possible mix up with ENMI
EAQL	*Encyonopsis aequalis*	2	1.3	1.4	
EOMI	*Eolimna minima*	51	1.2	1.3	
FSAP	*Fistulifera saprophila*	30	8.6	7.2	
FARC	*Fragilaria arcus* var. *arcus*	49	2.4	1.7	
FCAP	*Fragilaria capucina* var. *capucina*	48	4.6	5.1	There are taxonomists that motivate to group FCAP and FCVA into one taxon. Possible mix up can occur.
FCRP	*Fragilaria capucina* var. *rumpens*	37	1.1	0.9	
FCVA	*Fragilaria capucina* var. *vaucheriae*	84	9.5	8.5	There are taxonomists that motivate to group FCAP and FCVA into one taxon. Possible mix up can occur.
FCRO	*Fragilaria crotonensis*	51	2.5	2.6	
FGRA	*Fragilaria gracilis*	23	2.0	1.3	
FRAG	*FRAGILARIA* SPEC	7	0.9	0.5	
GANG	*Gomphonema angustatum*	13	0.9	0.9	
GCLF	*Gomphonema calcifugum*	17	3.2	3.4	possible mix up with GOOL
GCLE	*Gomphonema clevei*	5	7.6	7.4	
GOOL	*Gomphonema olivaceum* var. *olivaceoides*	68	7.0	9.9	possible mix up with GCLF
GOLI	*Gomphonema olivaceum* var. *olivaceum*	6	1.3	1.4	
GPAR	*Gomphonema parvulum* var. *parvulum*	102	2.4	1.9	
MAAT	*Mayamaea atomus*	8	1.9	1.9	
MAPE	*Mayamaea atomus* var. *permitis*	29	2.9	5.5	
MVAR	*Melosira varians*	93	6.5	7.8	
MCIR	*Meridion circulare* var. *circulare*	72	2.9	2.7	
NCTE	*Navicula cryptotenella*	15	1.6	2.3	
NGRE	*Navicula gregaria*	109	16.4	16.5	
NLAN	*Navicula lanceolata*	107	1.4	1.0	

Code	*Name*	*Number of occurences*	*Mean*	*std*	*Comment*
NMEN	*Navicula menisculus* var. *menisculus*	16	0.7	0.6	
NVIR	*Navicula viridula*	2	1.3	1.3	
NACI	*Nitzschia acicularis*	35	0.6	0.6	
NDIS	*Nitzschia dissipata* var. *dissipata*	107	2.2	1.8	
NDME	*Nitzschia dissipata* var. *media*	9	0.6	0.4	
NINC	*Nitzschia inconspicua*	39	0.9	1.0	
NZLT	*Nitzschia linearis* var. *tenuis*	6	0.6	0.4	
NPAL	*Nitzschia palea*	46	0.8	0.6	
NPAD	*Nitzschia palea* var. *debilis*	2	0.6	0.0	
NPAE	*Nitzschia paleacea*	22	0.9	0.7	
NITZ	*NITZSCHIA* SPEC	17	0.8	0.6	
NZSU	*Nitzschia supralitorea*	3	0.7	0.8	
NTUB	*Nitzschia tubicola*	31	0.8	0.7	
PPRO	*Parlibellus protracta*	24	0.7	0.9	
PCLT	*Placoneis clementis*	25	1.3	2.4	
PDAU	*Planothidium daui*	6	1.7	0.7	
PLFR	*Planothidium frequentissimum*	34	1.2	1.8	Possible mix-up with PTLA
PTLA	*Planothidium lanceolatum*	99	1.8	1.8	Possible mix-up with PLFR. This taxon no horseshoe-like structure in valve
PTPE	*Planothidium peragallii*	2	0.7	0.5	
PGDA	*Psammothidium grischunum* var. *daonensis*	33	0.8	0.7	
PROS	*Psammothidium rossii*	2	0.8	0.7	
PSAT	*Psammothidium subatomoides*	22	0.8	1.9	
RSIN	*Reimeria sinuata*	87	2.3	2.5	
SSVE	*Staurosira venter*	26	0.9	1.0	
SBRE	*Surirella brebissonii* var. *brebissonii*	74	1.2	1.0	Possible mix-up with SBRE
SBKU	*Surirella brebissonii* var. *kuetzingii*	18	1.0	0.6	Possible mix-up with SBKU
SOVI	*Surirella ovalis*	13	2.6	2.6	
UULN	*Ulnaria ulna* (= *Fragilaria ulna*)	65	3.5	4.0	