Standardisation of river classifications:

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

Contract No: EVK1-CT 2001-00089
7th deliverable (Paper version) due 30/11/04,
entitled:

Audit of Performance
Compiled by John Murray-Bligh
Partner No. 11 (Environment Agency, United Kingdom)
incorporating

Results of the La Bresse sampling and analysis workshop
Compiled by Johan van der Molen and Piet Verdonschot
Partner no 4 (Alterra, The Netherlands)
(5th deliverable (Part b), due 31/05/04)

A project under the 5th Framework Programme
Energy, Environment and Sustainable Development
Key Action 1: Sustainable Management and Quality of Water

PART 1

Audit of Performance
Work Package 9

J.A.D. Murray-Bligh (Partner 11, Environment Agency, United Kingdom), R.T. Clarke (Partner 1, CEH Dorset, United Kingdom), P.F.M. Verdonschot (Partner 4, Alterra, Netherlands), J.A. Sinkeldam (Partner 4, Alterra, Netherlands), M.W. van den Hoorn (Partner 4, Alterra, Netherlands), and J.S. van der Molen (Partner 4, Alterra, Netherlands)

Contents

1 Aims and Scope

1.1 This report

Part 1 of this report describes Work Package 9, the Audit of Performance. Part 2 describes the replicate sampling programme for diatoms, undertaken at the La Bresse workshop as a part of Work Package 5.

This part of the report covers the sub-sampling audit for STAR-AQEM samples and audit of laboratory sorting and identification of diatom and invertebrate samples. The identification audit of invertebrate samples had not been completed when this version of the report was written, so the results of that audit and the overall effect of sorting and identification errors on invertebrate metrics are not described. However, this report does describe the variance in metric values caused by sub-sampling STAR-AQEM samples together and its contribution to their overall variance between replicate field samples.

STAR Deliverable 8, *Inter-calibration and harmonisation of invertebrate methods*, (Sandin *et al.*, 2005) contains a more comprehensive analysis of (replicate) sampling variance. It also covers other sources of variability in metric values for both the STAR-AQEM method and other 'national' sampling methods.

In this report, the primary analysis is the original analysis by the laboratory that collected the sample. Data from these analyses is called primary data and they are the main survey results. Primary samples (including STAR-AQEM sub-samples) selected for audit are called audit samples. The re-analysis of these samples for the audit is the audit analysis, which produces the audit data. The person analysing a primary sample is a primary analyst and the person analysing an audit sample is an auditor.

1.2 The Audit of Laboratory Performance

The general objectives of the STAR project were to develop, evaluate and demonstrate methods:
- to provide an inter-calibration of European methods for assessing river quality
- to improve the quality control throughout Europe
- to improve the quantification of errors throughout Europe
- to integrate multi-source ecological data
- to describing the complementarity and redundancy of data
- to advise about cost effective monitoring.

There will always be uncertainty and errors in assessments of the ecological status of river sites that are based on biological samples. Most quantitative assessments are based on the values of biological indices (= metrics) derived from the taxonomic composition of the samples. These metrics are intended to measure either specific or general features of the biota and indicate either overall environmental quality or a specific aspect of it. An index or classification of ecological quality is of little value without knowing its degree of uncertainty (Clarke, 2000, Wallin *et al.*, 2003). This is because differences cannot be ascribed to river quality unless they exceed the uncertainty in the data. Uncertainty arises from *every* stage of sampling, sample analysis and data handling. It is caused by both the natural variability of the biota that is used to evaluate river quality

and by human error introduced by the analysts. The sources of error must be identified so that they can be either taken into account when the results are evaluated or steps taken to reduce them in future analyses.

Uncertainty from different sources are additive, so measures to reduce error from any source will improve the precision of the final results and sometimes their accuracy too.

You must take uncertainty into account when determining the best methods to use. Sampling and sub-sampling methods and biotic indices that are prone to considerable variation will provide less reliable estimates of ecological quality ratios and ecological status and have less power and confidence to detect changes in ecological quality. However, methods that provide the most precise results are usually the most time-consuming and therefore expensive. To find the best compromise between speed and precision you must understand uncertainty. You can reduce uncertainty by changing the taxa that are analysed and when, where and how samples are collected, but these are not considered in this report.

Audits can be used to test whether target or "acceptable" quality standards are being met. There were no such standards for STAR, although the results from the STAR audits would help to indicate the practical and achievable magnitude of such standard. However, the Water Framework Directive requires the level of confidence and precision of results provided by monitoring programmes to be given in the River Basin Management Plans (Annexe V, Section 1.3). As with all ecological analyses, it is more important to have moderate errors that have been quantified than to have very small errors but no estimate of their magnitude. The former allows significance of any differences to be determined whereas the latter does not. Monitoring for the Water Framework Directive in most, if not all, member states will be undertaken by environmental protection agencies, not research laboratories. Whoever undertakes the analysis, it is impossible to eliminate all errors from data based on field survey and laboratory analysis.

The aim of this work package was to quantify the sorting and identification errors associated with the laboratory analysis of macro-invertebrate and diatom samples by each partner. For invertebrates, the emphasis was placed on sorting and identification variations. For diatoms, the emphasis was on the identification and counting errors.

Sub-sampling the material collected in the field adds an extra source of variability, so this was also quantified in this work package. Only the STAR-AQEM invertebrate method involved sub-sampling to reduce the time and effort of sorting these large samples.

The effects of sampling variation on the value of biotic indices or metrics used to assess the ecological status of river sites can be estimated by comparing differences between replicate samples. Replicate sample values will vary because of inherent natural spatial heterogeneity in the fauna at a site, in addition to uncertainly caused by sample analysis and data handling.

The STAR project was designed to include extensive replicated sampling and, where relevant, sub-sampling within the main field sampling programme.

As part of the field sampling programme, each participating partner collected invertebrate samples by the STAR-AQEM method and their national method (or RIVPACS where this was the AQEM method) from all sites. They collected these samples in two seasons: spring and either summer or autumn - the actual months varied because of the climatic differences across Europe. To assess invertebrate sampling variability, each partner took a second replicate field sample using each invertebrate sampling method in each sampling season at (usually) six of their sites. The invertebrate replicate sampling programme was reported as a part of Work package 7, *Core Stream-Type Sampling*. Originally it was to have been a part of Work package 6, *Sampling Workshops*. The diatom replicate sampling is described in Part 2 of this report.

2 Methods

2.1 Overview

Instead of collecting and analysing separate audit samples, which is the ideal, samples already analysed were re-analysed for the audit. The exception was the STAR-AQEM sub-sampling audit, for which additional sub-samples were collected and analysed.

Originally, one partner was to act as the auditor for all analyses of a particular taxonomic group. The auditors were to be:
- Partner 1 (UK)   Invertebrate sorting audit except for UK samples
- Partner 2 (D)   Invertebrate sorting audit of UK samples
- Partner 4 (NL)   All diatom samples (this partner did not participate in Work Packages 7 and 8)

Most partners undertook some auditing for the invertebrate identification audit and all partners who collected STAR-AQEM samples also took part in the sub-sampling audit. All partners who collected samples took part in the replicate sampling programme.

The audit was not the only quality assurance scheme for STAR. The training workshops and the written sampling and laboratory protocols were also parts of the quality assurance for STAR. Partners were encouraged to supplement the STAR audits by their own quality assurance schemes, so long as these did not interfere with the STAR audits.

The audit in STAR was not an analytical quality control (AQC) scheme. Audits only measure errors; they do not control them. Larger laboratories may also have AQC procedures to ensure that errors are within "acceptable" limits. AQC was not included in the STAR project.

2.2 Development of the work package and changes to the planned programme

2.2.1 Development and changes to the invertebrate audit

The original plans for the project only included the sorting audit. The STAR-AQEM sub-sampling audit and the identification audit were added to the project after it had started. Each partner absorbed the additional work without additional funds. The

replicate sampling programme (Work Package 6), which complemented the audit, was also an addition that was not included in the original project plans and budget.

The audit of macro-invertebrate samples, as originally planned, was based on the principles and procedures used by the United Kingdom's environmental protection agencies for their ecological river quality monitoring programmes. This was the only audit scheme in Europe (and probably in the world) that was used to provide quantitative error data for comparing ecological quality for environmental management. It had been in use since 1990 and had undergone considerable testing and development under operational conditions. It was based on the re-analysis of a number of randomly selected samples to family. This was the taxonomic level used for operational assessment of river quality in the UK. A single auditing laboratory at CEH Dorset undertook all the audit analyses to ensure comparability of results between the audited laboratories. The same auditors were used for STAR to allow direct comparisons.

Although the operational assessment of river quality in many of the STAR partner countries was based on species-level analysis, we felt that an audit to family would provide sufficient information about error to demonstrate the importance uncertainty caused by analytical errors to river quality assessments. Experience in the UK indicated that most analytical errors were caused by sorting (recognising specimens of animals from detritus collected in the samples) and not by identification and that auditing to species-level was much more time-consuming and therefore expensive.

Plans and budgets in the original project proposal funded by the EU 5th Framework Research Programme were based on an invertebrate audit as outlined above.

The concept of auditing and the use of audit data when comparing ecological data for environmental quality assessments was introduced to partners in the project kick-off meeting at Dorchester in December 2001.

An initial version of the protocol for the audit was completed on 25 April 2002 and it was distributed to all partners on the audit discussion pages of the STAR web site the following day. This developed considerable debate as partners increased their understanding of the objectives of the work package and the procedures that were proposed to meet them. This delayed the agreement of the final protocol considerably.

The fact that the family-level audit did not cover all of the potential errors in the data generated in the project was of most concern. Many partners' national quality assessment methods were based on species analysis, as was the STAR-AQEM procedure that was used by all partners as the standard comparative method for the project.

The British audit method could not be adapted simply by altering the taxonomic level to which it was performed, because it depended on a single laboratory undertaking all the audit analyses. None of the laboratories in the project consortium was able to undertake species-level audit analyses across the whole of Europe. None had the capacity for such a large amount of work and, more importantly, none was sufficiently familiar with identifying species from rivers across the whole of Europe. UK experience had demonstrated the vital importance for the auditing laboratory to undertake audit analysis to a very high quality that was only possible if the auditors were experts in the sorting and identifying the organisms being audited (Dines & Murray-Bligh, 2000).

Furthermore, the cost of undertaking all the auditing at species-level would have been prohibitive to any individual partner, particularly as there was no budget for this additional work.

The invertebrate expert panel considered the protocol for auditing invertebrates during and after the first workshop. The family-level audit did provide a standardised measure of analytical quality for sorting, even though it could not provide a measure of error for all of the invertebrate environmental assessment methods that were likely to be evaluated in the project. We considered whether the family-level sorting audit should be applied only to samples analysed by methods that used a coarse level of taxonomic penetration, such as the British and southern European methods, and another type of audit to samples analysed by methods based on species or genera. A ring-test was also considered as a potentially less expensive approach for auditing species analyses. This was not adopted because it would only have been effective for the small proportion of species that are found in all partners' countries. The distribution of audit samples between sampling methods was also considered, in particular whether audit samples for STAR-AQEM and national methods should be matched. The taxonomic list against which the audit would be standardised was also debated. No decisions were made at this stage.

At the main sampling workshop held in La Bresse in April 2002, it became evident that the sub-sampling of AQEM samples was likely to cause variations that would be missed by the audit. The consortium agreed that this should be considered in the audit and so the STAR-AQEM sub-sampling audit was added to the project. Although there was no budget for the work, the additional cost was accepted because it would be distributed evenly between the partners.

The invertebrate audit was considered at the sub-committee meeting in Vienna in March 2002.

Further discussions took place at the North Europe AQEM sampling workshop held in Denmark in June 2002. CEH Dorset (Partner 1) described the invertebrate audit procedures that had been developed by that time. This was to undertake a family audit and take specimens from this to include in a species audit, and to allow all partners countries to share the additional work that this would involve. The replicate sampling programme was also discussed.

A special meeting was held on 16 August 2002 at CEH Dorset between the co-leaders of the audit work package (John Murray-Bligh from the UK Environment Agency and Mike Furse from CEH Dorset, and Daniel Hering from the University of Duisburg-Essen. The meeting thus included both the co-ordinating institutes; the sorting auditors for most partners (CEH Dorset) and the sorting auditors of the UK samples (University of Duisburg-Essen). Many important decisions were ratified and solutions proposed in this meeting. The most important was to devise a practical way of achieving the objectives of the work package and implementing a species-level audit without compromising the comparability of audit results.

The approach proposed in that meeting was to split the audit into two separate components:

- A sorting audit at family level, undertaken by a single auditing laboratory, to assess sorting errors across the whole project in a consistent and unified way.
- An identification audit undertaken by partners familiar with analysing invertebrates from similar environments. Generally, these were neighbouring countries from the same Ecoregion.

The sorting audit was essentially the audit that was originally planned for the project. The identification audit was completely new and involved additional work for all partners. This work was not included in their original project plans, nor had it been included in project budgets.

The identification auditors for each partner were decided in this meeting.

One way to reduce the impact of the additional identification audit on every partner's workloads was to reduce the number of audit samples from the 20 planned originally. Twenty was the minimum number of samples used for audit in the UK. It was decided that 12 samples from each country would undergo sorting audit: 6 AEQM samples and 6 collected and analysed by their national method. The distribution of these audit samples by quality class was also defined (see Table 1). The same samples would be used for the identification audit, which therefore also comprised 12 samples per country.

This decision involved a substantial compromise. It meant that the results of the audit would be very imprecise – so imprecise that they would be of little value in providing accurate measures of uncertainty and bias in the projects results. However, for the STAR project, the utility of the results was considered less important than testing and demonstrating the need for and potential use of audit data that covered every stage of analysis that could cause error.

The sub-sampling audit for AQEM samples was to be based on the samples collected for the replicate sampling programme. This would result in 12 samples per partner.

The protocol that was defined in this meeting was circulated in notes to all partners for comment and agreement at the workshop in Dorchester in September 2002.

The decision to select all the invertebrate audit samples from the replicate sampling programme was also made at the special meeting in Dorchester in August 2002. The replicate sampling programme was another additional work item that had not been planned or budgeted at the start of the project, but had been agreed by then. The partners were not told of this decision and it was not mentioned in the notes of the meeting that were circulated them. This was because it was important that their analysts did not know which samples would be audited. This would have affected the quality of the analysis of those samples and so have made the audit results unrepresentative of the project as a whole.

Partners agreed to adopt this approach to the invertebrate audit at the main project meeting in Dorchester in September 2002, despite the additional work and cost to all of them. A revised protocol incorporating the changes that were agreed was posted to the audit discussion pages of the project web site on 16 May 2003. This included a draft list of taxa to be included in the sorting audit.

The work-package co-leader from Partner 11 (Environment Agency) gave a presentation summarising the audit procedures at the fourth STAR project meeting in Arcidosso, Italy, in June 2003. This was to ensure that all the partners were familiar with the practical aspects and principles of the audit and to ensure that issues that needed to be resolved were identified and discussed. A discussion session was devoted to the invertebrate audit. The sorting and the identification audits were covered and a number of procedural details were agreed.

For the sorting audit, we agreed that all material that was sorted would be retained. When partners were told which samples are to be audited, the family level data from the project database (AQEMdip) would be sent to the auditors with the sample. The use of hand lenses in audit was discussed.   A number of additional taxa were considered for exclusion from the sorting audit, but the final list was to be agreed by the sorting auditors (CEH Dorset and Duisburg-Essen).

For the identification audit, we agreed that major groups of taxa should be sent to the auditors in separate vials but that individual species did not need to be separated. Some issues could not be resolved within the discussion session, in particular, what results the Data Analysts Sub-Group wanted from the identification audit for the work package to link different invertebrate methods (Work Package 11). This was important because practical problems, in particular the cost and time available for the additional work, were creating pressure to simplify the audit. Some partners wanted to restrict the identification audit to only Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddis-flies), often called the EPT taxa. Identification audit partners had to be chosen to audit samples from the new partners from Latvia, Poland & Slovakia.

We agreed that each partner would make their own courier arrangements for sending their samples to the auditors. A change agreed to the existing protocol was that the sorting auditor would to send specimens directly to the identification auditor, not back to the primary analyst. Primary analysts would have to make their own arrangements if they wanted specimens back from the auditors. A full report of the discussion on the audit at the fourth STAR project meeting was posted to the STAR web pages.

The co-leaders for this work package met at CEH Dorset in December 2003 to discuss the invertebrate audit. It was important that the primary analysts did not know which samples were to be audited before they completed their analyses and it must not be possible for data to be altered once the audit samples are selected. To ensure this, it was agreed that no partner would know which samples were to be selected for audit until all the primary analyses had been completed. At this stage, the audit was well behind schedule because few partners had completed the primary analysis of their macro-invertebrate samples and entered the results onto the project database, AQEMdip. The macro-invertebrate audit could not start until well into 2004. We realised that the identification audit must encompass every taxon used in partners' national assessment methods, so that the impact of analytical errors on national assessment methods could be evaluated in Work Package 11 on linking invertebrate methods.

Further details of the invertebrate audit were agreed in a breakout session of the project meeting in Lednice, Czech Republic in March 2004. Named quality controllers were agreed for every partner.

A revised version of the audit protocol was posted on the audit discussion pages of the project web site in May 2004 to reflect the changes agreed in Lednice.

A sorting audit results sheet was devised in Excel by the lead sorting auditor at CEH Dorset (Rick Gunn) to help the sorting auditors and to ensure that the results were recorded correctly (Appendix D).

Rick Gunn (CEH Dorset) devised a similar results sheet in consultation with John Murray-Bligh (Environment Agency) for the identification audit. Again, its purpose was to help the auditors from each partner to record their results correctly. An annotated example and a blank were sent to all partners' audit controllers in August 2004, to help their identification auditors (Appendix E and F).

Section 2.3 is a description of the methods that were finally adopted, including all the changes and adjustments made during the project.

Six new partners, from three of the Newly Associated States (Latvia, Poland and the Slovak Republic) joined the STAR project in its second year and their work was fully integrated into the invertebrate audit.

The audit of performance was originally planned to start in Month 7 and end in Month 21, with this report following 3 months later. Despite the considerable increase in the scope of the invertebrate audit from that originally planned, and its extension to include the partners that joined the project in its second year, there was no extension to the delivery date of this report.

2.2.2 Development and changes to the diatom audit

The concept of using audit data when comparing ecological data for environmental quality assessments was introduced to partners in the project kick-off meeting in Dorchester in December 2001.

When the project started, none of the regulatory agencies in any of the partner countries had adopted a standard procedure for auditing benthic diatom analyses, although a number of them were developing such methods. The *Comité Européen de Normalisation* (European Committee for Standardisation, CEN) had only just started to consider developing a standard for auditing diatoms. The methods for auditing diatoms in this the project therefore had to be designed from scratch. Alterra (Partner 4) led this development, which was responsible for undertaking the diatom audit, with help of the algal expert panel.

The protocol for the audit of benthic diatoms drawn up by Alterra was accepted by the partners during the first reporting period. It was placed on the public pages of the project web site as part of the field and laboratory protocol for this group.

The diatom audit procedures were discussed in the project workshop in La Bresse in April 2002.

The original plans for the diatom audit did not include the Polish and Slovak partners, who joined the project in its second year. However, their diatom slides were included in the audit.

Johan van der Molen from Alterra presented the preliminary results of the diatom ring test undertaken during the first sampling workshop in La Bresse at the diatom identification course in Wageningen in May 2002.

In addition, Bowburn Consultants (sub-contracted to Partner 11) audited some STAR diatom samples analysed by CEH Dorset. However, this was not a part of the STAR audit but was part of the training for CEH diatom analysts, who were following the training and audit system used operationally by the Environment Agency for its statutory work

2.3 Invertebrate audit detailed method

2.3.1   Scope

The invertebrate audit for STAR was based on practical methods that had been used operationally in the UK for the last 13 years, modified to take account of the analytical methods used by other project partners and EU Member States, in particular species-level analysis.

There were three different audits for invertebrate samples in STAR project:
1. **Sub-sampling audit** – for STAR-AQEM samples only
2. **Sorting audit** – to determine error caused by sorting
3. **Identification audit** – to determine error caused by misidentification

The objective of the sub-sampling audit was to estimate the variation caused by the sub-sampling procedures used in the STAR-AQEM protocol (AQEM Consortium, 2003). It complemented the investigation undertaken by Partner 4 (Alterra, NL) in Work Package 16 (Effectiveness and relative cost-efficiency of different field and laboratory protocols for macro-invertebrate samples).

Estimates or counts of the abundance of each taxon were not audited because there was insufficient time and budget. Auditing abundance data would have taken considerably more effort than was available to the auditors (particularly the sorting auditors). Also, it would not have been possible to undertake the identification and sorting audits in parallel, so a further extension to the project end date would have been necessary. However, variation in abundance data was estimated in the replicate-sampling programme.

Two parameters were used to measure analytical quality in these audits: **the number of gains** (taxa that were not recorded as being present in the sample but which the auditors found in the sample) and **the number of losses** (taxa that are recorded as being present but which were not found in the sample by the auditor).

Gains and losses were expressed as averages for all samples analysed by a particular partner, based on comparisons of the primary and auditor's results for all the audit samples for that partner. The precision of these statistics depended on the number of audit samples on which they were based and were independent of the total number of

samples analysed by the laboratory being audited. This is why the audits were based on a fixed number of audit samples from each partner and not a proportion of the total number of primary samples that they analysed.

Ideally, the auditor should be a competent laboratory, highly experienced in the analysis being audited and in which they are known to make very few errors. They should be completely independent of the laboratories being audited and have no interest in the results of the audit. The same audit laboratory should do all auditing of a particular analysis to ensure that the precision of the audit is consistent and therefore the results of the whole audit are all directly comparable.

Unfortunately, no laboratory had sufficient experience in analysing all the species found in the geographic area covered by the project to undertake the whole identification audit. Because of this, each partner's identification audit was done by one or more of its neighbours. Although this caused the quality of the identification audit to vary between partners, it ensured that it was more accurate. Just as importantly, no partner was over-burdened by extra work: the identification audit was not included in the original project plans.

Ring-tests to ensure competencies in identification were encouraged but were not a formal part of the audit.

2.3.2 Personnel

All those analysing samples for STAR were trained in the additional procedures needed for the audit (Section 2.3.4.2). They had read and understood all the instructions in this document.

Every laboratory appointed a quality controller who was responsible for the administration of the invertebrate audits. They did not need to be a biologist. They were responsible for sending the audit samples to the auditors and co-ordinating the results.

There was no need for individual analysts to be identified in the STAR audits, because the audits were not used for analytical quality control.

2.3.3 Samples subject to audit

It was important that primary analysts were aware that *all* invertebrate samples collected for STAR were subject to audit by having an equal chance of being selected as an audit sample. This included all replicate samples and replicate sub-samples. This ensured that they did not give any special attention to any particular samples so that the audit results reflected the quality of all the primary analyses. In an operational audit, this is very important, because different types of samples or samples analysed at different times may be subject to different quality of primary analysis. This was achieved by selecting audit samples randomly, but forcing the time component so that roughly equal numbers of spring and autumn samples were selected as audit samples.

The single exception was samples collected by the additional live-sorting methods adopted by Italy in addition to their main AQEM and national methods. These were not included in the audit.

A compromise was made in this project. Without the primary analysts' knowledge, the audit samples were not selected randomly but were selected from the samples included in the random sampling exercise. This was to allow the results of the audit to be analysed with data from the replicate sampling programme so that uncertainty from different sources could be partitioned.

To regain a measure of comparability, given the reduction in the number of samples to be audited, the audit samples were selected roughly evenly between seasons and included samples representing high, good and moderate ecological quality. For each combination of site and season, one sample collected by the STAR-AQEM protocol and one sample collected by the national survey protocol was chosen for audit.

The only parts of STAR-AQEM samples included in the audit were the sub-samples analysed for STAR. The rest of each STAR-AQEM sample, which some partners may have analysed for their own purposes, were not included in the audit.

Partners were not told which samples or sub-samples had been selected for audit until all of them had entered their primary data onto the project database, AQEMdip. Primary data could not be altered after the audit samples had been selected.

2.3.4 Procedures common to all invertebrate audits
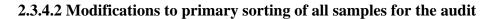
2.3.4.1 Taxonomic coverage of the audits

Only specimens and taxa that contributed to the primary results were included in the audit. Larval and pupal exuviae, empty caddis cases, and empty mollusc shells were excluded and the auditors ignored any in the audit samples. Specimens present only as posterior ends or as shells were excluded. For insects, a thorax plus abdomen was acceptable, but an abdomen alone was not. Pupae of Diptera and Trichoptera were included because they contributed to the primary results. Only species that live in rivers at some stage of their life cycle were included.

The mainly terrestrial families of beetles Chrysomelidae, Clambidae and Curculionidae have only a small number of aquatic species that are not easily identified were excluded. This was because it is difficult to differentiate the freshwater from the terrestrial species, with the result that a most records were likely to be wrong. This had been discovered already in the UK audit. These taxa do not contribute significantly to evaluations of environmental quality. Their exclusion followed the current British practice.

Families of Oligochaeta were not differentiated in the sorting audit because their identification often involved mounting them on temporary microscope slides which could not be transported to the auditors easily.

Some large and protected species were excluded because they were not removed from the field and were therefore not available for audit. These included pearl mussel *Margritifera margaritifera*, the medicinal leech *(Hirudo medicinalis)* and the white-clawed crayfish *Austropotamobius pallipes*.

2.3.4.2 Modifications to primary sorting of all samples for the audit

After sorting every sample or sub-sample, the primary analyst retained the following material for auditing:

- All specimens removed from the sample or sub-sample in one or more clearly labelled vials.
- All organic and inorganic material from the sorted national sample or STAR-AQEM sub-sample, together with any animals remaining in it. They threw way large leaves, large stones and twigs, but kept *all* other material. They kept material that had been washed in any sieves. They put this material into a clean storage jar with preservative and labelled it (see Section 2.3.4.3).

The sorting auditors re-sorted this material to check that the primary analyst had recorded (and in the case of AQEM samples, had removed) all the invertebrates.

It was important that no material from any samples or sub-samples was thrown-away until after the audit was fully completed.

The only material collected in the field that was not kept was the unsorted material that was not part of the STAR-AQEM sub-sample. This was the material in the cells of the sub-sampling device that had not been used to obtain the 700+ specimens. The effect of sub-sampling was investigated in the sub-sampling audit.

For STAR-AQEM samples, the primary analyst had to remove all the specimens from the sample. After analysis, these specimens were returned to a labelled vial or jar containing preservative and stored for the identification audit (see AQEM Consortium, 2003).

For other types of sample with protocols that did not demand that all specimens were removed during sorting, the primary analysts had to remove representatives (but not every specimen) of every taxon for the identification audit. The primary analysts removed up to three specimens of every invertebrate taxon. The taxa were based on the taxonomic level of the primary analysis: if the identification was to family, the taxa removed were families; if the sample was analysed to species, the taxa were species. The specimens removed had to be good quality examples and not simply the first ones that the analysts found in the sample. The analysts were recommended to remove *at least three* specimens of every flatworm taxon whenever possible, and preferably because they were so easily damaged. The primary analysts removed representatives of every aquatic life stage of every taxon.

Some protected species were not kept, but were returned to the river during sampling. These were the pearl mussel *Margritifera margaritifera* (Margaritiferidae), the medicinal leeches *(Hirudo medicinalis)* and the white-clawed crayfish *Austropotamobius pallipes* (Astacidae). These taxa were excluded from all invertebrate audits.

So long as they did not destroy any of the sample or labels, the primary analysts were allowed undertake any further analyses of the sample that they wanted to. However, they had to complete all analysis of the sample before indicating that data entry on the STAR database was complete and the samples were selected for audit. Once an audit sample

had been selected, no further analysis of any samples was allowed until the results of the audit were returned.

Once a sample or sub-sample had been selected for audit, the primary analyst was not allowed to re-analyse it in any way.

2.3.4.3 Preserving and labelling material for the audit

All the invertebrate samples had to be fixed and preserved so that they were in good condition for the auditors to re-analyse them.

Primary analysts had to follow their own laboratory's safe system of work for using formalin and alcohol. Some laboratories had special procedures for using formalin and alcohol and some did not allow formalin to be used. Primary analysts who could not use formalin had to use alcohol preservative alone, even though alcohol is an inadequate fixative.

For formaldehyde fixative to be effective, it had to be added to the live sample.

Samples were sent to the sorting auditors and identification auditors in 70% alcohol preservative, not in formalin. If the sample had been analysed in water, alcohol more concentrated than 70% had to be added to them in order to ensure that the final concentration was 70%.

Samples in industrial methylated spirit (IMS) are flammable and harmful. The outside of every container had to be labelled with the appropriate warning labels (see Murray-Bligh, 2003).

The primary analysts also put a non-sticky label inside every vial or container indicating:
>Partner number
>Partner name
>Sample collection date
>Sample analysis date
>Type of sample
>>(AQEM or national method, main or replicate sample)
>River name
>Site name

The primary analysts labelled the outside of containers in the same way, with alcohol-proof permanent ink. They could label lids, but if they did, they had to label the container too.

If primary analysts used the same type of container for storing specimens removed from the sample for the identification audit and the remaining material for the sorting audit, they marked which type of audit the contents were for.

If primary analysts used more than one container for the same sample, they labelled them as being container number
>'x' of 'n' containers

on the outside and on the labels inside the containers.

2.3.4.4 Primary results used for the audit

The primary results had to be entered onto the STAR database before the audit samples were selected. The results on the database could not be changed after the audit samples had been selected. The auditors took the primary results of audit samples from the project database as soon as they had been selected and these results were used to determine the gains and losses.

2.2.4.5 Selection of audit samples

Only STAR-AQEM samples were audited for sub-sampling variation. The sub-sampling audit used the STAR-AQEM samples collected in the replicate sampling programme. The primary analysts took replicate sub-samples from all the replicate STAR-AQEM samples from all sites in both seasons. This usually resulted in 12 extra STAR-AQEM sub-samples for each partner to analyse.

The Environment Agency (Partner 11) selected the audit samples for the sorting and identification audits. They were a co-leader for this work package but were not involved in the analysis of any samples. They did this for each partner after the primary analysts had entered all its primary results on the project database. The Environment Agency then told each partner's quality controller which samples or sub-samples to send to the auditors. The primary results did not influence the choice of audit samples.

Selecting audit samples after all the primary data had been entered onto the project database ensured that the primary analysts had no way of knowing which of their samples were to be audited whilst they were analysing the samples. It also ensured that the primary results on the STAR database could no be altered after the audit samples had been selected. Not only did the audit have to be fair; it had to be seen to be fair.

Both the sorting and identification audits were based on the same audit samples. There were six STAR-AQEM audit samples and six national method audit samples from each country. Primary analysts were aware of this from early in the project.

The sorting and identification audit samples were stratified across the whole project, to include a wide range of combinations of stream type and environmental quality. The audit samples were roughly stratified by season. Primary analysts were aware of this early in the project. National method audit samples matched the STAR-AQEM audit samples in site and season.

For each country, the sorting and identification audit samples covered:
>   3 x quality classes (moderate, good, high) where possible
>   2 x stream types
>   2 x methods (STAR-AQEM and national assessment)

Table 1 Distribution of numbers of invertebrate audit samples for each partner, as originally planned. Some partners were to audit core stream type 2 samples instead of samples from the additional stream type, and those sampling from only one stream type planned to audit 2 samples from each combination of quality class and sampling method.

Stream type	Quality class (anticipated quality based on pre-classification before samples were collected or analysed)	AQEM method samples	national method or RIVPACS samples	Total number of samples subject to audit/partner
core stream type 1	high	1	1	
core stream type 1	good	1	1	
core stream type 1	moderate	1	1	
core stream type 1	poor and bad			
additional stream type	high	1	1	12
additional stream type	good	1	1	
additional stream type	moderate	1	1	
additional stream type	poor and bad			

For countries that surveyed only one stream type, two audit samples or sub-samples were selected for each combination of environmental quality and sampling method. The distribution of audit samples planned for each partner is given in Table 1.

The final distribution of audit samples followed this distribution fairly closely, but there was inevitably some departure from it because the actual quality of sites was not always the quality anticipated.

2.3.5 The STAR-AQEM sub-sampling audit

2.3.5.1 Field and laboratory procedures for the STAR-AQEM sub-sampling audit

This was not an audit in the conventional sense. Instead, each laboratory estimated error variation by analysing a replicate sub-set of STAR-AQEM samples. The sub-sampling audit was therefore be done by each partner and not by specific auditors.

The STAR-AQEM method protocol involved a standardised method for sub-sampling. The sample material was spread out as evenly as possible on a tray marked out with a grid of 6 x 5 cells. The STAR-AQEM protocol required the analyst to select randomly five of the 30 grid cells and identify and count all of the macro-invertebrate specimens in these five cells. If they did not find at least 700 specimens in these five cells, they selected and sorted additional whole cells, one at a time, until they obtained 700 or more specimens. They retained the specimens that they removed for the identification audit

and they retained the rest of the material from the sub-sample for the sorting audit. They recorded the results on the STAR database.

This sub-sampling introduced an additional source of variation in the taxonomic composition, and hence in the values of metrics, recorded for the site. This variation had not been assessed in the AQEM project, which devised the AQEM sampling protocol (since modified to the STAR-AQEM protocol). Variation in taxonomic composition and metric values between two replicate field samples taken from the same site at the same time will be caused by both sampling (because of the spatial variation of animals in the field) and laboratory sub-sampling effects.

To quantify the sub-sampling variation, especially in relation to that caused by field sampling variability, the project partners took a second replicate sub-sample from one of the replicate STAR-AQEM samples for all or most of the sites at which two replicate samples were taken (Table 2). They did this by removing material from a different set of five grid cells (plus any additional cells needed to bring the total number of specimens removed from the sample to 700 or more). They treated the sub-sample in the same way that they would any other STAR-AQEM sample, in case it was selected for sorting and identification audit, and recorded the results as a replicate sub-sample (see Section 2.3.7.4).

Table 2 Number of sites (and their site codes) in each stream type and country for which two sub-samples ('main' and 'replicate') were taken from one sample in at least one season (1=spring, 2=summer, 3=autumn). Site code 'xxx.y' indicates replicate sub-sample only taken in season y at site xxx.

Country	Stream Type	Seasons sampled	n Sites	STAR site codes
Austria	A05	1 + 2	4	600 603 607 609.2
	A06	1 + 2	4	701 702.2 706 708.1
Czech Republic	C04	1 + 2	3	614 620 625
	C05	1 + 2	3	713 717 722
Germany	D03	1 + 2	2	649 659
	D04	1 + 2	2	627 634
	D06	1 + 2	2	816 821
France	F08	1 + 3	6	724 725 726 728 729 733.1
Italy	I05	1 + 2	3	849 855 856
Denmark	K02	1 + 2	6	662 663 665 667 671 673
Poland	O02	1 + 3	6	895 897.3 903 913 916.1 952.1
Portugal	P04	1 + 3	3	859 860 867
Sweden	S05	1 + 3	5	685.1 689.1 691 695.3 697.3
	S06	1 + 3	3	875 876 878
UK	U15	1 + 3	3	639 642 648
	U23	1 + 3	3	674 678 681

All cells not included in the first sub-sample were available for selection as a part of the replicate sub-sample.

The primary analysts did not start work on the second sub-sample until they had completed the first sub-sample. This was in case they had to select extra cells for the first sub-sample to remove at least 700 specimens. They had to keep the whole sample

moist while they worked on the first sub-sample, to prevent the second sub-sample becoming dry.

2.3.5.2 Statistical Methods used to quantify sub-sampling variability

The statistical analysis concentrated on assessing the sub-sampling variation in many of the most commonly used metrics based on macro-invertebrates. Hierarchical nested analysis of variance (ANOVA) techniques were used to estimate the variances in the observed metric values caused by laboratory sub-sampling and caused by replicate field sampling using the STAR-AQEM method. In particular

σ_U^2 = variance between replicate sub-samples within a sample

σ_R^2 = variance between replicate samples within a season within a site.

This approach correctly identified the part of the replicate sample variability that was merely the consequence of sub-sampling from that caused by real differences between the fauna obtained in the two samples. The relative importance (P_{sub}) of sub-sampling effects to sampling effects was assessed and measured by:

$$P_{sub} = 100\sigma_U^2 / (\sigma_U^2 + \sigma_R^2).$$

Because it was only possible to take replicate samples (and STAR-AQEM sub-samples) at a few (3-6) sites in each stream type of each STAR partner, estimates of the above variance components for individual stream types may be imprecise. Therefore, to obtain more robust estimates for a particular sampling method, the variance components (and their relative size) for a particular metric were also derived using all of the sites for which the method was used in a particular country, and also for all sites regardless of country. The variance components were quoted in the tables in their standard deviation (SD) form where SD is the square root of the variance, because SDs are in the same units as the metric values. For, example $SD_U = \sqrt{\sigma_U^2}$ denotes the SD caused by STAR-AQEM sub-sampling. When a SD is based on only two values (x_1 and x_2) then the SD is equal to the absolute value of their difference divided by the square root of two (i.e. $|x_1 - x_2|/\sqrt{2} = 0.71|x_1 - x_2|$).

In ecology, the replicate sampling variability in a biotic index of taxonomic abundance, richness or composition often increases with the value of the index. For example, Clarke *et al.* (2002) found that the variance in the number of macro-invertebrate taxa found in replicate RIVPACS samples increased roughly in proportion to the average number of taxa found in samples from the same site, but that by transforming the data, the replicate variability in the square root of the number of taxa was roughly constant and did not depend on the physical type or ecological quality of the sites.

A similar approach was used for the STAR data-set to determine whether, for a particular sampling method and metric, the sampling and other variances should be analysed and estimated using the metric's un-transformed or transformed values, using either a square root ($\sqrt{x}$) or double square root ($\sqrt{\sqrt{x}}$) transformation. See Sandin *et al.* (2005) for further details. For reasons of consistency and robustness, only one transformation was used for any single metric, regardless of method or stream type.

Many of the metrics selected were percentages (range 0-100) or proportions (0-1), which were based on the fraction of all individuals or of all taxa which were in a particular group or had particular characteristics. The replicate sample values of such metrics tend to be less variable when their values for a site are very low (near zero) or very high (near 100%) and most variable at intermediate values (20-80%). In such cases, the arcsine transformation of the square root of the proportions x (i.e. arcsine $(\sqrt{x})$) is the standard transformation used in statistical analyses to make the sampling variance more equitable (e.g. Sokal & Rohlf, 1995) and this was used for all such metrics in STAR.

2.3.6 The sorting audit

CEH Dorset undertook the sorting audit for all partners, except for their own primary samples, which were audited by the University of Duisburg-Essen.

Partners had to enter the results of all samples onto the STAR database before they were told which samples had been selected as audit samples. It was important that the primary analysts did not know which samples were audited before they analysed them, because this would have affected their primary analysis and make the audit results unrepresentative. Primary analysts are only human!

2.3.6.1 Sending audit samples to the sorting auditors

For STAR-AQEM samples, the quality controllers only sent to the sorting auditors the re-constituted material from the sub-sample that was sorted, i.e. only the substratum material from the cells used to obtain the 700-plus taxa. They did not send the animals removed from the sub-sample because these were used for the identification audit and had to be sent to the identification auditors. They did not send the unsorted fraction of the sample to the sorting auditor either.

For national method samples, the quality controllers sent the material from the whole sample after the primary analyst had sorted it. The sorting auditors needed all the substratum material even if the primary analysts had sorted only a sub-sample of it. They did not send the animals removed from the sample to the sorting auditor because these were used for the identification audit and had to be sent to the identification auditors.

The quality controllers did not have to send the primary results to the auditors because the auditors obtained them from the project database. However, they did send a list of the audit samples that they had sent, so that the auditors knew what to expect in each consignment, in case there was a problem with the labels.

The quality controllers had to pack the samples carefully. The sample containers had to be strong and airtight. They had to be packed securely in strong boxes with plenty of packaging so that they could survive mistreatment. The quality controllers were advised that it should be possible to drop the package without the samples being damaged.

The quality controllers labelled packs of samples in accordance with the appropriate transport regulations. The audit samples were in 70% alcohol, which is flammable and harmful. Every container had to be labelled with appropriate warning labels.

The partners followed the procedures for preserving and labelling samples in Section 2.3.4.3.

2.3.6.2 The analysis undertaken by the sorting auditors

The only identification undertaken by the sorting auditors was to identify the families that they found in the samples.

The sorting auditors recorded their results on a sorting audit results sheet that had been prepared beforehand with information to identify the sample and its primary results from the project database. The results sheet was designed mainly to help the sorting auditor to record their results in the laboratory, so it was designed to be printed, see Appendix D.

STAR-AQEM samples
The auditors re-sorted the whole sub-sample and removed any animals that they found. The sorting auditor sent these specimens to the identification auditor. In a few cases, the primary analysts asked for the samples to be returned to them so that they could check what they had missed before sending them to the identification auditors themselves.

National method samples
In all cases, national methods involved sorting the whole sample and removing only representative specimens of all of the taxa present.   Where partners sorted only a sub-sample, the auditors still checked the whole sample.

The auditors re-sorted the sample and removed from it all specimens of families missed by the primary analyst. They also removed up to three good quality specimens of every potentially different species that they found in the sample. The auditors put these specimens in a vial with 70% alcohol preservative. They put specimens of missed families into a separate vial. The sorting auditor sent these vials to the identification auditor unless the primary analysts asked for them to be returned to them so that they could check what they had missed before sending them to the identification auditors themselves.

2.3.7 The identification audit

2.3.7.1 Laboratory analysis

The same samples used for the sorting audit were also used for the identification audit.

All vials of specimens produced by the primary analyst and the sorting auditor were sent to the identification auditors. Material mounted on permanent microscope slides by the

primary analysts was also to be sent to the identification auditors. Temporary mounts could not be sent.

The identification audit was undertaken at the taxonomic level used for the calculation of the national metrics. In practice, this meant the levels of identification used by each partner's primary analysts. This was species, family or a mixed taxonomic level, depending on the partner. For core stream types, it was always species level, or as close as to species-level as was possible with existing keys and expertise.

Oligochaeta and Chironomidae were excluded from the identification audit. The partners were told that these might be included later, depending on a decision by the Analytical Sub-Group.

The identification auditors used the same method of identification that they used for their primary analysis. Partners who used experts for their primary analyses used the same experts for the identification audit.

The identification auditors produced a results sheet in Microsoft Excel for each sample that they audited, using the blank template (see Appendix E), the primary data for the sample from the project database AQEMdip and the sorting audit results sheet, following instructions provided to them.

On the identification audit results sheet, the identification auditors recorded a new list of taxa based on their identification of the animals in the vial(s) and slide mounts from the primary analyst (for identification errors) and the vials from the sorting auditor (for sorting errors). They returned completed identification audit results sheets to the primary analyst's quality controller and to one of the to work package co-leaders (John Murray-Bligh, Environment Agency) for collation.

2.3.7.2 Taxonomic adjustment of audit results

The auditor should have identified taxa to the same level of precision as that attempted by the primary analyst. In some cases this was to species but sometimes it was to genus or family. Auditors could determine the level of identification by looking at the primary analyst's results on the database. These were available to all partners on the STAR web site.

Both lists were subjected to the taxonomic adjustments first used by the primary analyst on their raw data. The audit results were therefore modified where necessary to replicate the exact taxonomic levels used by the primary analyst.

Chironomids and Oligochaeta were not audited. For these, the best-achieved level of identification was used. As many partners as possible based their pre- and post-audit metric values on the full ID levels for chironomids and Oligochaeta achieved by the primary analyst.

In making a general evaluation of the impact of audit on partners' metric values it was important that all partners results were compared, as far as possible, on the basis of a common level of identification.

2.3.7.3 Abundances recorded in the audit results

Even though the audit analysis was not quantitative, it was possible to provide abundance information in the audit results that allowed the impacts of sorting and identification variations to be investigated on metrics based on quantitative data.

If the identification auditor identified all audited specimens of a taxon as a different taxon to the primary analyst, it was assigned the abundance that the primary analyst recorded for the taxon that the auditor believed to have been misidentified.

When the identification auditor agreed with the primary analyst's determination of some, but not all specimens of a particular taxon, a different approach had to be used. The abundance of such a new taxon (gain) was recorded as simply the number of that taxon identified by the auditor (i.e. the abundance of the taxon in the vials provided to the identification auditor). The abundances recorded for these gains were therefore unlikely to have been the full abundances, because in most instances, the auditor would have been sent only a sub-set of those originally identified by the primary analyst. An equal number was subtracted from the abundance of the taxon that the primary analyst recorded for those specimens. *This applied only to the specimens in the vial provided by the primary analyst.* All specimens sent by the sorting auditor were regarded as new and previously unrecorded specimens and they were simply added to the audit results without changing any other results.

The abundance values for the primary analyst were deemed to be correct (although the auditor may have disputed some of the identifications) but the abundances in the auditor's list were inevitably less precise. They under-estimated the number of specimens of gains and over-estimated the abundances of taxa that the auditor considered the primary analyst to have misidentified.

Because of this, most of the metrics used to compare the two samples were based on presence/absence data. However, some quantitative metrics that used abundance categories could also be used. For example, the first abundance category in the German Saprobic Index is 1-7 taxa. In this case, most "new" taxa added by the auditor probably genuinely had no more than 7 specimens and comparisons of the values of these metrics would therefore give reliable information.

Wrong identifications with precise information are no more reliable than right identifications with imprecise abundances. The auditor's identifications were not necessarily more correct than the primary analyst's, so we did not worry too much about getting abundances exactly correct for these samples because they were essentially qualitative.

2.3.7.4 Entering audit results onto the project database

The Replicate sub-samples were identified in the database by unique coding in the second set of 8 digits of the sample analysis codes (the first 8 digits identified the sample):

Main samples, main sub-sample	ISM0 0CM0
Main samples, replicate sub-sample	ISM0 0CR0
Replicate samples, main sub-sample	ISR0 0CM0
Replicate samples, replicate sub sample	ISR0 0CR0

The final digit was either an '0' (as in the examples above) to indicate a primary analysis or an 'A' (for example 'ISR0 0CRA') to indicate an audit analysis.

The audit result for the sample only included the taxa identified by the auditor (plus the primary analyst's records of taxa that were excluded from the audit: Oligochaeta and Chironomidae and rare taxa were excluded from the audit, see Section 2.3.4.2). Because the primary analyst sent examples of all taxa that they recorded to the identification auditor, the audit results should include all taxa in a sample.

The primary analyst entered the results of the identification audit (which incorporated the results of the sorting audit) onto the project database, AQEMdip. Before they did this, they had to be satisfied that the identification audit had been undertaken correctly and that the results of the audit were valid (even if they disagreed with the identifications).

To enter the audit data into the database, the primary analyst created a replicate copy of the primary result. There was a special function in AQEMdip for doing this automatically. This copy was changed into the audit result by adding or deleting taxa. The sample now had two taxon lists, one from the primary analyst and one from the identification auditor.

2.3.7.5 Calculating ecological quality assessment metrics

The primary analysts calculated a range of ecological quality metrics from the pre-and post audit sample analyses using AQEM Assessment Software version 2.3 (also known as AQEMrap). This was available to all partners (as well as the public) as a downloadable package from the AQEM web site http://www.aqem.de/start.htm. Instructions for using this software were included with the software. (AQEM Consortium, 2004). A link to this site was included on the 'Links' page of the STAR project web site. NB: this software is to be upgraded and will be available from the STAR project web site when the project ends.

Detailed instructions for using the software were included with the software package, and are not repeated here.

The primary analysts produced a separate AQEMrap import file for the audit samples only, comprising the original primary results plus their equivalent audit results recorded on the project database. This file therefore had two results for each sample selected for audit. In this file, the taxa were adjusted to the common level of taxonomic precision shared by the primary analyst and the identification auditor, See Section 2.3.7.3.

The import file was presented to AQEMrap and the metrics results exported to Excel. In this file the primary and audit results for each sample pair were clearly identified by their full 16-digit sample code. Each pair of sample codes had exactly the same first 15

digits but the primary sample code ended with '0' and the audit sample code ended with 'A', see Section 2.3.7.4.

These Excel exports from AQEMrap were sent to one of the work package co-leaders (John Murray-Bligh, Environment Agency, Partner 11) for collation.

2.4 Diatom audit detailed method

2.4.1 Sample processing

Sample collection and processing
Diatom samples were collected by individual partners from core and additional stream types. The samples were collected from different habitats, such as stones, macrophytes, organic material, and sediment following the STAR diatom sampling protocol (Van der Molen & Verdonschot, 2002). Each partner made sure that the samples were analysed (i.e. identified and counted) by their respective laboratories. All samples from the sites that were selected for the audit were sent to the auditors.

Identification
Identification was to the most precise taxonomic level that was achievable.

The taxonomic nomenclature used for identification was taken from the taxa-list used in the OMNIDIA program (Lecointe *et al*., 2003). This list was widely accepted and used throughout Europe, and was kept up to date to allow for taxonomic revisions and new autecological information for individual taxa. The taxa-list generally used nomenclature following Krammer and Lange-Bertalot (1986-1998) and included information about synonyms.

During the project, it became evident that the above mentioned taxa-list did not cover all taxa and taxonomic levels used by the project partners. The list of all taxa found by the partners and the auditor was harmonised by converting synonyms and doubtful taxa to valid names, based on the OMNIDIA taxa-list and agreement between experts within the STAR consortium. This adjusted STAR diatom taxa-list was made available on the project web site.

Counting
Three-hundred valves (a valve is one half of a frustule) needed to be identified and counted from each slide, following the procedures described in the STAR diatom sampling, identification and counting protocol (Van der Molen & Verdonschot, 2002). The valves were identified and counted at 1000x magnification. The slide was searched in such way that as successive fields were examined, duplicate counting of the same field was avoided, and the randomly chosen fields were distributed over the whole slide, not just a small area of it.

The results were sent to the auditor: Alterra in Wageningen, the Netherlands (Partner 4).

Audit procedure
To perform the audit the following steps were undertaken:

1. All STAR partners that were involved drew-up a list of all the samples that they collected from core and additional stream sites. Slides of all samples were also sent to the auditor.

2. Thirty-eight percent of all core and additional stream samples were re-analysed by the auditor. The samples to be re-analysed were selected randomly from all the samples taken by each partner. This was done by numbering all the samples and selecting the numbers of the samples to be audited using a list of random digits.

3. Both the identity and count of specimens in the audit samples were checked in the auditing process.

4. Taxa were identified to the most precise taxonomic level that was achievable (species, varieties or forms). By exchanging the results amongst the STAR partners through a round of comments, the level of identification was raised and the results improved. After resolving nomenclatural differences, the results of taxa and counts obtained by the primary analysts and the auditor were compared to determine the error rates.

5. For comparing the number of taxa, counts, Bray-Curtis distance and the diatom metrics were calculated.

2.4.2 Data analysis

Bray-Curtis distance
The distance between samples was calculated with the Bray-Curtis distance measure (Bray & Curtis 1957):

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

where D_{ij} is the distance between samples i and j and x is the abundance of the kth taxon in sample i and j. The Bray-Curtis distance was calculated using the program MVSP (Kovach Computing Services, 2002). The Bray-Curtis distance between results of primary analysts and auditor was plotted for all samples per country. The plots illustrate the spread of values about the median.

Van der Molen & Verdonschot (2004) reported that a distance of 0.4 or lower would indicate natural variation and thus comparability of samples, based on the 'La Bresse' analysis (see Part 2 of this report).

Diatom metrics
The OMNIDIA program (Lecointe *et al.*, 2003) was used to calculate 14 different diatom metrics that are regularly used to assess several aspects of quality in flowing waters (Table 3). The programme standardised most results on a scale between 1 and 20. The objective was to analyse the degree of variation between the metrics calculated for samples originating from a primary analyst and those from the auditor.

Difference
A positive difference between the results of metrics based on primary analysts' and auditor's results implied a higher value for the primary analyst and a negative value implied a lower one.

Table 3 Diatom metrics available in the OMNIDIA program.

abbreviation	full name	year	reference
IPS	Specific Pollution Sensitivity Metric	1987	(Coste, 1987)
SLAD	Sládeček's pollution metric	1986	(Sládeček, 1986)
DESCY	Descy's pollution metric	1979	(Descy, 1979)
LMI	Leclercq & Maquet's pollution metric	1987	(Leclercq & Maquet, 1987)
SHE	Steinberg & Schiefele trophic metric	1988	(Steinberg & Schiefele, 1988)
WAT	Watanabe et al pollution metric	1990	(Lecointe et al., 2003)
TDI	Trophic Diatom metric	1995	(Kelly & Whitton, 1995)
%PT	% pollution tolerant taxa	1995	(Kelly & Whitton, 1995)
EPI_D	Pollution metric based on diatoms	1996	(Dell'Uomo, 1996)
ROTT	Trophic metric	1999	(Rott et al., 1999)
IDG	Generic Diatom Metric	1991	(Lecointe et al., 2003)
CEE	Commission for Economical Community metric	1991	(Descy & Coste, 1991)
IBD	Biological Diatom Metric	1999	(Prygiel & Coste, 1999)
IDAP	Indice Diatomique Artois Picardie	2002	(Lecointe et al., 2003)

Coefficient of determination (R^2)
The coefficient of determination (R^2; R = correlation coefficient) explained the relationship between two variables (X and Y) in a linear regression. It could be interpreted as a percentage (R^2 multiplied by 100) of the total variance of variable Y explained by variable X. Values of R^2 range from 1 (regression explains all) to 0 (regression explains nothing). Thus, considering X as the value of a metric obtained by the auditor and Y the corresponding value obtained by the primary analyst, R^2 indicates the percentage of variance of the primary analyst's data explained by the auditor. In other words, a high R^2 indicated that the results of both the primary analyst and the auditor showed an equal linear pattern.

Paired Student's t-test
A paired approach has a smaller sampling allowance and was therefore better suited to testing paired observations. The first step in the comparison between results obtained by the primary analyst and the auditor was to calculate the difference between the pairs of observations or metric results for each sample. The difference was then tested for significance. In these tests, the null hypothesis was that both ranges of observations or metrics showed the same distribution. If the probability (P) equalled or was less than 0.05, the results of primary analyst and auditor were considered to have differed significantly.

3 Results

3.1 Invertebrate audit results

3.1.1 STAR-AQEM sub-sampling audit results

The analyses reported here are for 27 metrics that represented a wide range of aspects and responses of the macro-invertebrate fauna (Table 2). The STAR database permitted similar analyses to be made for other metrics.

The differences between the results of the two replicate sub-samples are explored in Figures 1-6. These show the relationship between the average value of each pair of un-transformed metric and the difference (variation) between them. Each figure shows a different metric. Figure 1 shows the difference between the number of taxa found in the two replicate sub-samples from the same STAR-AQEM sample. In most instances, the difference was less than five and often less than two. However there were a few large differences, the most extreme of which was a sample from Denmark where 24 taxa were found in one sub-sample and 44 in the other. In terms of 'Number of Families' (Figure 2), the difference between sub-samples was no more than three in most cases, but there was a difference of nine families for one Swedish sample and of 10 for the Danish sample mentioned above.

Table 5 gives estimates of the standard deviation in un-transformed metric values from STAR-AQEM samples caused by sub-sampling, with separate estimates for each STAR stream type for which replicate sub-sample values were obtained. Table 6 gives the same information but, where indicated, using the transformed values of particular metrics.

The estimates in Table 6 can be used in the STARBUGS (STAR Bioassessment Uncertainty Guidance Software) package (produced within the STAR project and available from the STAR web-site) to assess the effect of sub-sampling variability in individual metric values on the uncertainty of multi-metric assessments of the ecological status of sites
.

Table 4 Key to stream type codes mentioned in tables and figures in this section

Type code	country	Stream type
A05	Austria	small-sized, shallow mountain streams
A06	Austria	small-sized crystalline streams of the ridges of the Central Alps
C04	Czech Republic	small-sized, shallow mountain streams
C05	Czech Republic	small-sized streams in the Central sub-alpine Mountains
D03	Germany	medium-sized lowland streams
D04	Germany	small-sized, shallow mountain streams
D06	Germany	small-sized Buntsandstein-streams
F08	France	small-sized, shallow headwater streams in Eastern France
H04	Greece	small-sized calcareous mountain streams in Western, Central and Southern Greece
H05	Greece	small-sized, silicious mountain streams in Northern Greece
H06	Greece	small-sized, silicious streams on the Aegean Islands
H07	Greece	medium-sized, calcareous streams in Southern Greece
I05	Italy	small-sized streams in the southern calcareous Alps
I06	Italy	small-sized calcareous streams in the Central Apennines
K02	Denmark	medium-sized lowland streams
L02	Latvia	medium-sized lowland streams
O02	Poland	medium-sized lowland streams
P04	Portugal	medium-sized streams in lower mountainous areas of Southern Portugal
S05	Sweden	medium-sized lowland streams
S06	Sweden	medium-sized streams on calcareous soils
U15	UK	small-sized, shallow lowland streams
U23	UK	medium-sized lowland streams
V01	Slovakia	small-sized calcareous mountain stream in the East Carpatians
V01	Slovakia	small-sizes silicious mountains streams in the West Carpathians

Figure 1 Difference between two STAR-AQEM method replicate sub-samples plotted against the average of the two values for un-transformed values of the metric 'Number of Taxa', for all available STAR sites and seasons with replicated sub-samples. Symbols denote STAR stream type of each site.
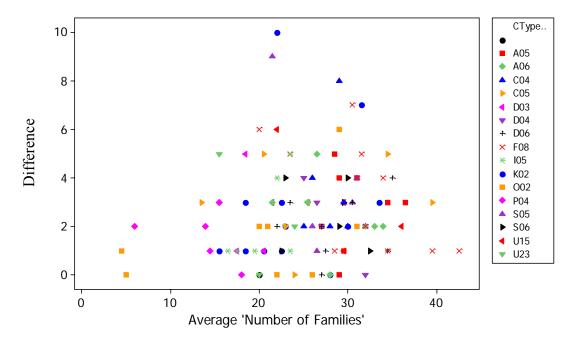
Figure 2 Difference between two STAR-AQEM method replicate sub-samples plotted against the average of the two values for un-transformed values of the metric 'Number of Families'.

(excludes two French samples with differences of 0.35 and 0.60)
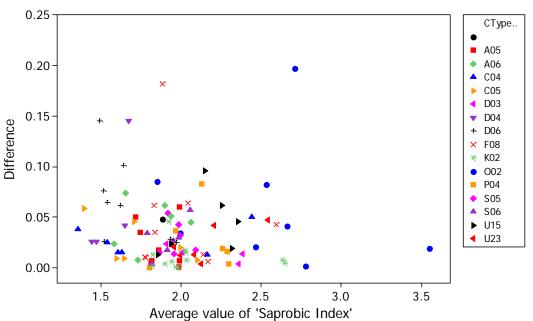
Figure 3   Difference between the two STAR-AQEM method replicate sub-samples plotted against the average of the two values for un-transformed values of the metric 'Saprobic Index (Zelinka & Marvan)', for all available STAR sites and seasons with replicated sub-samples. Symbols denote STAR stream type of each site.
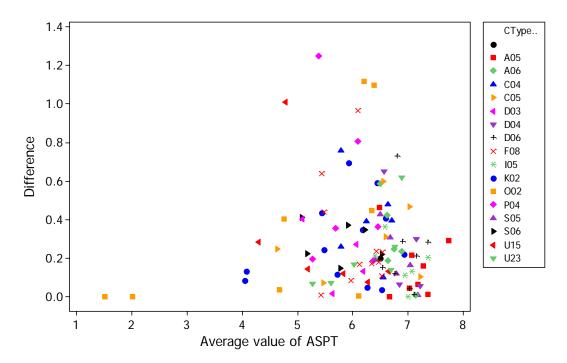
Figure 4 Difference between two STAR-AQEM method replicate sub-samples plotted against the average of the two values for un-transformed values of the metric 'Average Score per Taxon (ASPT)'.
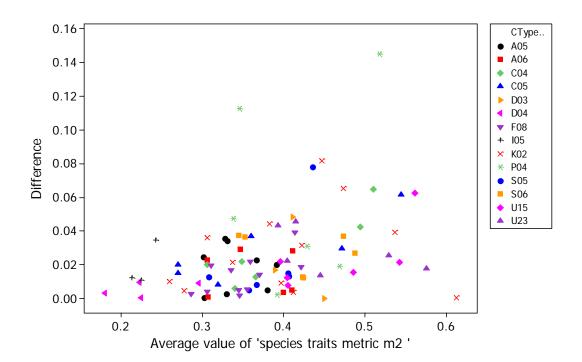
Figure 5 Difference between the two STAR-AQEM method replicate sub-samples plotted against the average of the two values for un-transformed values of traits metric 'Trait m2: % individuals with >1 reproductive cycle per year' for all available STAR sites and seasons with replicated sub-samples. Symbols denote STAR stream type of each site.
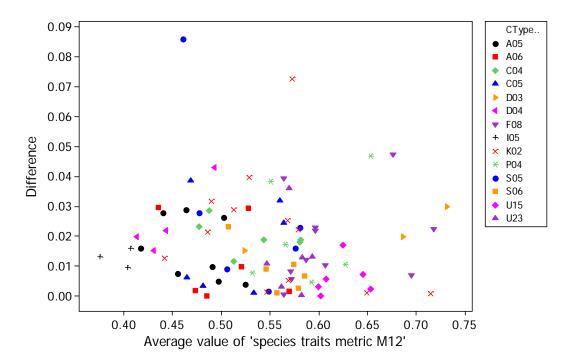
Figure 6 Difference between two STAR-AQEM method replicate sub-samples plotted against the average of the two values for un-transformed values of traits metric 'Trait m2: % individuals preferring current velocity < 25cm/s)'.

Table 5 Estimate of the standard deviation (SD$_U$) in (un-transformed) metric values caused by sub-sampling in the STAR-AQEM method, separately for each STAR stream type.

Metric	Stream Type															
	A05	A06	C04	C05	D03	D04	D06	F08	I05	K02	O02	P04	S05	S06	U15	U23
Abundance [ind/m²]	538	464	862	625	319	611	609	4160	398	1128	2930	141	893	866	1068	602
Number of Taxa	3.32	3.92	3.70	1.61	2.76	5.27	2.99	4.44	1.98	5.00	3.19	2.27	5.66	5.66	1.89	2.48
Number of Families	2.24	2.06	2.90	2.45	1.87	1.90	1.66	2.82	1.94	2.80	1.75	1.50	3.10	1.98	2.38	1.96
Number of EPT Taxa	2.21	1.71	2.43	2.48	1.77	3.34	2.52	2.01	0.96	2.50	2.11	1.12	2.14	1.80	1.04	0.96
Saprobic Index	0.022	0.035	0.021	0.023	0.011	0.055	0.054	0.155		0.011	0.059	0.027	0.023	0.023	0.037	0.020
German Saprobic new	0.035	0.024	0.076	0.087	0.041	0.051	0.013	0.025	0.020	0.046	0.073	0.030	0.056	0.040	0.079	0.034
Czech Saprobic	0.047	0.049	0.045	0.034	0.022	0.044	0.049	0.041	0.023	0.110	0.088	0.023	0.065	0.035	0.084	0.058
ASPT	0.154	0.237	0.315	0.251	0.178	0.255	0.221	0.282	0.145	0.247	0.420	0.461	0.172	0.214	0.311	0.206
IBE	0.304	0.700	0.770	0.548	0.458	0.854	0.357	0.742	0.356	0.735	0.760	0.796	1.098	0.600	0.735	0.404
IBE AQEM	0.304	0.700	0.770	0.532	0.458	0.854	0.357	0.785	0.356	0.639	0.350	0.785	1.320	0.702	0.721	0.469
Diversity SW	0.112	0.066	0.108	0.058	0.162	0.145	0.071	0.107	0.036	0.168	0.101	0.107	0.225	0.102	0.129	0.086
% Rheophilic	2.53	2.79	2.97	1.03	2.45	3.22	5.50	2.97	3.47	2.10	2.14	6.22	9.41	2.89	3.89	5.97
% Rheophilic (ab-class)	2.19	2.58	3.57	2.81	3.24	3.03	2.14	3.90		3.89	3.33	7.41	3.12	2.95	3.00	4.00
% Littoral	1.71	1.46	1.47	1.38	0.62	1.69	1.63	3.28		1.42	1.10	2.96	5.77	1.41	0.86	2.77
% Grazers/Scrapers	2.20	1.42	2.02	0.35	0.85	3.68	1.53	2.07	1.27	2.21	1.10	4.96	7.12	1.55	0.74	1.96
% Shredders	0.91	1.00	1.64	0.70	2.32	0.67	1.46	1.40		1.56	0.59	0.09	1.40	1.15	1.92	0.99
% Gatherers/Collectors	1.33	1.08	1.26	0.95	0.45	1.47	0.79	3.59		3.09	3.28	6.74	5.38	0.90	2.32	1.29
% Oligochaeta	1.26	1.57	1.57	0.44	0.39	0.20	0.22	3.02	0.06	2.06	4.76	2.30	0.74	0.83	2.20	3.12
% EPT individuals	2.94	1.97	3.86	1.32	3.40	4.06	2.10	2.57	3.91	2.19	1.06	3.57	16.36	3.22	1.04	2.79
% EPT (ab-class)	2.33	0.92	2.54	5.78	4.73	1.63	1.40	3.44		2.37	2.98	4.15	3.47	2.59	2.53	3.65
% EPT Taxa	3.23	1.98	4.60	7.97	5.22	1.48	2.51	6.29	3.42	3.44	4.05	6.00	2.19	2.67	3.26	4.27
RETI	0.020	0.016	0.015	0.015	0.022	0.019	0.016	0.030	0.013	0.026	0.017	0.068	0.086	0.019	0.021	0.012
1 –GOLD	0.034	0.018	0.034	0.010	0.030	0.026	0.018	0.038	0.030	0.036	0.027	0.036	0.176	0.043	0.032	0.034
Trait m1:max size ≤1cm	0.018	0.019	0.023	0.022	0.028	0.012		0.017	0.011	0.023		0.026	0.028	0.010	0.008	0.018
Trait m2 : >1 cycle	0.016	0.014	0.024	0.024	0.021	0.005		0.012	0.016	0.027		0.056	0.024	0.021	0.021	0.022
Trait m7 : crawler loco.	0.011	0.016	0.029	0.019	0.026	0.010		0.010	0.008	0.020		0.036	0.012	0.013	0.014	0.015
Trait m12:current<25cm	0.013	0.012	0.015	0.016	0.016	0.019		0.016	0.009	0.021		0.019	0.027	0.008	0.006	0.012

Table 6 Estimate of the standard deviation (SD$_U$) in transformed (f(x) metric values caused by sub-sampling in the STAR-AQEM method, separately for each STAR stream type

Metric	f(x)	A05	A06	C04	C05	D03	D04	D06	F08	I05	K02	O02	P04	S05	S06	U15	U23
											Stream Type						
Abundance [ind/m²]	√√x	0.320	0.243	0.256	0.229	0.281	0.425	0.288	0.804	0.134	0.390	0.544	0.129	0.928	0.347	0.486	0.282
Number of Taxa	√x	0.212	0.255	0.298	0.149	0.216	0.356	0.205	0.338	0.195	0.422	0.257	0.250	0.460	0.404	0.150	0.201
Number of Families	√x	0.203	0.202	0.273	0.251	0.217	0.188	0.155	0.272	0.204	0.286	0.176	0.214	0.321	0.191	0.244	0.226
Number of EPT Taxa	√x	0.261	0.212	0.335	0.283	0.245	0.320	0.230	0.288	0.138	0.348	0.280	0.356	0.260	0.213	0.232	0.134
Saprobic Index	x	0.022	0.035	0.021	0.023	0.011	0.055	0.054	0.155	0.011		0.059	0.027	0.023	0.023	0.037	0.020
German Saprobic new	x	0.035	0.024	0.076	0.087	0.041	0.051	0.013	0.025	0.020	0.046	0.073	0.030	0.056	0.040	0.079	0.034
Czech Saprobic	x	0.047	0.049	0.045	0.034	0.022	0.044	0.049	0.041	0.023	0.110	0.088	0.023	0.065	0.034	0.084	0.058
ASPT	x	0.154	0.237	0.315	0.251	0.178	0.255	0.221	0.282	0.145	0.247	0.420	0.461	0.172	0.214	0.311	0.206
IBE	x	0.304	0.700	0.770	0.548	0.458	0.854	0.357	0.742	0.356	0.735	0.760	0.796	1.098	0.600	0.735	0.404
IBE AQEM	x	0.304	0.700	0.770	0.532	0.458	0.854	0.357	0.785	0.356	0.639	0.350	0.785	1.320	0.702	0.721	0.469
Diversity SW	x	0.112	0.066	0.108	0.058	0.162	0.145	0.071	0.107	0.036	0.168	0.101	0.107	0.225	0.102	0.129	0.086
% Rheophilic	asin	0.033	0.029	0.032	0.013	0.026	0.036	0.058	0.034	0.036	0.036	0.031	0.090	0.100	0.030	0.042	0.070
% Rheophilic (ab-class)	asin	0.022	0.026	0.037	0.028	0.038	0.030	0.022	0.046		0.049	0.035	0.077	0.031	0.031	0.031	0.040
% Littoral	asin	0.018	0.015	0.017	0.015	0.014	0.017	0.016	0.037		0.019	0.015	0.034	0.069	0.015	0.010	0.031
% Grazers/Scrapers	asin	0.023	0.016	0.022	0.004	0.019	0.040	0.017	0.028	0.013	0.025	0.022	0.067	0.084	0.016	0.009	0.023
% Shredders	asin	0.022	0.014	0.019	0.011	0.023	0.013	0.018	0.019		0.025	0.011	0.007	0.044	0.017	0.022	0.015
% Gatherers/Collectors	asin	0.014	0.012	0.013	0.010	0.005	0.017	0.010	0.044		0.036	0.043	0.073	0.059	0.011	0.026	0.013
% Oligochaeta	asin	0.025	0.029	0.019	0.016	0.025	0.027	0.026	0.042	0.011	0.030	0.069	0.069	0.022	0.025	0.039	0.037
% EPT individuals	asin	0.035	0.022	0.046	0.014	0.048	0.042	0.022	0.032	0.042	0.026	0.014	0.044	0.178	0.036	0.017	0.033
% EPT (ab-class)	asin	0.028	0.010	0.029	0.059	0.052	0.017	0.014	0.039		0.026	0.033	0.088	0.035	0.027	0.032	0.038
% EPT Taxa	asin	0.037	0.022	0.049	0.081	0.057	0.015	0.025	0.070	0.034	0.037	0.043	0.117	0.022	0.028	0.042	0.044
RETI	asin	0.020	0.017	0.016	0.015	0.022	0.020	0.018	0.035	0.014	0.029	0.025	0.081	0.094	0.020	0.023	0.013
1 –GOLD	asin	0.036	0.020	0.038	0.011	0.046	0.031	0.024	0.040	0.041	0.037	0.032	0.047	0.190	0.044	0.041	0.035
Trait m1:max size ≤1cm	asin	0.018	0.019	0.023	0.022	0.028	0.012		0.017	0.011	0.023		0.031	0.030	0.010	0.008	0.018
Trait m2 : >1 cycle	asin	0.017	0.014	0.025	0.024	0.021	0.006		0.013	0.018	0.027		0.057	0.024	0.021	0.021	0.022
Trait m7: crawler loco.	asin	0.011	0.016	0.029	0.019	0.026	0.010		0.010	0.008	0.020		0.038	0.012	0.013	0.014	0.015
Trait m12:current<25cm	asin	0.013	0.012	0.015	0.016	0.017	0.019		0.017	0.009	0.021		0.019	0.028	0.008	0.006	0.012

It is important to be able to quantify the practical effect of sub-sampling a STAR-AQEM sample, i.e. identifying and counting the macro-invertebrate individuals from only a fraction (typically one-sixth) of the whole sample. The relative influence of sub-sampling effects to those caused by field sampling variation through small-scale spatial heterogeneity in habitat and macro-invertebrates within a site were assessed by calculating the percentage (P_{sub}) of the overall variance in metric values between replicate field samples that was caused specifically by sub-sampling variation (Table 7).

Table 7 Estimates of the average standard deviations (SD) in metric values caused by each of the hierarchical effects of sub-sampling (SD_U) and field sampling (SD_R) in STAR-AQEM samples. P_{sub} = percentage of the overall replicate field sampling variance in metric values caused by sub-sampling. Estimates are based on, and applicable to, transformed (f(x)) values of the metrics, as indicated. Based on all available sites from all available countries and averaged across stream types.

Metric	f(x)	SD_U	SD_R	P_{sub}
Abundance [ind/m²]	√√x	0.458	0.610	36
Number of Taxa	√x	0.297	0.271	55
Number of Families	√x	0.234	0.190	60
Number of EPT Taxa	√x	0.271	0.191	67
Saprobic Index	x	0.060	0.114	22
German Saprobic new	x	0.049	0.065	36
Czech Saprobic	x	0.061	0.124	19
ASPT	x	0.271	0.183	69
IBE	x	0.671	0.415	72
IBE AQEM	x	0.668	0.418	72
Diversity SW	x	0.120	0.221	23
% Rheophilic	asin	0.048	0.131	12
% Rheophilic (abund classes)	asin	0.039	0.092	15
% Littoral	asin	0.027	0.064	15
% Grazers/Scrapers	asin	0.032	0.047	32
% Shredders	asin	0.021	0.047	17
% Gatherers/Collectors	asin	0.034	0.068	20
% Oligochaeta	asin	0.037	0.098	12
% EPT individuals	Asin	0.052	0.078	31
% EPT (abund. classes)	Asin	0.038	0.030	62
% EPT Taxa	Asin	0.052	0.022	85
RETI	asin	0.036	0.064	24
1 –GOLD	asin	0.057	0.097	26
Trait m1 : max size ≤ 1cm	asin	0.021	0.029	34
Trait m2 : >1 cycle	asin	0.025	0.023	54
Trait m7 : crawler locomotion	asin	0.019	0.020	47
Trait m12 : current <25cm/s	asin	0.017	0.015	56
Average (range)				40 (12 – 85)

STAR-AQEM sub-sampling variation was relatively important for many metrics, and contributed more than 50% of the overall replicate sample variance for 10 of the 27 metrics analysed. In general, sub-sampling variance had a large effect on metrics that were based on the number of taxa present, such as number of families and number of EPT taxa. Sub-sampling variation also caused an estimated two-thirds (69%) of the total replicate sample variance in ASPT, at least when averaged across all stream types.

The metrics based on relative abundance (i.e. percentage composition) of one of more taxonomic groups seemed to be less prone to the effects of sub-sampling than replicate field sampling effects associated with spatial heterogeneity within a site.

Some stream types might be expected to give more 'nuisance' material of small-scale debris than others do, which might influence the analysts' ability to distribute the macro-invertebrates evenly between the grid cells. However, there were no obvious systematic consistent differences between stream types in the pattern and extent of sub-sampling variation in metric values (Figure 1-6).

3.1.2 Sorting audit results

Only gains could be detected in the sorting audit, and not all gains at that, because the sorting auditors did not see the vials produced by the primary analysts. It is likely that all specimens of taxa occurring in the sample as one, two or three individuals would be in this vial. Nevertheless, the sorting audit did provided comparable estimates of sorting errors across the project.

Table 8 Results of the sorting audit, mean and range of number of gains. Data for national method includes both sorted and unsorted fractions of a sample

	METHOD			
Partner	AQEM/STAR		NATIONAL	
	Mean	Range	Mean	Range
A	1.00	0 - 2	0.83*	0 - 3
B	0.83	0 - 3	0.83	0 - 1
C	4.17	0 - 11	3.50	1 - 7
D	1.83	0 - 3	4.83	2 - 11
E	1.67	1 - 3	-	-
F	3.50	0 - 9	3.17	0 - 6
G	0.33	0 - 1	0.83	0 - 2
H	4.25	3 - 6	8.25	5 - 12
I	1.00	0 - 2	2.75	2 - 3
J	1.00	0 - 3	8.83*	3 - 18
K	4.33	2 - 7	6.33	1 - 11
L	-	-	5.33*	2 - 11
M	1.33	0 - 3	1.50	0 - 3
N	2.67	0 - 4	5.67*	1 - 13
O	3.17	0 - 10	3.17	0 - 7

The number of sorting errors varied widely between different partners (Table 8). Most partners made few errors, but some made many. There was also a wide variation in the number of errors made in different samples analysed by the same partner.
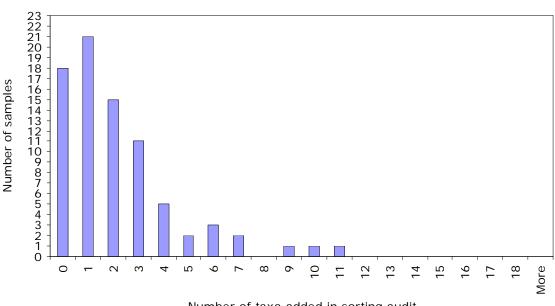
Some counties included taxa in their national macro-invertebrate assessment methods that others did not. There is no clear distinction between taxa that are always considered to be macro-invertebrates and those sometimes considered to be meiofauna. The distinction is really one of size rather than taxonomy and there is an overlap, particularly with juvenile stages. To ensure comparability of sorting audit results between partners, Table 8 is based only on the taxa included in Appendix G.

Table 9 'National' methods used by each partner. For those partners for whom the STAR-AQEM method was the national method, the UK version of RIVPACS was used.

Partner	National method
CEH (UK)	RIVPACS
Univ Duisburg-Essen (Germany)	RIVPACS
BOKU (Austria)	RIVPACS
SLU (Sweden)	Swedish
Water Inst Brno (CZ)	PERLA
HCMR - IIW (Greece)	RIVPACS
CNR-IRSA (Italy)	IBE
University of Evora (Portugal)	Portuguese
NERI (Denmark)	DSFI
University of Metz (France)	IBGN
Senckenberg (Germany)	RIVPACS
University of Lodz (Poland)	PP
University of Latvia (Latvia)	LVS 240:1999
Comenius Univ (Slovakia)	PERLA

The distribution of errors was highly skewed (Figures 7, 8 and 9). Care is needed when interpreting Figure 8 because it is based on a wide range of methods involving both species and family level primary analyses (see Table 9). Figure 9 shows only the RIVPACS and PERLA samples. The RIVPACS and PERLA samples are based on very similar laboratory analyses. Unfortunately, pattern in the data was not clear, possibly because of the lack of data.

AQEM samples

Figure 7 Distribution of errors in STAR-AQEM samples detected by the sorting audit

40

National (non-AQEM) samples

Figure 8 Distribution of errors in all non-STAR-AQEM samples detected by the sorting audit

RIVPACS - PERLA samples
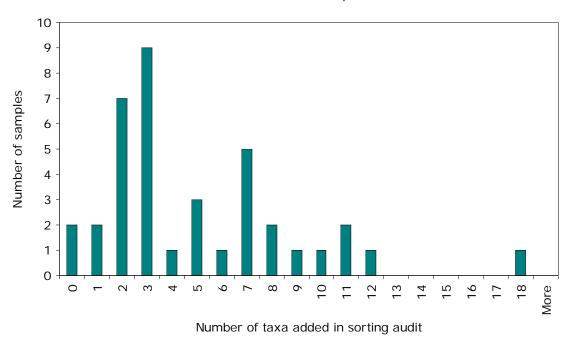
Figure 9 Distribution of errors in RIVPACS and PERLA samples detected by the sorting audit

The results for national methods were not directly comparable because the size of the samples produced by the different national sampling methods differed widely. Nevertheless, more errors were made in the analysis of samples collected and sorted by the national method than STAR-AQEM method. The difference was significant in the case of 3 partners (Table 10).

Seven partners analysed both STAR-AQEM and RIVPACS samples. There were more errors in samples analysed by the RIVPACS method than by the STAR-AQEM method.

Table 10 Significance of difference in number of errors in national method samples compared to STAR-AQEM samples detected by the sorting audit. 1-sample signed rank Wilcoxon test based on ranks of the absolute values of the differences for each site/season. Test statistic W = sum of ranks of positive differences (RIVPACS minus AQEM). Cases where p is an exact value not an estimation are indicated as such.

Partner	Median difference	n for test	W	p	
A	-1	5	5	0.75	
B	0	2	1	1	exact
C	0	6	10.5	1	exact
D	2	5	15	0.0625	exact
E	2	4	10	0.125	
F	0	4	5	1	
G	1	5	15	0.0625	exact
H	0	4	9	0.25	exact
I	0	4	10	0.125	exact
J	7	6	21	0.031	exact
K	3	5	13.5	0.187	exact
M	0	3	5	1	exact
N	2	5	13	0.187	exact
O	0	6	11.5	0.906	exact

The sorting protocol for STAR-AQEM samples required all specimens to be removed from the sample. The sorting protocols for most of the national methods, including RIVPACS, did not require every specimen to be removed, only representative specimens. It is likely that the STAR-AQEM sorting method produces fewer sorting errors at the expense of taking longer.

In order to get an impression of sorting error on biotic indices, the UK's BMWP-score, ASPT and number of scoring taxa (N-taxa) were calculated from the primary data and the primary data with the addition of gains found in the sorting audit (sorting-audit data). These indices were calculated by hand at CEH Dorset. This avoided the computational errors in these metrics that were know to occur in the current version of AQEM Assessment software (Version 2.3), but at the likely expense of transcription errors.

Indices of the BMWP-score system were well suited to the sorting audit because they were based on family-level analyses and did not take account of abundances. They included both an index of organic pollution (ASPT) and general stress (N-taxa) and were reasonably well known. N-taxa was particularly useful because it enabled sorting errors to be measured against a common list of families which ensured comparability between the results from different partners. It removed discrepancies between partners caused by different partners including different taxa in their primary analyses. Also, ASPT was a component of the proposed intercalibration common

metric index (ICMi) for the Water Framework Directive that was devised Andrea Buffagni (Partner 8) in another work package of this project.

As with the basic statistics relating to numbers of gains (Table 8), results from the audit of STAR-AQEM samples from different partners are more comparable than from the audit of national method samples, because of the greater differences between the size and nature of samples from the different national methods.

Table 11 Effect of sorting error in STAR-AQEM samples on BMWP indices as increases or (negative values) decreases in BMWP indices

Partner	Mean impact			SD of impact			Max impact		
	BMWP Score	N-taxa	ASPT	BMWP Score	N-taxa	ASPT	BMWP Score	N-taxa	ASPT
A	-3.167	-0.667	0.041	3.656	0.516	0.101	-10.000	-1.000	0.137
B	-4.667	-0.833	0.002	7.090	1.169	0.096	-18.000	-3.000	0.169
C	-17.500	-2.667	0.014	19.129	2.422	0.183	-46.000	-6.000	0.347
D	-7.000	-1.167	0.029	4.382	0.753	0.138	-10.000	-2.000	0.164
E	-3.500	-0.500	-0.048	5.431	0.837	0.101	-11.000	-2.000	-0.253
F	-13.333	-2.167	-0.050	12.565	2.041	0.080	-37.000	-6.000	-0.158
G	-2.500	-0.333	-0.016	4.183	0.516	0.065	-10.000	-1.000	-0.142
H	-12.250	-2.000	0.073	5.909	0.816	0.309	-20.000	-3.000	0.344
I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
J	-4.167	-0.500	-0.220	6.646	0.837	0.498	-15.000	-2.000	-1.233
K	-13.000	-2.500	0.145	11.454	2.074	0.209	-31.000	-5.000	0.443
M	-4.500	-0.667	-0.050	5.206	0.816	0.125	-11.000	-2.000	-0.271
N	-12.500	-2.000	-0.184	10.968	1.549	0.356	-27.000	-4.000	-0.730
O	-9.500	-1.500	0.018	12.227	2.258	0.204	-33.000	-6.000	0.403
All partners	-7.763	-1.263	-0.020	9.893	1.549	0.219	-46.000	-6.000	-1.233

Table 12 Effect of sorting error in national method samples on BMWP indices as increases or (negative values) decreases in BMWP indices

Partner	Mean impact			SD of impact			Max impact		
	BMWP Score	N-taxa	ASPT	BMWP Score	N-taxa	ASPT	BMWP Score	N-taxa	ASPT
A	-2.833	-0.500	-0.004	4.916	0.837	0.039	-12.000	-2.000	-0.071
B	-4.667	-0.500	-0.053	5.164	0.548	0.064	-10.000	-1.000	-0.142
C	-18.000	-2.333	-0.281	18.374	2.160	0.374	-43.000	-5.000	-0.765
D	-20.667	-2.833	-0.057	17.420	2.401	0.132	-50.000	-7.000	-0.211
F	-12.667	-2.000	-0.068	12.111	1.789	0.188	-29.000	-5.000	-0.417
G	-6.333	-1.000	-0.061	3.933	0.632	0.155	-11.000	-2.000	-0.375
H	-33.250	-5.250	0.108	18.839	2.630	0.109	-61.000	-9.000	0.235
I	-16.250	-2.000	-0.168	4.787	0.000	0.255	-20.000	-2.000	-0.465
J	-32.167	-7.000	0.270	14.770	2.966	0.854	-58.000	-12.000	-1.435
K	-24.167	-3.833	0.040	14.331	2.317	0.089	-44.000	-7.000	0.155
L	-20.333	-3.167	-0.003	20.186	2.639	0.205	-60.000	-8.000	0.345
M	-5.333	-0.833	-0.024	7.763	0.983	0.189	-20.000	-2.000	-0.351
N	-22.833	-3.333	-0.289	19.167	2.503	0.350	-57.000	-8.000	-0.927
O	-16.333	-2.167	-0.167	14.652	1.835	0.169	-34.000	-4.000	-0.417
All partners	-16.450	-2.575	-0.055	15.744	2.530	0.314	-61.000	-12.000	-1.435

As expected, sorting errors tended to have a greater impact on index values derived from national method samples than from STAR-AQEM samples (compare Tables 11 and 12). Sorting errors caused estimates of taxonomic richness to be biased (i.e. error always caused this metric to be underestimated). For some partners, the average bias in national samples was substantial (reductions of 5.25 and 7.00 families were the greatest deviations recorded).

Error in ASPT is more difficult to evaluate because the effects of sorting error are not biased, i.e. sorting errors can both decrease and increase its value and the effect is not independent of the number of errors. ASPT has many of the properties of a mean and it is much less susceptible to sampling and sorting error. The range of error (as indicated by standard deviation) is a better measure of error variation in ASPT and the

mean increase is not a particularly helpful statistic. Notwithstanding this, some partners' sorting error did cause substantial errors in ASPT derived from both STAR-AQEM and national method samples.

Tables 13 and 14 show the effects of sorting error as percentage impacts on BMWP indices, which may be easier to interpret by those unfamiliar with BMWP-indices. They give an indication of the potential impact on evaluations of ecological quality, but still require careful interpretation because a small error in a sample with few taxa (from a poor quality site) will result in a greater proportional impact on taxonomic richness.

Table 13 Impact of sorting error in STAR-AQEM samples as % increases or (negative numbers) decreases in BMWP indices

Partner Name	% average impact (STAR-AQEM)			min % impact (STAR-AQEM)			max % impact (STAR-AQEM)		
	BMWP Score	Ntaxa	ASPT	BMWP Score	Ntaxa	ASPT	BMWP Score	Ntaxa	ASPT
A	-1.75	-2.40	0.67	-5.03	-3.85	-1.96	0.00	0.00	2.13
B	-3.68	-3.66	-0.06	-15.25	-13.04	-2.54	0.00	0.00	2.58
C	-8.53	-8.87	0.35	-18.97	-16.67	-2.77	0.00	0.00	5.92
D	-4.58	-5.05	0.53	-8.06	-10.00	-2.27	0.00	0.00	2.61
E	-3.83	-3.15	-0.74	-12.36	-11.76	-3.76	0.00	0.00	0.00
F	-11.33	-10.44	-0.95	-26.62	-27.27	-3.06	0.00	0.00	0.90
G	-1.78	-1.54	-0.25	-5.88	-5.56	-2.26	0.00	0.00	0.79
H	-7.05	-7.97	1.09	-11.17	-12.50	-3.77	-3.45	-3.70	4.95
I	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J	-8.69	-4.01	-5.59	-43.48	-16.67	-32.17	0.00	0.00	0.00
K	-8.06	-9.91	2.19	-19.75	-19.23	-0.64	0.00	0.00	6.94
M	-5.16	-4.28	-0.93	-10.53	-11.11	-4.56	0.00	0.00	1.35
N	-17.37	-12.91	-6.15	-50.00	-33.33	-25.00	0.00	0.00	3.81
O	-6.69	-6.67	0.18	-20.50	-25.00	-3.18	0.00	0.00	6.00
All partners	-6.46	-5.87	-0.75	-43.48	-33.33	-32.17	0.00	0.00	6.94

Table 14 Impact of sorting error in national method samples as % increases or (negative numbers) decreases in BMWP indices

Partner Name	% average impact (national)			min % impact (national)			max % impact (national)		
	BMWP Score	Ntaxa	ASPT	BMWP Score	Ntaxa	ASPT	BMWP Score	Ntaxa	ASPT
A	-1.66	-1.55	-0.12	-8.00	-6.67	-1.43	0.00	0.00	0.72
B	-2.78	-2.02	-0.79	-6.33	-4.55	-2.07	0.00	0.00	0.00
C	-16.72	-12.99	-4.86	-37.62	-27.78	-13.63	0.00	0.00	1.72
D	-12.25	-11.47	-0.95	-30.30	-28.00	-3.20	0.00	0.00	2.23
F	-8.98	-7.89	-1.30	-20.71	-19.23	-7.69	0.00	0.00	2.31
G	-11.44	-9.09	-2.60	-17.70	-11.11	-7.41	-7.46	-7.41	2.28
H	-18.01	-19.30	1.61	-29.19	-30.00	-0.37	-11.86	-11.54	3.43
I	-11.44	-9.09	-2.60	-17.70	-11.11	-7.41	-7.46	-7.41	2.28
J	-25.30	-29.49	1.93	-80.56	-70.59	-33.89	-7.81	-11.11	11.20
K	-14.16	-14.66	0.62	-30.14	-30.43	-0.90	0.00	0.00	2.32
L	-12.17	-12.22	-0.03	-29.13	-25.81	-4.47	-5.30	-4.76	5.32
M	-4.12	-3.80	-0.33	-13.42	-9.52	-5.89	0.00	0.00	4.55
N	-20.60	-16.01	-6.02	-43.51	-30.77	-18.41	-7.80	-6.06	0.84
O	-16.23	-14.31	-2.56	-37.35	-33.33	-6.02	0.00	0.00	0.00
All partners	-12.00	-11.19	-1.25	-80.56	-33.33	-33.89	0.00	0.00	3.43

Taxa at the top of either column in Table 15 caused most errors. Taxa comprising species on the border, in terms of size, between macro-invertebrates and meiofauna, such as Hydracarina, Nematoda and Nematomorpha, were towards to top of these lists. They were not routinely identified by all partners in their macro-invertebrate analyses, and this could have accounted for their position in this list. These taxa were not included in the general analysis of sorting audit results (Appendix G). They are not widely used for environmental assessment.

Table 15 Frequency with which taxa caused sorting errors by number of audit samples and percentage of total gains. Taxa in lower case are not included in Appendix G.

STAR-AQEM samples			National method samples		
Taxa	Frequency	% Frequency	Taxa	Frequency	% Frequency
Hydrachnidia	29	16.20	Hydrachnidia	30	9.29
Nematoda	10	5.59	HYDRAENIDAE	15	4.64
HYDRAENIDAE	7	3.91	EMPIDIDAE	12	3.72
LEPTOCERIDAE	7	3.91	ELMIDAE	11	3.41
HYDROPTILIDAE	6	3.35	PLANORBIDAE	10	3.10
PSYCHODIDAE	6	3.35	PSYCHODIDAE	10	3.10
PLANARIIDAE	5	2.79	DYTISCIDAE	9	2.79
HYDROBIIDAE	5	2.79	Nematoda	8	2.48
PLANORBIDAE	5	2.79	SPHAERIIDAE	8	2.48
NEMOURIDAE	5	2.79	HYDROPTILIDAE	8	2.48
Oligochaeta	4	2.23	PSYCHOMYIIDAE	8	2.48
LEUCTRIDAE	4	2.23	CERATOPOGONIDAE	8	2.48
CERATOPOGONIDAE	4	2.23	EPHEMERELLIDAE	7	2.17
HYDRIDAE	3	1.68	GYRINIDAE	7	2.17
DUGESIIDAE	3	1.68	POLYCENTROPODIDAE	7	2.17
LYMNAEIDAE	3	1.68	PLANARIIDAE	6	1.86
VALVATIDAE	3	1.68	LYMNAEIDAE	6	1.86
SPHAERIIDAE	3	1.68	HYDROPHILIDAE	6	1.86
CAENIDAE	3	1.68	SCIRTIDAE	6	1.86
LEPTOPHLEBIIDAE	3	1.68	GOERIDAE	6	1.86
GOERIDAE	3	1.68	LEPTOCERIDAE	6	1.86
LEPIDOSTOMATIDAE	3	1.68	HYDROBIIDAE	5	1.55
POLYCENTROPODIDAE	3	1.68	GLOSSIPHONIIDAE	5	1.55
Nematomorpha	2	1.12	SIALIDAE	5	1.55
GLOSSIPHONIIDAE	2	1.12	LEPIDOSTOMATIDAE	5	1.55
GAMMARIDAE	2	1.12	SERICOSTOMATIDAE	5	1.55
CORIXIDAE	2	1.12	LIMONIIDAE	5	1.55
DYTISCIDAE	2	1.12	STRATIOMYIIDAE	5	1.55
ELMIDAE	2	1.12	LEPTOPHLEBIIDAE	4	1.24
GYRINIDAE	2	1.12	LEUCTRIDAE	4	1.24
BERAEIDAE	2	1.12	NEMOURIDAE	4	1.24
BRACHYCENTRIDAE	2	1.12	BERAEIDAE	4	1.24
LIMNEPHILIDAE	2	1.12	ACROLOXIDAE	3	0.93
SERICOSTOMATIDAE	2	1.12	GAMMARIDAE	3	0.93
DIXIDAE	2	1.12	CAENIDAE	3	0.93
DOLICHOPODIDAE	2	1.12	SIPHLONURIDAE	3	0.93
EPHYDRIDAE	2	1.12	PERLODIDAE	3	0.93
DENDROCOELIDAE	1	0.56	OSMYLIDAE	3	0.93
ACROLOXIDAE	1	0.56	CURCULIONIDAE	3	0.93
BITHYNIIDAE	1	0.56	GLOSSOSOMATIDAE	3	0.93
Oribatei	1	0.56	LIMNEPHILIDAE	3	0.93
ASELLIDAE	1	0.56	TABANIDAE	3	0.93
EPHEMERELLIDAE	1	0.56	TIPULIDAE	3	0.93
HEPTAGENIIDAE	1	0.56	DUGESIIDAE	2	0.62
PERLODIDAE	1	0.56	Nematomorpha	2	0.62
NEPIDAE	1	0.56	HEPTAGENIIDAE	2	0.62
VELIIDAE	1	0.56	TAENIOPTERYGIDAE	2	0.62
SIALIDAE	1	0.56	CORIXIDAE	2	0.62
HELOPHORIDAE	1	0.56	HELOPHORIDAE	2	0.62
LATHRIDIIDAE	1	0.56	ODONTOCERIDAE	2	0.62
SCIRTIDAE	1	0.56	RHYACOPHILIDAE	2	0.62
HYDROPSYCHIDAE	1	0.56	DIXIDAE	2	0.62
MOLANNIDAE	1	0.56	HYDRIDAE	1	0.31
PSYCHOMYIIDAE	1	0.56	VALVATIDAE	1	0.31
Diptera	1	0.56	UNIONIDAE	1	0.31
ATHERICIDAE	1	0.56	Oligochaeta	1	0.31
EMPIDIDAE	1	0.56	ASELLIDAE	1	0.31
PEDICIIDAE	1	0.56	POTAMIDAE	1	0.31
PTYCHOPTERIDAE	1	0.56	BAETIDAE	1	0.31
TABANIDAE	1	0.56	EPHEMERIDAE	1	0.31
TIPULIDAE	1	0.56	CALOPTERYGIDAE	1	0.31
			CORDULIIDAE	1	0.31
			GOMPHIDAE	1	0.31
			PLATYCNEMIDIDAE	1	0.31
			CHLOROPERLIDAE	1	0.31
			PERLIDAE	1	0.31
			PLEIDAE	1	0.31
			NEPIDAE	1	0.31
			STAPHYLINIDAE	1	0.31
			BRACHYCENTRIDAE	1	0.31
			HYDROPSYCHIDAE	1	0.31
			MOLANNIDAE	1	0.31
			PHILOPOTAMIDAE	1	0.31
			Lepidoptera	1	0.31
			PYRALIDAE	1	0.31
			EPHYDRIDAE	1	0.31
			MUSCIDAE	1	0.31
			RHAGIONIDAE	1	0.31
			SIMULIIDAE	1	0.31
Total	179	100	Total	323	100

45

Other taxa were undisputedly macro-invertebrates. Most were present only as one or two individuals. Many were small, cryptic and easily hidden or confused with detritus.

Lists of taxa causing error, ranked in order of frequency in sorting audit results, were prepared for each partner (Appendix H). The taxa missed during sorting depends on both the sampling method and the type of environment from which they were collected.

3.1.3 Identification audit

Neither the primary analyst's nor the identification auditor's species lists were considered to be definitive – they were considered simply as alternative views of the same data. Audit results were not used to correct the primary data. The identification audit mainly provided information about precision. In contrast, the sorting audit mainly provided information about bias, which inevitably implies error.

The metrics calculated by AQEM assessment software are explained in detail in the AQEM manual (AQEM consortium, 2002), which can be downloaded from the AQEM web site.

The results of this audit were not completed in time for inclusion in this report.

3.2 Diatom audit results

3.2.1 Samples

Table 16 Number of primary and audit samples for each partner.

Country	Institute	number of primary samples	number of audit samples
Austria	University of Agricultural Sciences (BOKU)	16	6
Czech Republic	Masaryk University	24	8
Germany	University of Duisburg-Essen	20	7
Germany	Research Institute Senckenberg	6	3
France	University of Metz	15	6
Italy	Labbio, Province of Bolzano	10	3
Italy	Istituto di Recerca sulle Acque (CNR-IRSA)	13	5
Denmark	National Environmental Research Institute (NERI)	21	4
Portugal	University of Evora	26	5
Sweden	Swedish University of Agricultural Sciences	25	10
United Kingdom	Centre for Ecology and Hydrology (CEH)	25	8
Slovakia	Institute of Zoology	24	10
Greece	National Centre for Marine Research	16	5
Poland	University of Łódź	44	14
	total	**285**	**94**

Table 16 lists the number of primary diatom samples (original samples taken and analysed by each partner) and audit samples for each partner. The number of samples included in the audit varied from three up to fourteen samples. Different partners took a different number of diatom samples from their core and additional streams.

3.2.1.1 Taxonomic adjustment

Before comparing the primary and audit results, a taxonomic adjustment was necessary because:
- for a number of taxa, species-level data were absent from the STAR diatom taxa-list, although data for varieties and forms were present,
- differences between the primary analysts' and auditor's results were sometimes caused by variations in the identification of varieties that exhibit a transition in features between each of the varieties and forms. The best example was *Cocconeis placentula* where different partners as well as the auditor identified different varieties; e.g., *var. placentula, var. lineata, var. euglypta, and var. pseudolineata*. These varieties show a transition in features and are therefore difficult to distinguish decisively. For this reason, all records of varieties and forms were adjusted to species level (in this example, to *Cocconeis placentula*).
- different primary analysts used different identification keys and therefore recorded different synonyms, confusing taxa or other deviations. For these taxa, species level was used.

The identification lists were adjusted to levels agreeable to all partners and experts in a taxonomic adjustment procedure based on expert knowledge and experience, and the primary analysts' responses to any decisions proposed.

The number of taxonomic differences between the primary analysts and the auditor are listed in Table 17. Most of the differences related to rare taxa and the use of different taxonomic levels and synonyms. Real misidentifications (identifications by the primary analysts that were corrected by the auditor) only occurred incidentally, for nine taxa in total.

Table 17 Summary of taxonomic differences after taxonomic adjustment. Taxonomic level too low indicates that the level listed was lower then the level agreed upon in the STAR taxa-list, taxonomic level too high indicates a higher level then agreed upon).

Taxonomic level too low by	partner	34
	auditor	37
Taxonomic level too high by	partner	5
	auditor	2
Identification error by	partner	9
	auditor	0
Taxon only identified by	partner	24
	auditor	20
	synonym	2
	difference caused by taxonomic confusion	3

In the last step, the taxonomic levels that were too low and the misidentification were corrected.

For data analysis, two taxonomic lists were used. The first referred to the STAR taxa-list and was called the valid data. The second incorporated the additional adjustments referred to above and was called the adjusted data. The latter reflected the 'definitive' audit.

3.2.1.2 Counts

According to the protocol (Van der Molen & Verdonschot, 2002), the slides should have been analysed by identifying and counting diatom valves until 300 valves had been counted. In most cases, more than 300 valves were present on the slide. In exceptional cases, slides contained fewer valves and the count was limited to 100-150 valves. To avoid the differences caused by the different counts, the results were also analysed as percentages.
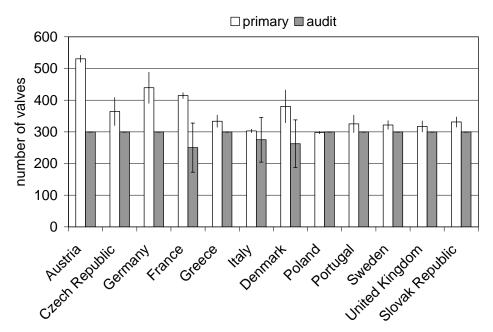
Figure 10 Number of valves counted by the primary analysts and the auditor (standard deviations of both numbers indicated).

The number of valves counted by the primary analysts usually exceeded the 300 prescribed in the protocol (Figure 10, Table 18). In a few cases the auditor counted 100-150 valves (one sample for France, one for Italy and one for Denmark), while the primary analysts exceeded this number. This was because the auditor only had one of the slides at his disposal at the time of the audit. Except for Portugal, Italy and Denmark, the counts differed significantly and for the latter two, the primary analysts and auditors counts were not correlated (Table 4). Because the auditor almost always counted exactly 300 valves, no correlation coefficient was calculated.

Table 18 Number of valves counted by primary analysts and the auditor. Significant differences indicated by Student's t-test and linear relationships indicated by the correlation co-efficient R^2 are highlighted.

	primary analysts		Auditor			
	average number of valves	standard deviation	Average number of valves	standard deviation	R^2	t-test
Austria	530.5	10.88	300.0	0.00	-	0.000
Czech Republic	364.0	44.19	300.0	0.00	-	0.005
Germany	439.2	48.91	300.0	0.00	-	0.000
France	414.3	9.29	250.0	77.46	0.00	0.004
Greece	333.6	19.27	300.0	0.00	-	0.018
Italy	302.9	5.28	275.0	70.71	0.05	0.295
Denmark	380.3	51.56	262.5	75.00	0.04	0.098
Poland	297.6	3.67	300.0	0.00	-	0.028
Portugal	325.0	27.50	300.0	0.00	-	0.112
Sweden	321.5	13.66	300.0	0.00	-	0.001
United Kingdom	317.0	17.22	300.0	0.00	-	0.027
Slovak Republic	330.9	15.95	300.0	0.00	-	0.000
Latvia	426.1	76.71	300.0	0.00	-	0.000

The higher number of valves counted indicated that the protocol was not strictly followed. This affected the audit results, because a higher number of counts increased the chance of recording a higher number of taxa.

3.2.1.3 Number of taxa

Both the number of valid taxa and the number of adjusted ones were compared (Figures 11 and 12; Table 19 and 20).
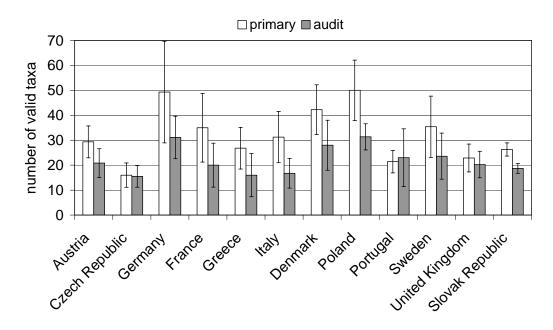
Figure 11 Number of valid taxa identified by primary analysts and the auditor (standard deviations of both numbers indicated).

The number of valid taxa identified by the primary analysts and the auditor differed (Figure 11). The number of valid as well as adjusted taxa was higher for all primary analysts, except for Portugal (a very small negative difference; Figure 11 and 12 and Table 19 and 20). The difference was statistically significant for all primary analysts except those from Czech Republic, Denmark, Portugal and United Kingdom. A significantly greater number of adjusted taxa was recorded by all primary analysts, except for Czech Republic and Portugal. Nevertheless, there was a linear relationship between the primary analysts' and the auditor's estimates of both the number of valid taxa and the adjusted taxa for Austria, Czech Republic, Germany, France, Greece, Portugal, Sweden, and (for valid taxa only) the United Kingdom
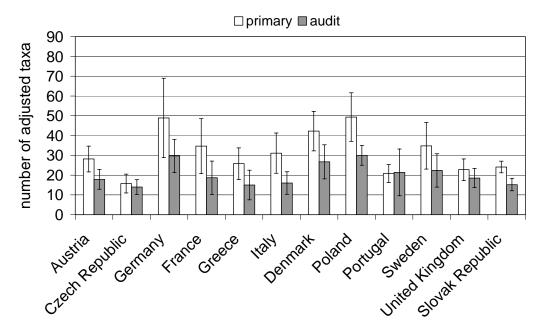
Figure 12 Number of adjusted taxa identified by primary analysts and the auditor (standard deviations of both numbers indicated).

Table 19 Number of valid taxa identified by the primary analysts and the auditor. Significant differences indicated by Student's t-test and linear relationships indicated by the correlation co-efficient R^2 are highlighted.

primary analysts			Auditor				
	average	standard deviation	average	standard deviation	difference	R^2	t-test
Austria	29.3	6.41	20.8	5.78	8.50	0.85	0.000
Czech Republic	16.0	4.87	15.5	4.34	0.50	0.70	0.613
Germany	49.3	20.36	31.1	8.53	18.20	0.76	0.002
France	35.0	13.78	20.0	8.79	15.00	0.65	0.007
Greece	26.8	8.35	16.0	8.63	10.80	0.83	0.003
Italy	31.3	10.26	16.8	5.92	14.50	0.22	0.003
Denmark	42.3	9.95	28.0	10.03	14.25	0.33	0.053
Poland	50.0	12.10	31.4	5.23	18.64	0.04	0.000
Portugal	21.4	4.51	23.0	11.58	-1.60	0.81	0.669
Sweden	35.4	12.26	23.6	9.24	11.80	0.65	0.001
United Kingdom	22.9	5.57	20.3	5.31	2.63	0.65	0.067
Slovak Republic	26.3	2.63	18.7	1.95	7.60	0.00	0.000

The higher number of valid and adjusted taxa again indicated that the protocol had not been followed strictly. A further investigation revealed that most of the taxa involved had a count of about 1. It is likely that the slides were surveyed for additional taxa after the count was completed. This is a procedure that is often followed in diatom research, but it was not a part of the STAR protocol. This could affect the audit results because the higher number of counts and taxa could affect the values of metrics calculated from the data.

Table 20 Number of adjusted taxa identified by the primary analysts and the auditor. Significant differences indicated by Student's t-test and linear relationships indicated by the correlation co-efficient R^2 are highlighted.

primary analysts			auditor				
	average	standard deviation	average	standard deviation	Difference	R^2	t-test
Austria	28.2	6.49	17.8	4.96	10.33	0.84	0.000
Czech Republic	15.8	4.77	14.0	3.70	1.75	0.75	0.082
Germany	48.9	20.04	29.7	8.41	19.20	0.75	0.001
France	34.7	13.87	18.7	8.41	16.00	0.70	0.005
Greece	25.8	7.98	15.0	7.48	10.80	0.85	0.001
Italy	31.1	10.18	16.0	5.68	15.13	0.23	0.002
Denmark	42.3	9.95	26.8	8.62	15.50	0.32	0.038
Poland	49.4	12.27	30.0	5.04	19.36	0.09	0.000
Portugal	20.8	4.55	21.4	11.80	-0.60	0.77	0.877
Sweden	34.8	11.75	22.4	8.42	12.40	0.65	0.000
United Kingdom	22.8	5.42	18.5	4.81	4.25	0.58	0.012
Slovak Republic	24.1	2.92	15.2	3.12	8.90	0.31	0.000

3.2.2 Bray-Curtis distance

The Bray-Curtis distance between the primary and the audit results for valid taxa per country (based on percentages) are shown in Figure 13. The dissimilarity between primary analysts' and auditor's samples of only one country was less than the threshold of 0.4 (a distance less then 0.4 is generally regarded as a good fit between primary analyst and auditor).

The distance between primary analysts' and auditor's results was less when based on adjusted taxa (Figure 14).

For the adjusted taxa list, the Bray-Curtis distance met the criterion of 0.4 for nine countries. Only Denmark, Germany, Poland and Portugal did not meet the critical level. The standard deviation for Greece was especially high.
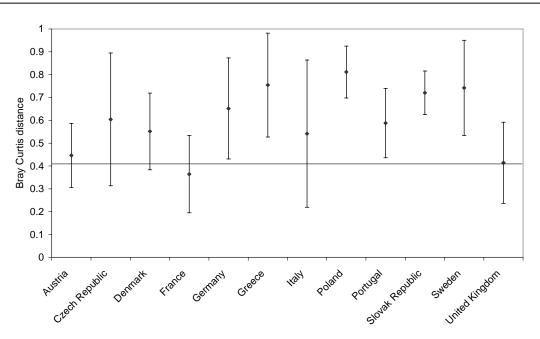
Figure 13 The Bray-Curtis distance (average and standard deviation) between primary analysts' and auditor's samples per country. A distance less then 0.4 indicates a generally good fit.
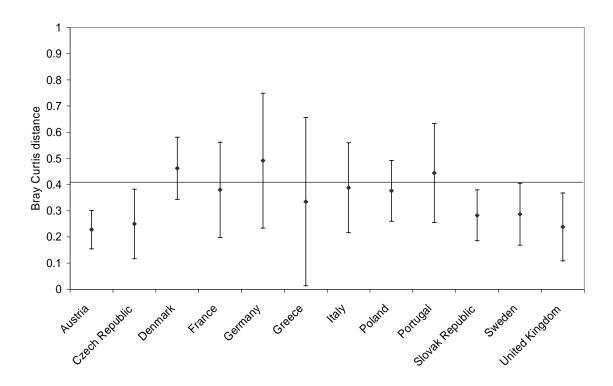
Figure 14 The Bray-Curtis distance (average and standard deviation) between primary analysts' and the auditor per country. A distance lower then 0.4 indicates a generally good fit.

3.2.3 Diatom metrics

There was a good correlation between the primary analysts' and auditor's values of metrics based on counts of the adjusted taxa (except for the DESCY, WAT, TDI, EPI-D, CEE, and IDAP, see Table 3 for an explanation of the abbreviations for diatom metrics). For the valid data, the primary analysts' and auditor's results were correlated only for SLAD, SHE, %PT and IDG (Table 21). There was a significant difference between the results of eight (57%) of the metrics based on counts of the valid taxa and only one metric (7%) for calculations based on the adjusted taxa.

There was a good correlation between the primary analysts' and auditor's values of metrics based on percentages of adjusted taxa (except for the DESCY, WAT, EPI-D, CEE, and IDAP metrics). For the valid data, the primary analysts' and auditor's results were only correlated for SHE, %PT and IDG (Table 21). These results were similar to the count based results. An example of a highly correlated diatom metric (SLAD) is shown in Figure 15, and an un-correlated one in Figure 16 (IDAP).

Eight (57%) of the metric results based on counts of valid taxa differed significantly between primary analyst and auditor and only one (7%) for calculations based on the adjusted taxa. The differences between primary analysts' and auditor's results based on adjusted data were therefore not significant.

Table 21 Overall correlation (R^2) and Student's t-test for all diatom metrics. Significant differences indicated by Student's t-test and linear relationships indicated by the correlation co-efficient R^2 are highlighted. See Table 3 for an explanation of data types.

data type	count					percentage			
	Adjusted	valid	adjusted	valid		adjusted	valid	adjusted	valid
	R^2	R^2	t-test	t-test		R^2	R^2	t-test	t-test
IPS	0.744	0.596	0.214	0.000		0.739	0.593	0.208	0.000
SLAD.	0.767	0.649	0.881	0.000		0.754	0.639	0.847	0.000
DESCY	0.576	0.414	0.670	0.061		0.583	0.375	0.406	0.130
L&M	0.701	0.567	0.819	0.750		0.695	0.554	0.824	0.799
SHE	0.760	0.651	0.167	0.304		0.757	0.651	0.151	0.393
WAT	0.624	0.125	0.293	0.000		0.620	0.134	0.298	0.000
TDI	0.600	0.286	0.721	0.196		0.528	0.229	0.855	0.232
%PT	0.913	0.907	0.431	0.025		0.905	0.900	0.593	0.044
EPI-D	0.636	0.506	0.001	0.007		0.612	0.525	0.002	0.006
ROTT	0.767	0.420	0.325	0.509		0.752	0.393	0.291	0.668
IDG	0.826	0.737	0.438	0.000		0.824	0.727	0.522	0.000
CEE	0.401	0.096	0.844	0.000		0.304	0.140	0.151	0.000
IBD	0.766	0.330	0.313	0.000		0.767	0.341	0.327	0.000
IDAP	0.135	0.037	0.196	0.669		0.002	0.001	0.240	0.652
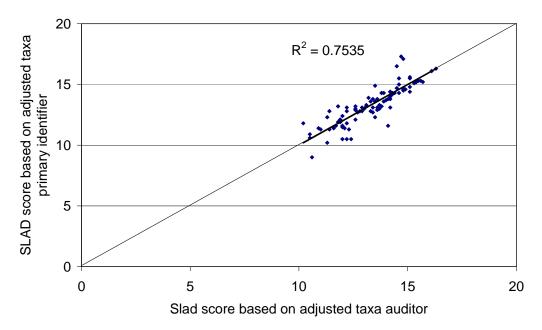
Figure 15 The SLAD score based on adjusted taxa for primary analysts and auditor

The correlations between diatom metric results based on primary analysts' and auditor's data for individual partners were generally good (Appendix M, N and O). The differences between the values of diatom metrics of the primary analysts and auditor were generally either positive or negative depending on the metric and country. Only the EPI-D and IPS always scored negatively (Appendix 7). For these metrics, the auditor's value was always higher then the primary analyst's one.

Most partners' diatom metrics results were highly correlated except for Greece and Germany. However, the IDAP was not correlated for almost any of the countries (Appendix N). These results were true for calculations based on both counts and percentages.
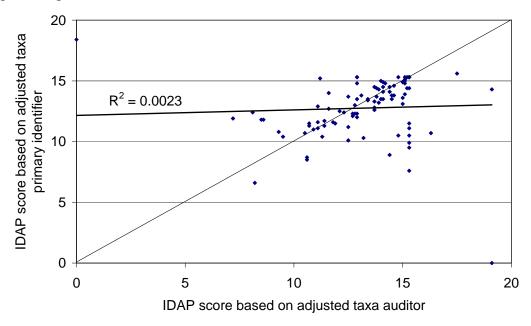
Figure 16 The IDAP score based on adjusted taxa for primary analysts and auditor

The values of most diatom metrics did not differ significantly between the primary analysts and the auditor. Exceptions were the comparisons between primary analysts and auditor for Slovak Republic, Sweden and to a lesser extent Poland (Appendix M). If we count all cases (country versus metric), 87% (count based) and 89% (percentage based) of the primary analysts' results appeared not to differ significantly with those from the auditor.

4   Discussion and conclusions

Audit data was used only to measure error variation. Audit results do not replace the primary data in the main survey results. If they did, the audit results would not be an accurate measure of the precision or accuracy of the survey.

Ideally, auditing should involve independent sampling, sorting and analysis. This was impractical and too expensive both for this project, as it would be for operational use for the Water Framework Directive monitoring. Instead, the audit was restricted to the analysis of the samples in the field and laboratory. Instead of collecting and analysing separate audit samples, samples already analysed were re-analysed for the audit.

Replicate sampling was used to assess variation caused by sampling, see Sandin *et al.* 2005. This assumed that all those taking part in the replicate sampling exercise were following the sampling procedures correctly. This is not a safe assumption, based on the experience of environmental protection agencies in the UK and the AQEM partners during the sampling workshop for this project (Murray-Bligh, 2004). The only practical solution is to ensure that all those involved in the survey receive the same instructions, by practical demonstrations covering all aspects of sampling (and analysis), before the start of the survey. Furthermore, experience has shown that written instructions alone are not sufficient: field workshops are necessary. Because those who take part are already fully trained professionals, these workshops are more appropriately termed calibration workshops (the aim being to re-calibrate surveyors' understanding of the survey methods and to ensure a common understanding of concepts and updates). Such workshops were included in the STAR project as Work package 7, described in Murray-Bligh (2004).

The ultimate purpose of the audit was to enable the accuracy and precision of the final results of ecological assessments of river quality to be calculated. Unfortunately, there is no single method available to measure overall data quality, i.e. the combined effects of all different sources of error (Cao *et al.*, 2003). The results of the audits can be combined with information about sampling error from replicate sampling programmes to provide a comprehensive estimate of uncertainty of ecological quality class. This uncertainty can be quantified as confidence limits and bias for the biological metrics and ecological quality classifications. (Bias is the non-random effect of errors on accuracy.) This can be done using the STARBUGS software produced in this project by Ralph Clarke and available from the project web-site. The aim of STAR was to develop methods for comparing data from different biological elements (invertebrate, plants, fish, etc) from different stream types and from different countries using

different classification schemes. Confidence limits and bias are crucial to any such comparisons because they enable the real differences to be distinguished from differences caused by statistical, analytical and sampling variation.

The audit results can be used to determine the sensitivity of classification indices used by member states. A detailed approach would be to base this on modelling, by investigating the effect of sequential removal of taxa and their effect on the indices. The audit results presented here provide a realistic indication of the numbers to remove in such an analysis.

4.1 Invertebrate sub-sampling audit

In most cases, the number of taxa and number of families removed from replicate sub-samples of STAR-AQEM samples were similar, but in some cases there were very large differences. Sub-sampling variation is relatively important for some metrics, contributing more than 50% of the replicate sample variance (i.e. it causes greater uncertainty than sampling error). It has a large impact on metrics based on the number of taxa present and on ASPT, but less on metrics based on the relative abundance (i.e. percentage abundance) of one or more taxonomic groups.

Estimates of standard deviations in metrics caused by sub-sampling variation, shown in Tables 6 and 7 can be used in the STARBUGS software package to assess the effects of sub-sampling variability in individual metrics on the uncertainty of multi-metric assessments of ecological status. STARBUGS can be downloaded from the STAR web-site.

Sub-sampling variation is a significant and sometimes a major part of the replicate sample variability in many commonly used metrics. Sorting and identifying a larger fraction of the sample would reduce this source of variation and sorting the whole sample would eliminate it. However, all extra analysis increases costs. It is only possible to determine the cost-effectiveness of extra laboratory effort by sorting all 30 tray cells of a STAR-AQEM sample and selecting repeated random combinations of increasing numbers of cells by computer to assess the rate of reduction in sub-sampling variance.

The results highlight the importance of always trying to spread and distribute the sample material as evenly as possible amongst the 30 grid cells on a sorting tray for any STAR-AQEM macro-invertebrate sample.

4.2 Invertebrate sorting and identification audit

Most partners made few sorting errors, but some made many. There was also a wide variation in the number of errors made in different samples analysed by the same partner.

The STAR-AQEM sorting method produced fewer errors than national methods, including RIVPACS/PERLA. However, the STAR-AQEM samples took longer to

sort and they were subject to additional sub-sampling errors that did not affect the other national methods.

The results of the identification audit were not ready in time for inclusion in this report.

The invertebrate sorting and identification audits were based on a very small number of samples (generally 6 samples for each method undertaken by each partner). This was necessary to allow for the additional identification, sub-sampling and replicate sampling programmes that were not included in the original plans and were therefore not budgeted for. Because of this, the results were imprecise and so the conclusions from the audit for any particular partner must be treated with caution.

Analysis of sorting errors by the regulatory agencies in the UK indicated that these errors follow a Poisson distribution (Kinley & Ellis, 1991) and they appeared to follow a similar distribution in this project. Most samples had few errors but a few samples had many errors. This skewed distribution makes estimating the overall rate of error difficult and a relatively large number of samples are needed to estimate statistics with a useful degree of precision. When the UK's operational audit started, CEH Dorset concluded that 60 samples were needed for a sufficiently accurate estimation of mean number of errors. Since then, analytical quality in the Environment Agency has improved (Table 22), but the statistics are still based on 20 audit samples for each laboratory.

The number and frequency of sorting errors surprised some partners, but they were consistent with experiences in UK where the quality of sorting has been audited as an integral part of the regulatory authority's river quality assessment for almost 15 years (Table 22). The range of errors was similar to the Environment Agency's errors recorded in the first year (and in particular, the first batch of results that each laboratory received in that first year).

Table 22 Sorting audit results from the UK's Environment Agency as mean numbers of taxa that were 'gained' following audits of RIVPACS samples in successive years from 1990 to 2003. N = 20 except for results marked by an asterisk (*). This is an update of a table in Dines & Murray-Bligh (2000). Dashes (-) indicate gaps caused by the amalgamation of Regions (from ten to eight).

Region	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
A	3.55	1.4	2.7	2.38	1.73	2.25	1.85	2.03	1.75	1.67	1.85	2.56*	2.48	1.97
B	3.32	1.12	0.71	0.65	-	-	-	-	-	-	-	-	-	-
C	-	-	-	0.8	1.48	1.52	1.38	1.5	1.43	1.5	1.18	1.13	1	0.92
D	1.4	1.18	1.12	-	-	-	-	-	-	-	-	-	-	-
E	4.39	3.68	2.77	2.3	2.54	2.52	2.62	1.64	1.5	1.78	1.15	1.30*	1.4	1.85
F	3.75	1.88	2.1	2.48	1.83	1.84	2.2	1.91	1.8	1.93	1.68	1.96	1.75	2.57
G	1.9	1.28	1.77	1.37	1.13	1.33	1.95	1.31	1.16	1.42	1.73	1.53	1.5	1.17*
H	1.9	1.08	1.53	1.32	1.13	-	-	-	-	-	-	-	-	-
I	-	-	-	-	-	1.52	1.13	0.72	0.95	1.11*	0.84	1.09*	0.87	1.01
J	3.66	1.73	1.88	2.28	2.38	-	-	-	-	-	-	-	-	-
K	2.74	1.98	2.2	2.07	2.42	2.05	2.03	1.82	2.13	2.88	2.3	1.41*	1.67	1.63
L	2.37	1.48	2.08	1.95	1.3	1.98	1.88	2.3	1.32	1.57	1.78	1.13*	1.75	1.78

Errors were far greater in the first year of the Environment Agency's audit than subsequently. Audit results from other laboratories in the UK, including other government agencies and commercial contractors, have shown a similar pattern. Poor results are common when a laboratory is first audited but improve very rapidly thereafter. Poor initial audit results can happen in well-established laboratories as well as newly established ones.

Biologists often receive no training in sorting and unless someone points-out their mistakes, they will remain unaware of any shortcomings. Sorting is conceptually very simple and the task is sometimes left to the most junior and inexperienced biologists. The audit results demonstrate that sorting is a more skilled task than has often been recognised in the past.

If analysts pay particular attention to taxa high in the lists in Table 15 and Appendix H, they could prevent a large proportion of their sorting errors in the future.

After the initial improvements that result from this 'training', improving analytical quality still further can take considerable effort. My experience is that, once the biologists are fully trained, errors then relate to the time and effort that is invested in sorting rather than analysing a greater number of samples. The laboratory manager, not the analyst, determines the amount of work and the time available for analysing each sample, so it is difficult for individual analysts to improve analytical quality further without their manager's support.

The effect of sorting errors can be substantial, even in research laboratories.

Those partners whose audit results were much poorer than expected are unlikely to have similarly poor results if they are audited again. Because of this, the errors recorded in this project may not be representative of errors in laboratories working under operational conditions and which have been audited for some time.

Sorting error not only contributes to uncertainty, which affects the precision of results, but it also affects the accuracy of the results. (Accuracy means how close observed values are from the true value whereas precision means how close repeated measurements are from each other, Sokal & Rohlf, 1995). This is because sorting errors cause bias, i.e. they are uni-directional. Sorting error always causes gains, only very rarely losses. This always reduces the number of taxa recorded and the numerical values of metrics based on taxonomic richness. This bias also means that the only way to detect sorting error is by audit, using expert auditors that are known to produce very few sorting errors. Sorting errors cannot be detected by replicate sampling or analysis by the primary analysts. The audit results will only be as accurate as the auditor's analysis. Any errors that the auditor makes will be hidden. Unfortunately, it is impossible to eliminate sorting error, even from an audit.

Although it involves more work, the STAR-AQEM protocol for sorting, whereby every specimen is removed, makes auditing easier, although it also makes the job of the auditor less interesting.

4.3 Diatom audit

A small set of diatom samples was audited because only a small number of samples were taken during the project. Within the STAR project, the taxonomy of diatoms was often a point of discussion between the specialists involved. These discussions resulted in a STAR diatom list, which could be improved even further for assessment purposes.

The use of the adjusted taxa-list versus the valid one showed that adjustment is really necessary to obtain comparable results between different analysts.

A number of partners counted more then the prescribed 300 valves and this led to a bias between auditor and primary analyst. A higher count also led to a greater number of taxa.

The Bray-Curtis dissimilarity for adjusted data indicated that, on average, 75% of the partners provided results comparable with those of the auditor. This was consistent with the hypothesis that the difference in number of taxa was caused mainly by a number of rare taxa.

The adjusted data also performed best for the diatom metrics. Only 11% to 13% of the metric results differed significantly between primary analyst and auditor. This also indicated that the extra count and search through the slide for extra taxa are not necessary for assessment purposes. Rare taxa have only a slight influence on diatom metrics.

In general, the differences between results based on counts and percentages were small.

The audit of diatoms revealed several areas were standardisation needs further attention. They concern taxonomy and the protocol.

The taxonomy of diatoms is not easy and will be subject to discussion between experts. The large number of varieties and forms and the indistinct transition within species between these varieties, forma and sub-species puts limits their usefulness for environmental assessments. To date, most metrics have been based on taxa lists consisting mainly of varieties and forms, their taxonomy strongly influences the value of metrics, and hence the outcome of environmental assessments, based on them. During the STAR diatom audit, there was much discussion about appropriate taxonomic levels and the ability of several varieties and forms to be identified. Compromises were necessary. Metrics to be used in future assessments should be based on higher taxonomic levels (often species, but sometimes genus). Furthermore, an agreement on which identification keys should become part of the European standard will be benefit the use of diatoms all over Europe as a common assessment.

The sampling and identification protocol was established between partners during the workshop in La Bresse and a web-site discussion. The audit demonstrated that the majority of partners did not follow this protocol. The analyses usually involved many more than the 300 valves specified in the protocol and slides were also scanned for

additional taxa. Both of these deviations had a negative impact on the audit results. Stressing the importance of following the protocol most easily solves this problem.

It is not yet clear whether these deviations in methodology would affect water quality assessments. To confirm this, the diatom metrics results should be translated into the five ecological quality classes of the Water Framework Directive, when the classification methods for the Water Framework Directive have been decided.

5 References

AQEM consortium (2002). *Manual for the Application of the AQEM system. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive*. Contract No. EVK1-CT1999-00027. Downloadable from http://www.aqem.de/start.htm.

AQEM consortium (2003). *Revised AQEM/STAR sorting protocol*. Downloadable from http://www.eu-star.at.

AQEM Consortium (2004). *AQEM European stream assessment programme Version 2,3, released on April 2004*. Downloadable from http://www.aqem.de/start.htm.

Bray J.R. & Curtis J.T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs*, **27**: 325-349.

Cao, Y, C.P. Hawkins and M.R. Vinson (2003). Measuring and controlling data quality in biological assemblage surveys with special references to stream benthic surveys. *Freshwater Biology*, **48**: 1898-1911.

Clarke, R.T. (2000). Uncertainty in estimates of river quality based on RIVPACS. In: Wright, J.F., D.W. Sutcliffe and Furse, M.T. (eds). *Assessing the biological quality of freshwaters: RIVPACS and similar techniques*. pp 39-54. Freshwater Biological Association, Ambleside.

Clarke, R.T. (2004). 7th STAR deliverable. *Error/uncertainty module software STARBUGS (STAR Bio Assessment Uncertainty Guidance Software) User Manual*.

Coste, M. (1987). *Etude des méthodes biologique quantitatives d'appréciation de la qualité des eaux, Rapport Division Qualité des Eaux Lyon*. Agence de l'Eau Rhône: 28.

Dell'Uomo, A. (1996). Assessment of water quality of an Apennine river as a pilot study for diatom-based monitoring of Italian water courses. In: B. A. Whitton & E. Rott. *Use of Algae for Monitoring Rivers II*. Innsbruck, Institut für Botanik, Universität Innsbruck: 64-72.

Descy, J-P. & M. Coste (1991). A test of methods for assessing water quality based on diatoms. *Verh.int.Verein.Limnol.*, **24**: 2112-2116.

Descy, J-P. (1979). A new approach to water quality estimation using diatoms. *Nova Hedwigia* **64**: 305-323.

Dines, R.A. & JAD Murray-Bligh (2000). Quality assurance and RIVPACS. In: Wright, J.F., D.W. Sutcliffe and M.T. Furse, *Assessing the biological quality of fresh waters: RIVPACS and other techniques*. Ambleside, Freshwater Biological Association.

Kelly, M. G. & B. A. Whitton (1995). The Trophic Diatom Index: a new index for monitoring eutrophication in rivers. *Journal of Applied Phycology*, **7**: 433-444.

Kinley, R.D amd J.C. Ellis (1991) *The application of statistical quality control methods to macroinvertebrate sampling.* Report for the National Rivers Authority, Thames Region. WRc Report No. CO2905. Medmenham: Water Research Centre.

Kovach Computing Services (2002). *Multi-Variate Statistics Package. v. 3.13b*.
Krammer, K., H. Lange-Bertalot, N. Bate, A. Podzorski & J. Bukowska (1986-1998). *Bacillariophyceae.* Stuttgart, Fischer Verlag. 5 parts.

Lecointe, C., M. Coste & J. Prygiel (2003). *Omidia 3.2. Diatom index software including diatom database with taxonomic names, references and codes of 11645 diatom taxa.*

Leclercq, L. & B. Maquet (1987). Deux noveaux metrics diatomique et the qualite chimique des eaux courantes. Comparison avec differents metrics existants. *Cah. Biol. Mar.,* 28: 303-310.

Murray-Bligh, JAD (2003). *UK invertebrate sampling and analysis procedure for STAR project.* Downloadable from http://www.aqem.de/start.htm.

Murray-Bligh, J.A.D (2004). Sampling workshops – an overview. Star (ETK1-CT-2001-00089) Deliverable 5a.

Prygiel, J. & M. Coste (1999). Progress in the use of diatoms for monitoring rivers in France. In: J. Prygiel, B. A. Whitton & J. Bukowska. *Use of algae for monitoring rivers III*. Douai, Agence de l'Eau Artois-Picardie: 165-179.

Prygiel, J., P. Carpentier, S. Almeida, M. Coste, J. C. Druart, L. Ector, D. Guillard, M. A. Honore, R. Iserentant, P. Ledeganck, C. Lalanne-Cassou, C. Lesniak, I. Mercier, P. Moncaut, M. Nazart, N. Nouchet, F. Peres, V. Peeters, F. Rimet, A. Rumeau, S. Sabater, F. Straub, M. Torrisi, L. Tudesque, B. Van de Vijver, H. Vidal, J. Vizinet & N. Zydek (2002). Determination of the biological diatom index (IBD NF T 90-354): results of an intercomparison exercise. *Journal of Applied Phycology*, **14** (1): 27-39.

Rott, E., P. Pfister, H. Van Dam, E. Pipp, K. Pall, N. Binder & K. Ortler (1999). *Indikationslisten für aufwuchsalgen.* Wien, Bundesministerium für Land - und Forstwirtschaft: 248.

Sandin, L., Friberg, N, Clarke, R. T. and Furse, M.T. (2005). Inter-calibration and harmonisation of invertebrate methods. Star (ETK1-CT-2001-00089) Deliverable 8.

Sládeček, V. (1986). Diatoms as indicators of organic pollution. *Acta Hydrochimica et Hydrobiologica*, **14** (5): 555-566.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry: the principles and practice of statistics in biological research.* 3rd edition Freeman and Company, New York.

Steinberg, C. & S. Schiefele (1988). Biological indication of trophy and pollution of running waters. *Z. Wasser-Abwasser-Forsch.*, 21: 227-234.

Van der Molen, J.S. & P.F.M. Verdonschot (2002*). Sampling protocol and audit benthic diatoms. STAR sampling protocol*. Downloadable from http://www.eu-star.at. Wageningen: 15 pp.

Wallin, M. Wiederholm, T. & Johnson R.K. (2003). *REFCOND: Guidance on establishing reference conditions and ecological status class boundaries for inland surface waters.*

APPENDIX A PLANNED DISTRIBUTION OF INVERTEBRATE AUDIT SAMPLES AND IDENTIFICATION AUDITORS

Stream type group	Stream type/country	Primary analyst	Auditor
Mountain streams Central Europe: core stream type 1, additional stream types from France, Germany, Sweden, CZ, UK	Additional stream type UK	Partner 1 (UK)	Partner 5 (S)
	Core stream type 1 (Germany)	Partner 2 (Germany)	Partner 22 (SK)
	Core stream type 1 (Austria)	Partner 3 (Austria)	Partner 6 (CZ)
	Additional stream type (Sweden)	Partner 5 (Sweden)	Partner 1 (UK)
	Core stream type 1 (Czech Republic)	Partner 6 (CZ)	Partner 22 (SK)
	Additional stream type (Czech Republic)	Partner 6 (CZ)	Partner 3 (A)
	Additional stream type (France)	Partner 14 (France)	Partner 15 (D)
	Additional stream type (Germany)	Partner 15 (Germany)	Partner 14 (F)
	Core stream type 1a (West Carpathians) quality class 3 4 & 5 (Slovakia)	Partner 22 (Slovakia)	Partner 6 (CZ)
	Core stream type 1a (West Carpathians) - quality class 1 & 2 (Slovakia)	Partner 22 (Slovakia)	Partner 2 (D)
Core stream type 2	Core stream type 2 (UK)	Partner 1 (UK)	Partner 2 (D)
	Core stream type 2 (Germany)	Partner 2 (Germany)	Partner 1 (UK)
	Core stream type 2 (Sweden)	Partner 5 (Sweden)	Partner 10 (DK)
	Core stream type 2 (Denmark)	Partner 10 (Denmark)	Partner 5 (S)
	Core stream type 2a (Poland)	Partner 17 (Poland)	Partner 20 (L)
	Core stream type 2b (Poland)	Partner 17 (Poland)	Partner 20 (L)
	Core stream type 2a (Latvia)	Partner 20 (Latvia)	Partner 17 (Pl)
	Core stream type 2b (Latvia)	Partner 20 (Latvia)	Partner 17 (Pl)
Mediterranean streams	Additional stream type (Greece)	Partner 7 (Greece)	Partner 8 (I)
	Additional stream type (Italy, Apennines)	Partner 8 (Italy)	Partner 9 (P)
	additional stream type (Portugal)	Partner 9 (Portugal)	Partner 7 (GR)
Alpine streams	additional stream type (Austria)	Partner 3 (Austria)	Partner 13 (I)*
	additional stream type (Italy, Alps)	Partner 13 (Italy)*	Partner 3 (A)

Partner 13 was unable to audit samples – Austrian additional stream type samples were audited by Partner 6 (CZ)

Samples from Partner 13 were not audited because no replicate samples were taken by this partner

APPENDIX B QUALITY CONTROLLERS FOR THE INVERTEBRATE AUDIT

For all partners, the quality controller is also the contact for the identification audit.

Partner	Country	Quality Controller
1 CEH Dorset	UK	Rick Gunn
2 University of Duisburg-Essen	D	Sandra Kramm
3 BOKU	A	Ilse Stubauer
4 Alterra	NL	N/A
5 SLU	S	Lars Eriksson
6 Masaryk University	CZ	Libuse Opatrilova
7 National Centre for Marine Research	GK	Kostantinos Gritzalis
8 ISRA-CNR	I	Marcello Cazzola
9 University of Evora	Pt	Elsa Mourinha
10 NERI	DK	Jens Skrivner
11 Environment Agency	UK	N/A
12 Masaryk Water Research Agency	CZ	N/A
13 Province of Bolzano	I	Anna Mutschlechner
14 University of Metz	F	Virginie Archaimbault
15 Research Institute Senckenberg	D	Peter Haase
16 CEN		N/A
17 University of Łodz	PL	Malgorzata Slabiak
18 Agricultural University Poznan	PL	N/A
19 Inst. Environmental Protection, Warszaw	PL	N/A
20 University of Latvia	L	Elga Parele
21 Slovak Academy of Sciences	SK	Ferdinand Sporka
22 Comenius University	SK	Ilja Krno

APPENDIX C COMPLETE LIST OF INVERTEBRATE AUDIT SAMPLES AND THEIR IDENTIFICATION AUDITORS

- • = sorting audit only, not identification audit

Partner No.	Sample type	Season	Site No.	Sample Number	River	Site	Sample date	ID auditor	*
1 (UK)	AQEM (Main)	Autumn	674	U2310763	Clun	Marlow	28/09/2002	2 (D)	
1 (UK)	AQEM (Main)	Spring	678	U2310181	Ogmore	Bridgend	09/04/2003	2 (D)	
1 (UK)	AQEM (Main)	Autumn	681	U2310833	Sirhowy	Ynysddu	27/09/2002	2 (D)	
1 (UK)	RIVPACS (Main)	Autumn	674	U2311073	Clun	Marlow	28/09/2002	2 (D)	
1 (UK)	RIVPACS (Main)	Spring	678	U2310491	Ogmore	Bridgend	09/04/2003	2 (D)	
1 (UK)	RIVPACS (Main)	Autumn	681	U2311143	Sirhowy	Ynysddu	27/09/2002	2 (D)	
1 (UK)	AQEM (Main)	Spring	639	U1510011	Ecchinswell Brook	Headley	07/04/2003	5 (S)	
1 (UK)	AQEM (Main)	Autumn	642	U1510663	Westbury Brook	Westbury	08/10/2002	5 (S)	
1 (UK)	AQEM (Main)	Spring	648	U1510101	Cliff Brook	Crowton	13/04/2003	5 (S)	
1 (UK)	RIVPACS (Main)	Spring	639	U1510321	Ecchinswell Brook	Headley	07/04/2003	5 (S)	
1 (UK)	RIVPACS (Main)	Autumn	642	U1510973	Westbury Brook	Westbury	08/10/2002	5 (S)	
1 (UK)	RIVPACS (Main)	Spring	648	U1510411	Cliff Brook	Crowton	13/04/2003	5 (S)	
2 (D)	AQEM (Main)	Spring	649	D0300201	Stepenitz	Near Putlitz	10/04/2003	1 (UK)	
2 (D)	AQEM (Main)	Summer	649	D0300202	Stepenitz	Near Putlitz	15/07/2002	1 (UK)	
2 (D)	RIVPACS (Main)	Spring	649	D0300351	Stepenitz	Near Putlitz	10/04/2003	1 (UK)	
2 (D)	RIVPACS (Main)	Summer	649	D0300352	Stepenitz	Near Putlitz	15/07/2002	1 (UK)	
2 (D)	AQEM (Main)	Spring	634	D0400461	Salwey	Niedersalwey	25/03/2003	22 (SK)	
2 (D)	AQEM (Main)	Summer	627	D0400392	Wehebach	Wehebachtalsperre	29/06/2002	22 (SK)	
2 (D)	RIVPACS (Main)	Spring	634	D0400581	Salwey	Niedersalwey	25/03/2003	22 (SK)	
2 (D)	RIVPACS (Main)	Summer	627	D0400512	Wehebach	Wehebachtalsperre	29/06/2002	22 (SK)	
3 (A)	AQEM (Main)	Spring	600	A0500261	Sarmingbach	Wolfsschlucht	16/04/2003	6 (CZ)	
3 (A)	AQEM (Main)	Spring	603	A0500291	Grosse Ysper	near Altenmarkt	16/04/2003	6 (CZ)	
3 (A)	AQEM (Main)	Summer	607	A0500332	Sarmingbach	Waldhausen	09/07/2002	6 (CZ)	
3 (A)	AQEM (Main)	Spring	701	A0600141	Wildbach	near Kramermirtl	28/05/2003	6 (CZ)	
3 (A)	AQEM (Main)	Summer	706	A0600192	Stullneggbach	near Aichegg	30/07/2002	6 (CZ)	
3 (A)	AQEM (Main)	Summer	708	A0600232	Stullneggbach	near Mainsdorf	30/07/2002	6 (CZ)	
3 (A)	RIVPACS (Main)	Spring	600	A0500431	Sarmingbach	Wolfsschlucht	16/04/2003	6 (CZ)	
3 (A)	RIVPACS (Main)	Spring	603	A0500462	Grosse Ysper	near Altenmarkt	16/04/2003	6 (CZ)	
3 (A)	RIVPACS (Main)	Summer	607	A0500502	Sarmingbach	Waldhausen	09/07/2002	6 (CZ)	
3 (A)	RIVPACS (Main)	Spring	701	A0600341	Wildbach	near Kramermirtl	28/05/2003	6 (CZ)	
3 (A)	RIVPACS (Main)	Summer	706	A0600392	Stullneggbach	near Aichegg	30/07/2002	6 (CZ)	
3 (A)	RIVPACS (Main)	Summer	708	A0600432	Stullneggbach	near Mainsdorf	30/07/2002	6 (CZ)	
5 (S)	AQEM (Main)	Autumn	875	S0601193	Forsmarksan	Johannisfors	30/10/2002	1 (UK)	
5 (S)	AQEM (Main)	Autumn	876	S0601293	Hågaån	Lurbo	19/11/2002	1 (UK)	
5 (S)	AQEM (Main)	Spring	878	S0601561	Strömaran	Not known	22/05/2003	1 (UK)	
5 (S)	Swedish (Main)	Autumn	875	S0602153	Forsmarksan	Johannisfors	30/10/2002	1 (UK)	
5 (S)	Swedish (Main)	Autumn	876	S0602253	Hågaån	Lurbo	19/11/2002	1 (UK)	
5 (S)	Swedish (Main)	Spring	878	S0602521	Strömaran	Not known	22/05/2003	1 (UK)	
5 (S)	AQEM (Main)	Spring	685	S0501351	Nittälven	D/S Nordtjärnsälven	04/06/2003	10 (DK)	
5 (S)	AQEM (Main)	Autumn	689	S0501063	Sävälven	Upstream Sävefors	23/10/2002	10 (DK)	
5 (S)	AQEM (Main)	Spring	691	S0501431	Hörksälven	Brattforsen	04/06/2003	10 (DK)	
5 (S)	Swedish (Main)	Spring	685	S0502311	Nittälven	D/S Nordtjärnsälven	04/06/2003	10 (DK)	
5 (S)	Swedish (Main)	Autumn	689	S0502023	Sävälven	Upstream Sävefors	23/10/2002	10 (DK)	
5 (S)	Swedish (Main)	Spring	691	S0502391	Hörksälven	Brattforsen	04/06/2003	10 (DK)	
6 12 (CZ)	AQEM (Main)	Spring	614	C0401621	Velka Hana	Rychtarov	04/04/2003	22 (SK)	
6 12 (CZ)	AQEM (Main)	Spring	620	C0401701	Nectava	Brezinky	27/03/2003	22 (SK)	
6 12 (CZ)	AQEM (Main)	Summer	625	C0401172	Umori	Zbraslavec	19/07/2002	22 (SK)	
6 12 (CZ)	PERLA (Main)	Spring	614	C0403561	Velka Hana	Rychtarov	04/04/2003	22 (SK)	
6 12 (CZ)	PERLA (Main)	Spring	620	C0403631	Nectava	Brezinky	27/03/2003	22 (SK)	
6 12 (CZ)	PERLA (Main)	Summer	625	C0403152	Umori	Zbraslavec	19/07/2002	22 (SK)	
6 12 (CZ)	AQEM (Main)	Summer	713	C0501212	Huntava	Valsovsky dul	26/07/2002	3 (A)	
6 12 (CZ)	AQEM (Main)	Summer	717	C0501272	Luha	Sloup	22/07/2002	3 (A)	
6 12 (CZ)	AQEM (Main)	Spring	722	C0501941	Trebuvka	Borsov	09/04/2003	3 (A)	
6 12 (CZ)	PERLA (Main)	Summer	713	C0503182	Huntava	Valsovsky dul	26/07/2002	3 (A)	
6 12 (CZ)	PERLA (Main)	Summer	717	C0503232	Luha	Sloup	22/07/2002	3 (A)	
6 12 (CZ)	PERLA (Main)	Spring	722	C0503831	Trebuvka	Borsov	09/04/2003	3 (A)	
7 (GR)	AQEM (Replicate)	Summer	735	H0400222	Peristeria	Artiki	29/07/2002	8 (I)	
7 (GR)	AQEM (Replicate)	Summer	737	H0400242	Tsouraki	Tsouraki	01/08/2002	8 (I)	
7 (GR)	AQEM (Main)	Spring	738	H0400051	Tsouraki	SL 98	21/05/2003	8 (I)	
7 (GR)	AQEM (Main)	Spring	739	H0400031	Krathis	Tsivlos	22/05/2003	8 (I)	
7 (GR)	AQEM (Replicate)	Summer	753	H0400262	Gadouras	Gadouras	24/08/2002	8 (I)	
7 (GR)	AQEM (Main)	Spring	756	H0400011	Gorgopotamos	Gorgopotamos Bridge	18/05/2003	8 (I)	
7 (GR)	RIVPACS (Replicate)	Summer	735	H0400282	Peristeria	Artiki	29/07/2002	8 (I)	
7 (GR)	RIVPACS (Replicate)	Summer	737	H0400302	Tsouraki	Tsouraki	01/08/2002	8 (I)	
7 (GR)	RIVPACS (Main)	Spring	738	H0400151	Tsouraki	SL 98	21/05/2003	8 (I)	
7 (GR)	RIVPACS (Main)	Spring	739	H0400131	Krathis	Tsivlos	22/05/2003	8 (I)	
7 (GR)	RIVPACS (Replicate)	Summer	753	H0400322	Gadouras	Gadouras	24/08/2002	8 (I)	
7 (GR)	RIVPACS (Main)	Spring	756	H0400111	Gorgopotamos	Gorgopotamos Bridge	18/05/2003	8 (I)	
8 (I)	AQEM (Main)	Spring	836	I0601205	Albegna	Roccalbegna	01/05/2003	9 (P)	
8 (I)	AQEM (Main)	Spring	837	I0609205	Merse	Monticiano	07/05/2003	9 (P)	
8 (I)	AQEM (Main)	Spring	840	I0611205	Senna	Piancastagnano	02/05/2003	9 (P)	
8 (I)	AQEM (Main)	Spring	843	I0606205	Fiora	Cellena	05/05/2003	9 (P)	
8 (I)	AQEM (Main)	Spring	845	I0612205	Zancona	Zancona	04/05/2003	9 (P)	
8 (I)	AQEM (Main)	Spring	842	I0607204	Fiora	Fiora downstream farm S. Fiora (GR)	30/04/2003	9 (P)	
8 (I)	IBE (Main)	Spring	836	I0601405	Albegna	Roccalbegna	01/05/2003	9 (P)	
8 (I)	IBE (Main)	Spring	837	I0609405	Merse	Monticiano	07/05/2003	9 (P)	
8 (I)	IBE (Main)	Spring	840	I0611405	Senna	Piancastagnano	02/05/2003	9 (P)	
8 (I)	IBE (Main)	Spring	843	I0606405	Fiora	Cellena	05/05/2003	9 (P)	
8 (I)	IBE (Main)	Spring	845	I0612405	Zancona	Zancona	04/05/2003	9 (P)	
8 (I)	IBE (Main)	Spring	842	I0607404	Fiora	Fiora downstream farm S. Fiora (GR)	30/04/2003	9 (P)	

Complete list of invertebrate audit samples and their identification auditors, cont.

Partner No.	Sample type	Season	Site No.	Sample Number	River	Site	Sample date	ID auditor	*
9 (PT)	AQEM (Main)	Autumn	863	P0411313	Xévora	Xévora	03/02/2003	7 (GR)	
9 (PT)	AQEM (Main)	Autumn	864	P0411133	Tripeiro	Tripeiro	06/12/2002	7 (GR)	
9 (PT)	AQEM (Main)	Spring	865	P0411121	Taveiró	Taveiró	13/05/2003	7 (GR)	
9 (PT)	AQEM (Main)	Autumn	866	P0411213	Alpreade	Alpreade	05/12/2002	7 (GR)	
9 (PT)	AQEM (Main)	Spring	867	P0411321	Ponsul	Ponsul	14/05/2003	7 (GR)	
9 (PT)	AQEM (Main)	Spring	868	P0411221	Baságueda	Baságueda	14/05/2003	7 (GR)	
9 (PT)	National? (Main)	Autumn	863	P0431313	Xévora	Xévora	03/02/2003	7 (GR)	
9 (PT)	National? (Main)	Autumn	864	P0431133	Tripeiro	Tripeiro	06/12/2002	7 (GR)	
9 (PT)	National? (Main)	Spring	865	P0431121	Taveiró	Taveiró	13/05/2003	7 (GR)	
9 (PT)	National? (Main)	Autumn	866	P0431213	Alpreade	Alpreade	05/12/2002	7 (GR)	
9 (PT)	National? (Main)	Spring	867	P0431321	Ponsul	Ponsul	14/05/2003	7 (GR)	
9 (PT)	National? (Main)	Spring	868	P0431221	Baságueda	Baságueda	14/05/2003	7 (GR)	
10 (DK)	AQEM (Main)	Spring	662	K0201011	Karstoft	Noerre Grene	01/04/2003	5 (S)	
10 (DK)	AQEM (Main)	Spring	667	K0206011	Kastbjerg	Edderup	01/04/2003	5 (S)	
10 (DK)	AQEM (Main)	Spring	668	K0209011	Skibsted	Skibstedbro	07/04/2003	5 (S)	
10 (DK)	AQEM (Main)	Summer	663	K0202012	Mattrup	Stids Moelle	06/08/2002	5 (S)	
10 (DK)	AQEM (Main)	Summer	668	K0207012	Fjederholt	Okkels	08/08/2002	5 (S)	
10 (DK)	AQEM (Main)	Summer	671	K0210012	Skals	Faarup	12/08/2002	5 (S)	
10 (DK)	DSFI (Main)	Spring	662	K0201021	Karstoft	Noerre Grene	01/04/2003	5 (S)	
10 (DK)	DSFI (Main)	Spring	667	K0206021	Kastbjerg	Edderup	01/04/2003	5 (S)	
10 (DK)	DSFI (Main)	Spring	668	K0209021	Skibsted	Skibstedbro	07/04/2003	5 (S)	
10 (DK)	DSFI (Main)	Summer	663	K0202022	Mattrup	Stids Moelle	06/08/2002	5 (S)	
10 (DK)	DSFI (Main)	Summer	668	K0207022	Fjederholt	Okkels	08/08/2002	5 (S)	
10 (DK)	DSFI (Main)	Summer	671	K0210022	Skals	Faarup	12/08/2002	5 (S)	
14 (F)	AQEM (Main)	Autumn	724	F0800013	Aube	Aubepierre-sur-Aube	25/09/2002	15 (D)	
14 (F)	AQEM (Main)	Spring	725	F0800021	Seine	Ermitage du Val de Seine	15/04/2003	15 (D)	
14 (F)	AQEM (Main)	Spring	726	F0800041	Aujon	u/s Giey-sur-Aujon	25/05/2003	15 (D)	
14 (F)	AQEM (Main)	Autumn	728	F0800063	Ornain	d/s Abainville	30/09/2002	15 (D)	
14 (F)	AQEM (Main)	Autumn	729	F0800073	Meuse (Bassoncourt)	Between Daillecourt & Bassoncourt	10/10/2002	15 (D)	
14 (F)	AQEM (Main)	Spring	733	F0800111	Mouzon	Sartes	09/04/2003	15 (D)	
14 (F)	IBGN (Main)	Autumn	724	F0800193	Aube	Aubepierre-sur-Aube	25/09/2002	15 (D)	
14 (F)	IBGN (Main)	Spring	725	F0800201	Seine	Ermitage du Val de Seine	15/04/2003	15 (D)	
14 (F)	IBGN (Main)	Spring	726	F0800221	Aujon	u/s Giey-sur-Aujon	25/05/2003	15 (D)	
14 (F)	IBGN (Main)	Autumn	728	F0800243	Ornain	d/s Abainville	30/09/2002	15 (D)	
14 (F)	IBGN (Main)	Autumn	729	F0800253	Meuse (Bassoncourt)	Between Daillecourt & Bassoncourt	10/10/2002	15 (D)	
14 (F)	IBGN (Main)	Spring	733	F0800291	Mouzon	Sartes	09/04/2003	15 (D)	
15 (D)	AQEM (Main)	Spring	816	D0600021	Ilme	Above Relliehausen	26/03/2003	14 (F)	*
15 (D)	AQEM (Main)	Summer	816	D0600022	Ilme	Above Relliehausen	21/06/2002	14 (F)	
15 (D)	AQEM (Main)	Spring	821	D0600071	Klingbach	Above Hausen	11/03/2003	14 (F)	
15 (D)	AQEM (Main)	Summer	821	D0600072	Klingbach	Above Hausen	06/06/2002	14 (F)	*
15 (D)	RIVPACS (Main)	Spring	816	D0600121	Ilme	Above Relliehausen	26/03/2003	14 (F)	*
15 (D)	RIVPACS (Main)	Summer	816	D0600122	Ilme	Above Relliehausen	21/06/2002	14 (F)	
15 (D)	RIVPACS (Main)	Spring	821	D0600171	Klingbach	Above Hausen	11/03/2003	14 (F)	
15 (D)	RIVPACS (Main)	Summer	821	D0600172	Klingbach	Above Hausen	06/06/2002	14 (F)	*
17 (PL)	AQEM (Main)	Spring	895	O0200021	Dobrzyca (profile Czapla)	Czapla	16/05/2003	20 (L)	
17 (PL)	AQEM (Main)	Autumn	897	O0201443	Pliszka (profile Konotop)	Drzewce	08/11/2003	20 (L)	
17 (PL)	AQEM (Main)	Spring	903	O0200101	Ner (profile Lutomiersk)	Lutomiersk	28/05/2003	20 (L)	
17 (PL)	AQEM (Main)	Autumn	913	O0202253	lutownia (profile Pogorzelce)	Stara Bialowieza	14/10/2003	20 (L)	
17 (PL)	AQEM (Main)	Autumn	1036	O0203643	Lesna Prawa (Hajnowka)	Hajnowka	14/10/2003	20 (L)	
17 (PL)	AQEM (Main)	Spring	916	O0200881	Rospuda (profile Jozefowo)	Jozefowo	03/05/2003	20 (L)	
17 (PL)	PP (Main)	Spring	895	O0200191	Dobrzyca (profile Czapla)	Czapla	16/05/2003	20 (L)	
17 (PL)	PP (Main)	Autumn	897	O0201693	Pliszka (profile Konotop)	Drzewce	08/11/2003	20 (L)	
17 (PL)	PP (Main)	Spring	903	O0200591	Ner (profile Lutomiersk)	Lutomiersk	28/05/2003	20 (L)	
17 (PL)	PP (Main)	Autumn	913	O0202613	lutownia (profile Pogorzelce)	Stara Bialowieza	14/10/2003	20 (L)	
17 (PL)	PP (Main)	Autumn	1036	O0203653	Lesna Prawa (Hajnowka)	Hajnowka	14/10/2003	20 (L)	
17 (PL)	PP (Main)	Spring	916	O0201361	Rospuda (profile Jozefowo)	Jozefowo	03/05/2003	20 (L)	
20 (L)	AQEM (Main)	Autumn	997	L0201233	Kekava	In the park area of Kekava village	01/10/2003	17 (PL)	
20 (L)	AQEM (Main)	Autumn	1006	L0200953	Tumsupe	Above Podkajas farmstead	09/09/2003	17 (PL)	
20 (L)	AQEM (Main)	Spring	1007	L0200621	Veseta	Nearby Vietalva	18/06/2003	17 (PL)	
20 (L)	AQEM (Main)	Spring	1017	L0200301	Age	Lower part of river in Saulkrasti town	03/06/2003	17 (PL)	
20 (L)	AQEM (Main)	Spring	1010	L0200401	Raunis	Lower part	08/06/2003	17 (PL)	
20 (L)	AQEM (Main)	Autumn	1016	L0201213	Strikupe	Lower part	30/09/2003	17 (PL)	
20 (L)	LVS 240:1999 (Main)	Autumn	997	L0201533	Kekava	In the park area of Kekava village	01/10/2003	17 (PL)	
20 (L)	LVS 240:1999 (Main)	Autumn	1006	L0201253	Tumsupe	Above Podkajas farmstead	09/09/2003	17 (PL)	
20 (L)	LVS 240:1999 (Main)	Spring	1007	L0200931	Veseta	Nearby Vietalva	18/06/2003	17 (PL)	*
20 (L)	LVS 240:1999 (Main)	Spring	1017	L0200781	Age	Lower part of river in Saulkrasti town	03/06/2003	17 (PL)	
20 (L)	LVS 240:1999 (Main)	Spring	1010	L0200841	Raunis	Lower part	08/06/2003	17 (PL)	
20 (L)	LVS 240:1999 (Main)	Autumn	1016	L0201513	Strikupe	Lower part	30/09/2003	17 (PL)	
22 (SK)	AQEM (Main)	Autumn	988	V0100423	Hostiansky potok	pri Pod Javorom	16/09/2003	2 (D)	
22 (SK)	AQEM (Main)	Autumn	989	V0100443	Hostiansky potok	pod Obecným vrchom	16/09/2003	2 (D)	
22 (SK)	AQEM (Main)	Autumn	984	V0100473	Bystrica	pod Vel'kou skalou	17/09/2003	2 (D)	
22 (SK)	PERLA (Main)	Autumn	988	V0100433	Hostiansky potok	pri Pod Javorom	16/09/2003	2 (D)	
22 (SK)	PERLA (Main)	Autumn	989	V0100453	Hostiansky potok	pod Obecným vrchom	16/09/2003	2 (D)	
22 (SK)	PERLA (Main)	Autumn	984	V0100483	Bystrica	pod Vel'kou skalou	17/09/2003	2 (D)	
22 (SK)	AQEM (Main)	Autumn	990	V0100563	Hostiansky potok	nad Topolèiankami	18/09/2003	6 (CZ)	
22 (SK)	AQEM (Main)	Autumn	986	V0100493	Bystrica	Horná domovina	17/09/2003	6 (CZ)	
22 (SK)	AQEM (Main)	Autumn	987	V0100513	Bystrica	Bystrièany	17/09/2003	6 (CZ)	
22 (SK)	PERLA (Main)	Autumn	990	V0100463	Hostiansky potok	nad Topolèiankami	18/09/2003	6 (CZ)	
22 (SK)	PERLA (Main)	Autumn	986	V0100503	Bystrica	Horná domovina	17/09/2003	6 (CZ)	
22 (SK)	PERLA (Main)	Autumn	987	V0100523	Bystrica	Bystrièany	17/09/2003	6 (CZ)	

APPENDIX D BLANK INVERTEBRATE SORTING AUDIT RESULTS SHEETS

Invertebrate sorting audit results sheet for normal analyses

STAR SORTING AUDIT		Auditor:	Audit Date:

Found by auditor	Taxa	In AQEM-DIP	Additions	Found by auditor	Taxa	In AQEM-DIP	Additions

"Families" found in audit including additions to the database

Invertebrate sorting audit results sheet, for analyses where only a fraction of the sample has been sorted

STAR SORTING AUDIT										**Auditor:**					**Audit Date:**

Method:		**STAR Partner/Country:**		**Partner No:**

River:		**Site:**		**Sample Date:**

Site Code:		**Sample Code:**	

Found by auditor	**Taxa**	In AQEM-DIP	Additions	*Sorted part (All)* Found by auditor *Unsorted part*	**Taxa**	In AQEM-DIP	Additions
	"Families" found in audit including additions to the database						

APPENDIX E BLANK INVERTEBRATE IDENTIFICATION AUDIT RESULTS SHEET

STAR IDENTIFICATION AUDIT										
						Auditor:			**Audit Date:**	
Method:				**STAR Partner/Country:**					**Partner No:**	
River:				**Site:**					**Sample Date:**	
Site Code:				**Sample Code:**				**Counted taxa:**		
Taxa	In primary analyst's vial	In sorting auditor's vial	Primary results in AQEM-DIP	Additions		**Taxa**	In primary analyst's vial	In sorting auditor's vial	Primary results in AQEM-DIP	Additions
	Leave this space blank									Sheet 1 of 1
	Do not add anything outside this border because It will not print-out on A4 size paper									

APPENDIX F INVERTEBRATE IDENTIFICATION AUDIT RESULTS SHEET EXEMPLAR

STAR IDENTIFICATION AUDIT					Auditor: XX		Audit Date: 28.7.2004		

Method: AQEM	STAR Partner/Country: ABCD (UK)						Partner No: 0		

River: Beautiful River		Site: Utopia					Sample Date: 1/4/2003		

Site Code: 000	Sample Code: U1234567				Counted taxa: Oligochaeta, Simuliidae				

Taxa	In primary analyst's vial	In sorting auditor's vial	Primary results in AQEM-DIP	Gains & Losses	Taxa	In primary analyst's vial	In sorting auditor's vial	Primary results in AQEM-DIP	Gains & Losses
Dendrocoelum lacteum		✔		G	Sericostoma personatum	✔		✔	
Radix labiata	✔		✔		Ceratopogonidae Gen. sp.	✔		✔	
Pisidium sp.	✔		✔		Chironomidae	✔	✔	✔	
Oligochaeta	✔	(✔)	✔		Hemerodromia-Gr. Gen. sp.	✔		✔	
Helobdella stagnalis	✔		✔		Dicranota sp.	✔		✔	
Erpobdella octoculata	✔			G	Simulium angustitarse/lundstromi	✔		✔	
Hirudo medicinalis			✔	(L)	Simulium aureum-Gr.	✔	(✔)	✔	
Hydrachnidia Gen. sp.	✔		✔		Simulium cryophilum-Gr.	✔		✔	
Gammarus pulex	✔	✔	✔		Simulium erythrocephalum	✔			G
Baetis rhodani	✔		✔		Simulium ornatum-Gr.	✔		✔	
Baetis vernus	✔		✔						
Ephemera sp.	✔	✔		G					
Paraleptophlebia submarginata	✔		✔						
Calopteryx sp.		✔	✔						
Leuctra fusca	✔		✔						
Nemoura avicularis	✔		✔						
Hesperocorixa sahlbergi	✔		✔						
Sialis nigripes	✔		✔						
Curculionidae		[✔]		[G]					
Platambus maculatus Ad.	✔		✔						
Platambus maculatus Lv.	✔		✔						
Elmis aenea Lv.	✔		✔						
Esolus parallelepipedus Lv.	✔		✔						
Limnius volckmari Lv.	✔		✔						
Oulimnius sp. Lv.		✔	✔	G					
Hydraena gracilis Ad.	✔		✔						
Agapetus sp.	✔		✔						
Goera pilosa	✔		✔						
Hydropsyche siltalai	✔		✔						
Ithytrichia sp.	✔		✔						
Lasiocephala basalis	✔		✔						
Lepidostomatidae Gen. sp.	✔		✔						
Athripsodes albifrons	✔		✔						
Athripsodes bilineatus	✔		✔						
Athripsodes cinereus	✔		✔						
Athripsodes sp.	✔		✔						
Mystacides azurea	✔			G					
Mystacides nigra			✔	L					
Hydatophylax infumatus	✔		✔						
Limnephilus fuscicornis	✔		✔						
Potamophylax cingulatus/latipenni	✔		✔						

Leave this space blank							Sheet 1 of 1	

Do not add anything outside this border because It will not print-out on A4 size paper			

APPENDIX G LIST OF TAXA INCLUDED IN THE INVERTEBRATE SORTING AUDIT ANALYSES

TaxaGroup	ID_FAM	Family	Audit taxon	Taxa excluded
Araneae	689	[Ord:Aranea]		
Araneae	192	ARGYRONETIDAE	ARGYRONETIDAE	
Bivalvia	547	[Kl:Bivalvia]		
Bivalvia	697	CORBICULIDAE	CORBICULIDAE	
Bivalvia	256	DREISSENIDAE	DREISSENIDAE	
Bivalvia	335	MARGARITIFERIDAE	MARGARITIFERIDAE	*Margaritifera margaritifera*
Bivalvia	411	SPHAERIIDAE	SPHAERIIDAE	
Bivalvia	433	UNIONIDAE	UNIONIDAE	
Bryozoa	605	[Kl:Bryozoa]		
Coelenterata	559	[Kl:Hydrozoa]		
Coelenterata	694	[St:Coelenterata]		
Coelenterata		OLINIIDAE	OLINIIDAE *Craspedacusta*	
Coleoptera	557	[Ord:Coleoptera]		
Coleoptera	257	DRYOPIDAE	DRYOPIDAE	
Coleoptera	259	DYTISCIDAE	DYTISCIDAE	
Coleoptera	261	ELMIDAE	ELMIDAE	
Coleoptera	281	GYRINIDAE	GYRINIDAE	
Coleoptera	283	HALIPLIDAE	HALIPLIDAE	
Coleoptera	287	HELOPHORIDAE	HELOPHORIDAE	
Coleoptera	296	HYDRAENIDAE	HYDRAENIDAE	
Coleoptera	299	HYDROCHIDAE	HYDROCHIDAE	
Coleoptera	302	HYDROPHILIDAE	HYDROPHILIDAE	
Coleoptera	308	HYGROBIIDAE	HYGROBIIDAE	
Coleoptera	352	NOTERIDAE	NOTERIDAE	
Coleoptera	512	PSEPHENIDAE	PSEPHENIDAE	
Coleoptera	440	SCIRTIDAE	SCIRTIDAE	
Coleoptera	409	SPERCHEIDAE	SPERCHEIDAE	
Coleoptera	713	SPHAERIUSIDAE	SPHAERIUSIDAE	
Crustacea	556	[Kl:Crustacea]		
Crustacea	596	[Ord:Amphipoda]		
Crustacea	585	[Ord:Anostraca]		
Crustacea	691	[Ord:Conchostraca]		
Crustacea	602	[Ord:Decapoda]		
Crustacea	601	[Ord:Isopoda]		
Crustacea	693	[Ord:Mysidacea]		
Crustacea	586	[Ord:Notostraca]		
Crustacea	630	[UKl:Copepoda]		
Crustacea	690	[UOrd:Cladocera]		
Crustacea	191	ARGULIDAE	ARGULIDAE	
Crustacea	672	ARTEMIIDAE	ARTEMIIDAE	
Crustacea	577	ASELLIDAE	ASELLIDAE	
Crustacea	196	ASTACIDAE	ASTACIDAE	*Austropotamobius pallipes*
Crustacea	588	ATYIDAE	ATYIDAE	
Crustacea	207	BOGIDIELLIDAE	BOGIDIELLIDAE	
Crustacea	471	BRANCHINECTIDAE	BRANCHINECTIDAE	
Crustacea	213	BRANCHIPODIDAE	BRANCHIPODIDAE	
Crustacea	216	CAMBARIDAE	CAMBARIDAE	
Crustacea	476	CHIROCEPHALIDAE	CHIROCEPHALIDAE	
Crustacea	235	COROPHIIDAE	COROPHIIDAE	
Crustacea	671	CRANGONIDAE	CRANGONIDAE	
Crustacea	480	CRANGONYCITIDAE	CRANGONYCITIDAE	
Crustacea	243	CYTHERIDAE		
Crustacea	244	CYZICIDAE	CYZICIDAE	
Crustacea	715	GAMMARACANTHIDAE		
Crustacea	272	GAMMARIDAE	GAMMARIDAE	
Crustacea	280	GRAPSIDAE	GRAPSIDAE	
Crustacea	448	HAUSTORIIDAE	HAUSTORIIDAE	
Crustacea	573	IDOTEIDAE	IDOTEIDAE	
Crustacea	493	IMNADIIDAE	IMNADIIDAE	

List of taxa included in invertebrate sorting audit analyses, cont.

TaxaGroup	ID_FAM	Family	Audit taxon	Taxa excluded
Crustacea	579	JANIRIDAE	JANIRIDAE	
Crustacea	314	LEPTESTHERIIDAE	LEPTESTHERIIDAE	
Crustacea	553	LIGIIDAE	LIGIIDAE	
Crustacea	322	LIMNADIIDAE	LIMNADIIDAE	
Crustacea	499	LYNCEIDAE	LYNCEIDAE	
Crustacea	501	MYSIDAE	MYSIDAE	
Crustacea	533	PALAEMONIDAE	PALAEMONIDAE	
Crustacea	669	POLYARTEMIIDAE		
Crustacea	714	PONTOPOREIIDAE		
Crustacea	456	PORTUNIDAE		
Crustacea	535	POTAMIDAE		
Crustacea	572	SPHAEROMATIDAE	SPHAEROMATIDAE	
Crustacea	518	STREPTOCEPHALIDAE	STREPTOCEPHALIDAE	
Crustacea	668	TALITRIDAE	TALITRIDAE	
Crustacea	427	TRIOPSIDAE	TRIOPSIDAE	
Crustacea	463	XANTHIDAE		
Diptera	542	[Ord:Diptera]		
Diptera	695	[UOrd:Brachycera]		
Diptera	698	[UOrd:Nematocera]		
Diptera	607	ANTHOMYIIDAE	ANTHOMYIIDAE	
Diptera	197	ATHERICIDAE	ATHERICIDAE	
Diptera	203	BIBIONIDAE	BIBIONIDAE	
Diptera	206	BLEPHARICERIDAE	BLEPHARICERIDAE	
Diptera	667	CECIDOMYIIDAE	CECIDOMYIIDAE	
Diptera	221	CERATOPOGONIDAE	CERATOPOGONIDAE	
Diptera	223	CHAOBORIDAE	CHAOBORIDAE	
Diptera	224	CHIRONOMIDAE	CHIRONOMIDAE	
Diptera	238	CULICIDAE	CULICIDAE	
Diptera	241	CYLINDROTOMIDAE	CYLINDROTOMIDAE	
Diptera	253	DIXIDAE	DIXIDAE	
Diptera	254	DOLICHOPODIDAE	DOLICHOPODIDAE	
Diptera	263	EMPIDIDAE	EMPIDIDAE	
Diptera	486	EPHYDRIDAE	EPHYDRIDAE	
Diptera	660	FANNIIDAE	FANNIIDAE	
Diptera	328	LIMONIIDAE	LIMONIIDAE	
Diptera	343	MUSCIDAE	MUSCIDAE	
Diptera	688	PEDICIIDAE	PEDICIIDAE	
Diptera	395	PSYCHODIDAE	PSYCHODIDAE	
Diptera	397	PTYCHOPTERIDAE	PTYCHOPTERIDAE	
Diptera	400	RHAGIONIDAE	RHAGIONIDAE	
Diptera	514	SCATOPHAGIDAE	SCATOPHAGIDAE	
Diptera	515	SCIOMYZIDAE	SCIOMYZIDAE	
Diptera	406	SIMULIIDAE	SIMULIIDAE	
Diptera	415	STRATIOMYIIDAE	STRATIOMYIIDAE	
Diptera	416	SYRPHIDAE	SYRPHIDAE	
Diptera	417	TABANIDAE	TABANIDAE	
Diptera	420	THAUMALEIDAE	THAUMALEIDAE	
Diptera	424	TIPULIDAE	TIPULIDAE	
Ephemeroptera	558	[Ord:Ephemeroptera]		
Ephemeroptera	663	AMELETIDAE	AMELETIDAE	
Ephemeroptera	664	AMETROPODIDAE	AMETROPODIDAE	
Ephemeroptera	194	ARTHROPLEIDAE	ARTHROPLEIDAE	
Ephemeroptera	199	BAETIDAE	BAETIDAE	
Ephemeroptera	200	BEHNINGIIDAE	BEHNINGIIDAE	
Ephemeroptera	214	CAENIDAE	CAENIDAE	
Ephemeroptera	265	EPHEMERELLIDAE	EPHEMERELLIDAE	
Ephemeroptera	266	EPHEMERIDAE	EPHEMERIDAE	
Ephemeroptera	289	HEPTAGENIIDAE	HEPTAGENIIDAE	
Ephemeroptera	309	ISONYCHIIDAE	ISONYCHIIDAE	
Ephemeroptera	318	LEPTOPHLEBIIDAE	LEPTOPHLEBIIDAE	
Ephemeroptera	666	METREPODIDAE	METREPODIDAE	
Ephemeroptera	532	NEOEPHEMERIDAE	NEOEPHEMERIDAE	

List of taxa included in invertebrate sorting audit analyses, cont.

TaxaGroup	ID_FAM	Family	Audit taxon	Taxa excluded
Ephemeroptera	359	OLIGONEURIIDAE	OLIGONEURIIDAE	
Ephemeroptera	365	PALINGENIIDAE	PALINGENIIDAE	
Ephemeroptera	386	POLYMITARCYIDAE	POLYMITARCYIDAE	
Ephemeroptera	389	POTAMANTHIDAE	POTAMANTHIDAE	
Ephemeroptera	393	PROSOPISTOMATIDAE	PROSOPISTOMATIDAE	
Ephemeroptera	407	SIPHLONURIDAE	SIPHLONURIDAE	
Gastropoda	589	[Kl:Gastropoda]		
Gastropoda	699	"ANCYLIDAE"	"ANCYLIDAE"	
Gastropoda	180	ACROLOXIDAE	ACROLOXIDAE	
Gastropoda	696	ASSIMINAEIDAE		
Gastropoda	204	BITHYNIIDAE	BITHYNIIDAE	
Gastropoda	679	ELLOBIIDAE		
Gastropoda	298	HYDROBIIDAE	HYDROBIIDAE	
Gastropoda	677	HYDROCENIDAE		
Gastropoda	332	LYMNAEIDAE	LYMNAEIDAE	
Gastropoda	580	MELANOPSIDAE	MELANOPSIDAE	
Gastropoda	676	MICROMELANIIDAE	MICROMELANIIDAE	
Gastropoda	351	NERITIDAE	NERITIDAE	
Gastropoda	371	PHYSIDAE	PHYSIDAE	
Gastropoda	377	PLANORBIDAE	PLANORBIDAE	
Gastropoda	509	PLEUROCERIDAE	PLEUROCERIDAE	
Gastropoda	678	RISSOIDAE		
Gastropoda	421	THIARIDAE	THIARIDAE	
Gastropoda	675	TRUNCATELLIDAE		
Gastropoda	435	VALVATIDAE	VALVATIDAE	
Gastropoda	437	VIVIPARIDAE	VIVIPARIDAE	
Heteroptera	594	[Ord:Heteroptera]		
Heteroptera	189	APHELOCHEIRIDAE	APHELOCHEIRIDAE	
Heteroptera	716	BELOSTOMATIDAE	BELOSTOMATIDAE	
Heteroptera	234	CORIXIDAE	CORIXIDAE	
Heteroptera	274	GERRIDAE	GERRIDAE	
Heteroptera	285	HEBRIDAE	HEBRIDAE	
Heteroptera	301	HYDROMETRIDAE	HYDROMETRIDAE	
Heteroptera	336	MESOVELIIDAE	MESOVELIIDAE	
Heteroptera	347	NAUCORIDAE	NAUCORIDAE	
Heteroptera	350	NEPIDAE	NEPIDAE	
Heteroptera	353	NOTONECTIDAE	NOTONECTIDAE	
Heteroptera	710	OCHTERIDAE	OCHTERIDAE	
Heteroptera	381	PLEIDAE	PLEIDAE	
Heteroptera	436	VELIIDAE	VELIIDAE	
Hirudinea	546	[Kl:Hirudinea]		
Hirudinea	178	ACANTHOBDELLIDAE	ACANTHOBDELLIDAE	
Hirudinea	268	ERPOBDELLIDAE	ERPOBDELLIDAE	
Hirudinea	275	GLOSSIPHONIIDAE	GLOSSIPHONIIDAE	
Hirudinea	447	HAEMADIPSIDAE	HAEMADIPSIDAE	
Hirudinea	282	HAEMOPIDAE	HAEMOPIDAE	
Hirudinea	293	HIRUDINIDAE	HIRUDINIDAE	*Huirudo medicinalis*
Hirudinea	374	PISCICOLIDAE	PISCICOLIDAE	
Hirudinea	538	SALIFIDAE	SALIFIDAE	
Hydrachnidia	581	[Ph:Hydrachnidia]		
Lepidoptera	548	[Ord:Lepidoptera]		
Lepidoptera	612	CRAMBIDAE		
Lepidoptera	543	PYRALIDAE	PYRALIDAE	
Megaloptera	604	[Ord:Megaloptera]		
Megaloptera	404	SIALIDAE	SIALIDAE	
Nematoda	550	[Kl:Nematoda]		
Nematoda	584	MERMITHIDAE	MERMITHIDAE	
Nematomorpha	625	[Kl:Nematomorpha]		
Nematomorpha	615	GORDIIDAE	GORDIIDAE	
Odonata	591	[Ord:Odonata]		
Odonata	593	[UOrd:Anisoptera]		
Odonata	592	[UOrd:Zygoptera]		

List of taxa included in invertebrate sorting audit analyses, cont.

TaxaGroup	ID_FAM	Family	Audit taxon	Taxa excluded
Odonata	183	AESHNIDAE	AESHNIDAE	
Odonata	215	CALOPTERYGIDAE	CALOPTERYGIDAE	
Odonata	229	COENAGRIONIDAE	COENAGRIONIDAE	
Odonata	232	CORDULEGASTRIDAE	CORDULEGASTRIDAE	
Odonata	233	CORDULIIDAE	CORDULIIDAE	
Odonata	526	EUPHAEIDAE	EUPHAEIDAE	
Odonata	279	GOMPHIDAE	GOMPHIDAE	
Odonata	319	LESTIDAE	LESTIDAE	
Odonata	321	LIBELLULIDAE	LIBELLULIDAE	
Odonata	555	PLATYCNEMIDIDAE	PLATYCNEMIDIDAE	
Oligochaeta	549	[Kl:Oligochaeta]		
Planipennia	503	NEURORTHIDAE		
Planipennia	506	OSMYLIDAE	OSMYLIDAE	
Planipennia	408	SISYRIDAE	SISYRIDAE	
Plecoptera	560	[Ord:Plecoptera]		
Plecoptera	608	[UOrd:Filipalpia]		
Plecoptera	609	[UOrd:Setipalpia]		
Plecoptera	218	CAPNIIDAE	CAPNIIDAE	
Plecoptera	225	CHLOROPERLIDAE	CHLOROPERLIDAE	
Plecoptera	320	LEUCTRIDAE	LEUCTRIDAE	
Plecoptera	349	NEMOURIDAE	NEMOURIDAE	
Plecoptera	367	PERLIDAE	PERLIDAE	
Plecoptera	368	PERLODIDAE	PERLODIDAE	
Plecoptera	418	TAENIOPTERYGIDAE	TAENIOPTERYGIDAE	
Polychaeta	590	[Kl:Polychaeta]		
Polychaeta	582	[Ord:Archiannelida]		
Porifera	692	[Stamm:Porifera]		
Trematoda	718	[UeKl:Trematoda]		
Trichoptera	603	[Ord:Trichoptera]		
Trichoptera	680	APATANIIDAE	APATANIIDAE	
Trichoptera	190	ARCTOPSYCHIDAE	ARCTOPSYCHIDAE	
Trichoptera	201	BERAEIDAE	BERAEIDAE	
Trichoptera	210	BRACHYCENTRIDAE	BRACHYCENTRIDAE	
Trichoptera	651	CALAMOCERATIDAE	CALAMOCERATIDAE	
Trichoptera	260	ECNOMIDAE	ECNOMIDAE	
Trichoptera	277	GLOSSOSOMATIDAE	GLOSSOSOMATIDAE	
Trichoptera	278	GOERIDAE	GOERIDAE	
Trichoptera	527	HELICOPSYCHIDAE	HELICOPSYCHIDAE	
Trichoptera	304	HYDROPSYCHIDAE	HYDROPSYCHIDAE	
Trichoptera	305	HYDROPTILIDAE	HYDROPTILIDAE	
Trichoptera	313	LEPIDOSTOMATIDAE	LEPIDOSTOMATIDAE	
Trichoptera	315	LEPTOCERIDAE	LEPTOCERIDAE	
Trichoptera	324	LIMNEPHILIDAE	LIMNEPHILIDAE	
Trichoptera	340	MOLANNIDAE	MOLANNIDAE	
Trichoptera	356	ODONTOCERIDAE	ODONTOCERIDAE	
Trichoptera	369	PHILOPOTAMIDAE	PHILOPOTAMIDAE	
Trichoptera	370	PHRYGANEIDAE	PHRYGANEIDAE	
Trichoptera	383	POLYCENTROPODIDAE	POLYCENTROPODIDAE	
Trichoptera	396	PSYCHOMYIIDAE	PSYCHOMYIIDAE	
Trichoptera	401	RHYACOPHILIDAE	RHYACOPHILIDAE	
Trichoptera	403	SERICOSTOMATIDAE	SERICOSTOMATIDAE	
Trichoptera	530	UENOIDAE	UENOIDAE	
Turbellaria	541	[Kl:Turbellaria]		
Turbellaria	583	[UOrd:Tricladida]		
Turbellaria	249	DENDROCOELIDAE	DENDROCOELIDAE	
Turbellaria	258	DUGESIIDAE	DUGESIIDAE	
Turbellaria	375	PLANARIIDAE	PLANARIIDAE	

APPENDIX H FREQUENCY OF INVERTEBRATE SORTING ERRORS CAUSED BY TAXA AS NUMBER OF AUDIT SAMPLES AND PERCENTAGE OF GAINS

Austria

STAR-AQEM

Taxa	Frequency	% Gains
PLANARIIDAE	2	16.67
Nematoda	2	16.67
HYDRIDAE	1	8.33
HYDROBIIDAE	1	8.33
NEMOURIDAE	1	8.33
SIALIDAE	1	8.33
GOERIDAE	1	8.33
HYDROPTILIDAE	1	8.33
LEPIDOSTOMATIDAE	1	8.33
DIXIDAE	1	8.33
Total	12	100.00

RIVPACS

Taxa	Frequency	% Gains
Hydrachnidia	4	13.33
PLANORBIDAE	2	6.67
HELOPHORIDAE	2	6.67
HYDROPTILIDAE	2	6.67
LEPIDOSTOMATIDAE	2	6.67
PSYCHODIDAE	2	6.67
DUGESIIDAE	1	3.33
PLANARIIDAE	1	3.33
Nematoda	1	3.33
GAMMARIDAE	1	3.33
HEPTAGENIIDAE	1	3.33
CALOPTERYGIDAE	1	3.33
PERLODIDAE	1	3.33
OSMYLIDAE	1	3.33
Curculionidae	1	3.33
DYTISCIDAE	1	3.33
HYDRAENIDAE	1	3.33
GOERIDAE	1	3.33
LEPTOCERIDAE	1	3.33
PSYCHOMYIIDAE	1	3.33
CERATOPOGONIDAE	1	3.33
EMPIDIDAE	1	3.33
Total	30	100.00

Czech Republic

STAR-AQEM

Taxa	Frequency	% Gains
Hydrachnidia	5	50.00
Nematoda	2	20.00
DYTISCIDAE	1	10.00
HYDROPTILIDAE	1	10.00
LEPIDOSTOMATIDAE	1	10.00
Total	10	100.00

PERLA

Taxa	Frequency	% Gains
Hydrachnidia	4	11.76
Nematoda	2	5.88
CURCULIONIDAE	2	5.88
POLYCENTROPODIDAE	2	5.88
PSYCHODIDAE	2	5.88
Nematomorpha	1	2.94
LYMNAEIDAE	1	2.94
SPHAERIIDAE	1	2.94
GLOSSIPHONIIDAE	1	2.94
CAENIDAE	1	2.94
EPHEMERIDAE	1	2.94
NEMOURIDAE	1	2.94
PERLODIDAE	1	2.94
TAENIOPTERYGIDAE	1	2.94
SIALIDAE	2	5.88
OSMYLIDAE	2	5.88
HYDRAENIDAE	1	2.94
SCIRTIDAE	1	2.94
GOERIDAE	1	2.94
PHILOPOTAMIDAE	1	2.94
PSYCHOMYIIDAE	1	2.94
CERATOPOGONIDAE	1	2.94
EMPIDIDAE	1	2.94
STRATIOMYIIDAE	1	2.94
TIPULIDAE	1	2.94
Total	34	100.00

Frequency of invertebrate sorting errors caused by taxa as number of audit samples and percentage of gains, cont.

Denmark

STAR-AQEM

Taxa	Frequency	% Gains
Nematomorpha	2	9.09
HYDROBIIDAE	2	9.09
CAENIDAE	2	9.09
GYRINIDAE	2	9.09
HYDRIDAE	1	4.55
GLOSSIPHONIIDAE	1	4.55
EPHEMERELLIDAE	1	4.55
LEPTOPHLEBIIDAE	1	4.55
NEMOURIDAE	1	4.55
ELMIDAE	1	4.55
GOERIDAE	1	4.55
HYDROPSYCHIDAE	1	4.55
LEPTOCERIDAE	1	4.55
LIMNEPHILIDAE	1	4.55
POLYCENTROPODIDAE	1	4.55
CERATOPOGONIDAE	1	4.55
EMPIDIDAE	1	4.55
PEDICIIDAE	1	4.55
Total	22	100.00

DSFI

Taxa	Frequency	% Gains
EMPIDIDAE	3	16.67
LIMONIIDAE	2	11.11
LYMNAEIDAE	1	5.56
PLANORBIDAE	1	5.56
Hydrachnidia	1	5.56
LEPTOPHLEBIIDAE	1	5.56
SIALIDAE	1	5.56
ELMIDAE	1	5.56
GYRINIDAE	1	5.56
SCIRTIDAE	1	5.56
BERAEIDAE	1	5.56
GLOSSOSOMATIDAE	1	5.56
GOERIDAE	1	5.56
LEPIDOSTOMATIDAE	1	5.56
CERATOPOGONIDAE	1	5.56
Total	18	100.00

France

STAR-AQEM

Taxa	Frequency	% Gains
Nematoda	1	16.67
LYMNAEIDAE	1	16.67
VALVATIDAE	1	16.67
SPHAERIIDAE	1	16.67
GLOSSIPHONIIDAE	1	16.67
HEPTAGENIIDAE	1	16.67
Total	6	100.00

IBGN

Taxa	Frequency	% Gains
Nematomorpha	1	20.00
DYTISCIDAE	1	20.00
HYDRAENIDAE	1	20.00
HYDROPHILIDAE	1	20.00
MOLANNIDAE	1	20.00
Total	5	80.00

Germany (Duisburg-Essen)

STAR-AQEM

Taxa	Frequency	% Gains
Hydrachnidia	2	50.00
Nematoda	1	25.00
PSYCHODIDAE	1	25.00
Total	4	100.00

RIVPACS

Taxa	Frequency	% Gains
HYDRAENIDAE	2	20.00
SERICOSTOMATIDAE	2	20.00
CERATOPOGONIDAE	2	20.00
HEPTAGENIIDAE	1	10.00
CHLOROPERLIDAE	1	10.00
DYTISCIDAE	1	10.00
PSYCHODIDAE	1	10.00
Total	10	100.00

Frequency of invertebrate sorting errors caused by taxa as number of audit samples and percentage of gains, cont.

Germany (Senckenburg)

AQEM

Taxa	Frequency	% Gains
Hydrachnidia	4	23.53
PLANARIIDAE	2	11.76
Nematoda	1	5.88
SPHAERIIDAE	1	5.88
Oligochaeta	1	5.88
DYTISCIDAE	1	5.88
BRACHYCENTRIDAE	1	5.88
GOERIDAE	1	5.88
LEPTOCERIDAE	1	5.88
CERATOPOGONIDAE	1	5.88
EPHYDRIDAE	1	5.88
PSYCHODIDAE	1	5.88
PTYCHOPTERIDAE	1	5.88
Total	17	100.00

RIVPACS

Taxa	Frequency	% Gains
SPHAERIIDAE	3	9.09
Hydrachnidia	3	9.09
PLANARIIDAE	2	6.06
PLANORBIDAE	2	6.06
ODONTOCERIDAE	2	6.06
PSYCHOMYIIDAE	2	6.06
LIMONIIDAE	2	6.06
Oligochaeta	1	3.03
EPHEMERIDAE	1	3.03
SIPHLONURIDAE	1	3.03
PERLIDAE	1	3.03
PERLODIDAE	1	3.03
SIALIDAE	1	3.03
HYDRAENIDAE	1	3.03
HYDROPHILIDAE	1	3.03
SCIRTIDAE	1	3.03
GLOSSOSOMATIDAE	1	3.03
GOERIDAE	1	3.03
POLYCENTROPODIDAE	1	3.03
RHYACOPHILIDAE	1	3.03
DIXIDAE	1	3.03
EMPIDIDAE	1	3.03
PSYCHODIDAE	1	3.03
TABANIDAE	1	3.03
Total	33	100.00

Greece

STAR-AQEM

Taxa	Frequency	% Gains
Nematoda	2	10.00
Hydrachnidia	2	10.00
LEUCTRIDAE	2	10.00
DIXIDAE	2	10.00
VALVATIDAE	1	5.00
Oligochaeta	1	5.00
GAMMARIDAE	1	5.00
LEPTOPHLEBIIDAE	1	5.00
CURCULIONIDAE	1	5.00
HYDROCHIDAE	1	5.00
LEPTOCERIDAE	1	5.00
PSYCHOMYIIDAE	1	5.00
[Ord.Diptera]	1	5.00
ATHERICIDAE	1	5.00
DOLICHOPODIDAE	1	5.00
TIPULIDAE	1	5.00
Total	20	100.00

RIVPACS

Taxa	Frequency	% Gains
HYDROPTILIDAE	2	10.53
PSYCHOMYIIDAE	2	10.53
Hydrachnidia	1	5.26
POTAMIDAE	1	5.26
EPHEMERELLIDAE	1	5.26
LEPTOPHLEBIIDAE	1	5.26
SIPHLONURIDAE	1	5.26
LEUCTRIDAE	1	5.26
NEMOURIDAE	1	5.26
HYDROPHILIDAE	1	5.26
HYDROPSYCHIDAE	1	5.26
RHYACOPHILIDAE	1	5.26
CERATOPOGONIDAE	1	5.26
EMPIDIDAE	1	5.26
LIMONIIDAE	1	5.26
PSYCHODIDAE	1	5.26
RHAGIONIDAE	1	5.26
Total	19	100.00

Frequency of invertebrate sorting errors caused by taxa as number of audit samples and percentage of gains, cont.

Italy

STAR-AQEM

Taxa	Frequency	% Gains
Hydrachnidia	6	20.69
DUGESIIDAE	2	6.90
HYDROBIIDAE	2	6.90
PLANORBIDAE	2	6.90
NEMOURIDAE	2	6.90
HYDRAENIDAE	2	6.90
LYMNAEIDAE	1	3.45
Oligochaeta	1	3.45
Oribatei	1	3.45
CORIXIDAE	1	3.45
VELIIDAE	1	3.45
LATHRIDIDAE	1	3.45
STAPHYLINIDAE	1	3.45
HYDROPTILIDAE	1	3.45
LEPIDOSTOMATIDAE	1	3.45
LEPTOCERIDAE	1	3.45
POLYCENTROPODIDAE	1	3.45
CERATOPOGONIDAE	1	3.45
PSYCHODIDAE	1	3.45
Total	29	100.00

IBE

Taxa	Frequency	% Gains
Hydrachnidia	6	15.79
EMPIDIDAE	4	10.53
HYDROBIIDAE	3	7.89
DYTISCIDAE	2	5.26
HYDROPHILIDAE	2	5.26
BERAEIDAE	2	5.26
HYDROPTILIDAE	2	5.26
POLYCENTROPODIDAE	2	5.26
STRATIOMYIIDAE	2	5.26
LYMNAEIDAE	1	2.63
SPHAERIIDAE	1	2.63
CAENIDAE	1	2.63
SIPHLONURIDAE	1	2.63
PLATYCNEMIDIDAE	1	2.63
GYRINIDAE	1	2.63
HYDRAENIDAE	1	2.63
BRACHYCENTRIDAE	1	2.63
LEPTOCERIDAE	1	2.63
LIMNEPHILIDAE	1	2.63
PSYCHOMYIIDAE	1	2.63
CERATOPOGONIDAE	1	2.63
PSYCHODIDAE	1	2.63
Total	38	100.00

Latvia

STAR-AQEM

Taxa	Frequency	% Gains
Hydrachnidia	3	12.00
PSYCHODIDAE	3	12.00
HYDRIDAE	1	4.00
DENDROCOELIDAE	1	4.00
Nematomorpha	1	4.00
LYMNAEIDAE	1	4.00
PLANORBIDAE	1	4.00
SPHAERIIDAE	1	4.00
GAMMARIDAE	1	4.00
CAENIDAE	1	4.00
LEPTOPHLEBIIDAE	1	4.00
LEUCTRIDAE	1	4.00
CORIXIDAE	1	4.00
NEPIDAE	1	4.00
HYDRAENIDAE	1	4.00
BERAEIDAE	1	4.00
HYDROPTILIDAE	1	4.00
LEPTOCERIDAE	1	4.00
POLYCENTROPODIDAE	1	4.00
SERICOSTOMATIDAE	1	4.00
TABANIDAE	1	4.00
Total	25	100.00

LVS 240:1999

Taxa	Frequency	% Gains
EPHEMERELLIDAE	4	18.18
ELMIDAE	4	18.18
LEUCTRIDAE	3	13.64
HYDRIDAE	1	4.55
PLANARIIDAE	1	4.55
GLOSSIPHONIIDAE	1	4.55
Hydrachnidia	1	4.55
CAENIDAE	1	4.55
TAENIOPTERYGIDAE	1	4.55
HYDROPTILIDAE	1	4.55
LEPIDOSTOMATIDAE	1	4.55
LEPTOCERIDAE	1	4.55
PSYCHOMYIIDAE	1	4.55
Lepidoptera	1	4.55
Total	22	100.00

Frequency of invertebrate sorting errors caused by taxa as number of audit samples and percentage of gains, cont.

Poland

STAR-AQEM

Taxa	Frequency	% Gains
Hydrachnidia	3	18.75
PLANARIIDAE	1	6.25
DUDESIIDAE	1	6.25
ACROLOXIDAE	1	6.25
BITHYNIIDAE	1	6.25
PLANORBIDAE	1	6.25
ASELLIDAE	1	6.25
NEMOURIDAE	1	6.25
ELMIDAE	1	6.25
HYDRAENIDAE	1	6.25
BERAEIDAE	1	6.25
HYDROPTILIDAE	1	6.25
LEPTOCERIDAE	1	6.25
MOLANNIDAE	1	6.25
Total	16	100.00

PP

Taxa	Frequency	% Gains
Nematoda	3	8.82
Hydrachnidia	3	8.82
PLANARIIDAE	2	5.88
HYDRAENIDAE	2	5.88
LEPTOCERIDAE	2	5.88
HYDROBIIDAE	1	2.94
LYMNAEIDAE	1	2.94
PLANORBIDAE	1	2.94
GAMMARIDAE	1	2.94
LEPTOPHLEBIIDAE	1	2.94
CORDULIIDAE	1	2.94
NEMOURIDAE	1	2.94
PLEIDAE	1	2.94
DYTISCIDAE	1	2.94
HYDROPHILIDAE	1	2.94
BERAEIDAE	1	2.94
HYDROPTILIDAE	1	2.94
LEPIDOSTOMATIDAE	1	2.94
LIMNEPHILIDAE	1	2.94
POLYCENTROPODIDAE	1	2.94
SERICOSTOMATIDAE	1	2.94
PYRALIDAE	1	2.94
EMPIDIDAE	1	2.94
EPHYDRIDAE	1	2.94
PSYCHODIDAE	1	2.94
STRATIOMYIIDAE	1	2.94
TABANIDAE	1	2.94
Total	34	100.00

Portugal

STAR-AQEM

Taxa	Frequency	% Gains
Hydrachnidia	3	37.5
Oligochaeta	1	12.5
LEUCTRIDAE	1	12.5
PERLODIDAE	1	12.5
HYDROPTILIDAE	1	12.5
EPHYDRIDAE	1	12.5
Total	8	100.00

PMP

Taxa	Frequency	% Gains
Hydrachnidia	4	44.44
LYMNAEIDAE	1	11.11
UNIONIDAE	1	11.11
GLOSSIPHONIIDAE	1	11.11
LEPTOPHLEBIIDAE	1	11.11
LEPTOCERIDAE	1	11.11
Total	9	100.00

Frequency of invertebrate sorting errors caused by taxa as number of audit samples and percentage of gains, cont.

Slovakia

STAR-AQEM

Taxa	Frequency	% Gains
Nematoda	1	16.67
Hydrachnidia	1	16.67
HYDRAENIDAE	1	16.67
BRACHYCENTRIDAE	1	16.67
SERICOSTOMATIDAE	1	16.67
CERATOPOGONIDAE	1	16.67
Total	6	100.00

PERLA

Taxa	Frequency	% Gains
ELMIDAE	6	11.32
HYDRAENIDAE	6	11.32
GYRINIDAE	5	9.43
PLANORBIDAE	4	7.55
SPHAERIIDAE	3	5.66
Hydrachnidia	3	5.66
SCIRTIDAE	3	5.66
DYTISCIDAE	2	3.77
TIPULIDAE	2	3.77
DUGESIIDAE	1	1.89
Nematoda	1	1.89
HYDROBIIDAE	1	1.89
LYMNAEIDAE	1	1.89
GLOSSIPHONIIDAE	1	1.89
GAMMARIDAE	1	1.89
BAETIDAE	1	1.89
NEMOURIDAE	1	1.89
NEPIDAE	1	1.89
SIALIDAE	1	1.89
GOERIDAE	1	1.89
LIMNEPHILIDAE	1	1.89
POLYCENTROPODIDAE	1	1.89
CERATOPOGONIDAE	1	1.89
MUSCIDAE	1	1.89
PSYCHODIDAE	1	1.89
SIMULIIDAE	1	1.89
STRATIOMYIIDAE	1	1.89
TABANIDAE	1	1.89
Total	53	100.00

Sweden

STAR-AQEM

Taxa	Frequency	% Gains
VALVATIDAE	1	20.00
PLANORBIDAE	1	20.00
HYDRAENIDAE	1	20.00
LEPTOCERIDAE	1	20.00
LIMNEPHILIDAE	1	20.00
Total	5	100.00

Swedish

Taxa	Frequency	% Gains
Nematoda	1	20.00
VALVATIDAE	1	20.00
GOMPHIDAE	1	20.00
GOERIDAE	1	20.00
SERICOSTOMATIDAE	1	20.00
Total	5	100.00

Frequency of invertebrate sorting errors caused by taxa as number of audit samples and percentage of gains, cont.

UK

STAR-AQEM

Taxa	Frequency	% Gains
Total	0	100.00

RIVPACS

Taxa	Frequency	% Gains
ACROLOXIDAE	3	23.08
CORIXIDAE	2	15.38
GLOSSIPHONIIDAE	1	7.69
ASELLIDAE	1	7.69
EPHEMERELLIDAE	1	7.69
DYTISCIDAE	1	7.69
STAPHYLINIDAE	1	7.69
GLOSSOSOMATIDAE	1	7.69
SERICOSTOMATIDAE	1	7.69
DIXIDAE	1	7.69
Total	13	100.00

APPENDIX I CODED LIST OF SAMPLES ANALYSED IN THE DIATOM AUDIT PER COUNTRY

Country/Institute	Sample	audit sample
Austria	A0510262	ST02420020801B
Austria	A0510282	ST02020020801B
Austria	A0510312	ST02520020801B
Austria	A0510352	ST02120020801B
Austria	A0510691	ST02220020801B
Austria	A0610212	ST02320020820B
Czech Republic	C0402501	ST07020030411B
Czech Republic	C0402551	ST07120030410B
Czech Republic	C0402571	ST07220030328B
Czech Republic	C0402601	ST07320030401B
Czech Republic	C0402611	ST07420030401B
Czech Republic	C0402621	ST07520030328B
Czech Republic	C0502691	ST07620030418B
Czech Republic	C0502721	ST07720030400X
Germany UE	D0301232	ST00920020801F
Germany UE	D0301262	ST00820020729F
Germany UE	D0301282	ST00720020715F
Germany UE	D0301302	ST01320020712F
Germany UE	D0401442	ST01020020708B
Germany S	d0600023	ST00520020828B
Germany S	d0600083	ST00620020815B
Germany S	d0600103	ST00420020819B
France	F08003D1	ST06620030414X
France	F08004D1	ST06720030415X
France	F08010D1	ST06420030408X
France	F08014D1	ST06820030415X
France	F08016D1	ST06920030416X
France	F08018D1	ST06520030409X
Italy Labbio	I0500292	ST01720020723X
Italy Labbio	I0500302	ST01820020723X
Italy Labbio	I0500342	ST01920020723X
Italy CNR-IRSA	I0603607	ST06020020718X
Italy CNR-IRSA	I0608608	ST06120020803X
Italy CNR-IRSA	I0609608	ST05920020718X
Italy CNR-IRSA	I0610608	ST06220020806X
Italy CNR-IRSA	I0612608	ST06320020807X
Denmark	K0204062	ST01520020815F
Denmark	K0207062	ST01420020808F
Denmark	K0209062	ST01620020820F
Portugal	P0441111	ST05620030409B
Portugal	P0441121	ST05720030513B
Portugal	P0441321	ST05820030514B
Portugal	P0451411	ST05420030408A
Portugal	P0461311	ST05520030408F
Sweden	S0503003	ST03020020903B
Sweden	S0503033	ST02920020903B
Sweden	S0503073	ST03520020916B
Sweden	S0503243	ST03220020911B

Country/Institute	Sample	audit sample
Sweden	S0603093	ST03320020912B
Sweden	S0603103	ST03120020906B
Sweden	S0603123	ST03420020912B
Sweden	S0603153	ST02620020821B
Sweden	S0603183	ST02720020822B
Sweden	S0603193	ST02820020829B
United Kingdom	U0291421	ST03920030512B
United Kingdom	U0291431	ST04120030513B
United Kingdom	U0291441	ST03820030410B
United Kingdom	U0291451	ST04020030518B
United Kingdom	U0291471	ST04220030515B
United Kingdom	U1591251	ST04320030520B
United Kingdom	U1591261	ST03720030403B
United Kingdom	U1591291	ST03620030402B
Slovak Republic	V01D0041	ST04420030407X
Slovak Republic	V01D0051	ST04520030407X
Slovak Republic	V01D0091	ST04620030408X
Slovak Republic	V01D0101	ST04720030408X
Slovak Republic	V01D0121	ST04820030408X
Slovak Republic	V01D0131	ST05220030426X
Slovak Republic	V01D0181	ST04920030423X
Slovak Republic	V01D0201	ST05120030425X
Slovak Republic	V01D0221	ST05320030426X
Slovak Republic	V01D0241	ST05020030423X
Germany UE	XXXX	ST01120020000F
Germany UE	XXXX	ST01220020000A
Denmark	XXXX	ST01520020815A
Greece	H0400671	ST07820030522B
Greece	H0400651	ST07920030518B
Greece	H0400731	ST08020030515B
Greece	H0400801	ST08120030515B
Greece	H0400741	ST08220030510B
Greece	O0200112	ST09620030828X
Poland	O0200162	ST09720030828X
Poland	O0200172	ST09820030828X
Poland	O0200192	ST09920030827X
Poland	O0200242	ST10020030826X
Poland	O0200262	ST10120030826X
Poland	O0200292	ST10220030828X
Poland	O0200312	ST10320030828X
Poland	O0200352	ST10420030821X
Poland	O0200402	ST10520030820X
Poland	O0200412	ST10620030820X
Poland	O0200432	ST10720030818X
Poland	O0200502	ST10820030820X
Poland	O0200532	ST10920030820X

APPENDIX J STAR DIATOM TAXON CODE ADJUSTMENT BETWEEN PARTNER AND AUDITOR

	taxon partner	taxon adjusted	taxon audit	taxon adjusted	taxonomic level too low by		taxonomic level too high by		identification error by		taxon only identified by		synonym	difference caused by taxonomic confusion
					part-ner	audi-tor	part-ner	audi-tor	part-ner	audit-or	part-ner	audi-tor		
Austria	GOOL		-								1			
Austria	-		GPAR									1		
Austria	ADBI	ADMI	ADMI		1									
Austria			ABTH	ADMI		1								
Austria			CPLI	CPLA		1								
Austria			CPPL	CPLA		1								
Austria	FSAP	MAPE	MAPE						1					
Czech Republic	-		ABTH	ADMI		1								
Czech Republic	ADSU	ADMI	ADBI	ADMI	1	1								
Czech Republic	ADBI	ADMI	ADBI	ADMI	1									
Czech Republic	-		DVUL									1		
Czech Republic	DMON		-								1			
Czech Republic	GANG	GPUM											1	
Czech Republic			GMIC	GPUM		1								
Czech Republic			MCCO	MCIR		1								
Czech Republic			CPLI	CPLA		1								
Czech Republic			CPPL	CPLA		1								
Czech Republic			FCVA	FCAP		1								
Czech Republic	PHEL	PCHL	PCHL						1					
Germany Senck.			CPPL	CPLA		1								
Germany Senck.	GCLE	GRHB	GRHB											1
Germany Senck.	PTDU	PTLA	PTLA											1
Germany Senck.	NPSB		-								1			
Germany UE	COCO	CPLA					1							
Germany UE			CNDI	CPLA		1								
Germany UE			CPLI	CPLA		1								
Germany UE			CPPL	CPLA		1								
Germany UE	GCLE	GRHB												1
France			SUMI	SBRE		1								
France	RUNI	RSIN			1									
France	FCVA	FCAP	FCVA	FCAP	1	1								
France												1		
Italy Labbio		CPLA	CPLI	CPLA		1								
Italy Labbio	GTER	GSTA	GSTA						1					
Italy CNR-IRSA	-		ADSA									1		

86

	taxon partner	taxon adjus-ted	taxon audit	taxon adjus-ted	taxonomic level too low by		taxonomic level too high by		identification error by		taxon only identified by		syno-nym	difference caused by taxono-mic confusion
					part-ner	audi-tor	part-ner	audi-tor	part-ner	audit-or	part-ner	audi-tor		
Italy CNR-IRSA	AVLA	BVIT	BVIT		1									
Italy CNR-IRSA	CPLE	CPLA	CPLI	CPLA	1	1								
Italy CNR-IRSA	CMCR	ENCM	ENCM		1									
Italy CNR-IRSA	DTCR	DTEN	DTEN		1									
Italy CNR-IRSA	EADN		-								1			
Italy CNR-IRSA	FCVA	FCAP	FCCP	FCAP	1	1								
Denmark	FCVA	FCAP	FCAP		1									
Denmark	PTDU	PTLA	PTLA		1									
Denmark	SCON	SSVE	SSVE				1							
Denmark			CPLI	CPLA		1								
Denmark	PTLA	PLFR	PLFR				1							
Denmark			ALFF	PLFR				1						
Denmark	-		AULA									1		
Denmark	NRCH	NCRY	NCRY						1					
Denmark	NFON		-								1			
Denmark	-		NDIS									1		
Portugal	ADMF	ADMI	ADMI		1									
Portugal			CPLI	CPLA		1								
Portugal			CPPL	CPLA		1								
Portugal	CHAL		-								1			
Portugal	-		EOMI									1		
Portugal	FUAC		-								1			
Portugal	GCLE		-								1			
Portugal	-		GPUM									1		
Portugal	NCTE		-								1			
Portugal	NRHY		-								1			
Portugal	-		PTLA									1		
Portugal	SCON	SSVE	SSVE				1							
Sweden	-		ADSA									1		
Sweden	AUPD	AULA	AULA		1									
Sweden	AUSU	AULA	AULA		1									
Sweden	AUVA	AULA	AULA		1									
Sweden			CPLI	CPLA		1								
Sweden	FGRA	FCAP	FCAP		1									
Sweden	PABD		-								1			
Sweden	SRPI		-								1			
Sweden	-		SCON									1		
Sweden	ADCT	ADMI	ADMI		1									
Sweden			FHSU	FHEI				1						
Sweden	SPAV	SHAN	SHAN		1									

	taxon partner	taxon adjusted	taxon audit	taxon adjusted	taxonomic level too low by		taxonomic level too high by		identification error by		taxon only identified by			difference caused by taxonomic confusion
					part-ner	audi-tor	part-ner	audi-tor	part-ner	audit-or	part-ner	audi-tor	syno-nym	
UK			ADSA	ADMI		1								
UK			ADSU	ADMI		1								
UK			ADBI	ADMI		1								
UK	CPLE	CPLA	CPLI	CPLA	1	1								
UK			CPPL	CPLA		1								
UK	FCVA	FCAP	FCAP			1								
UK	MAAT		-								1			
UK	NCIN		-								1			
UK	SBRE		-								1			
UK	-		SBKU									1		
UK	ESLE		-								1			
UK	-		ENMI									1		
Slovakia	ADBI	ADMI	ADBI	ADMI	1	1								
Slovakia			ADSU	ADMI		1								
Slovakia			CPLI	CPLA		1								
Slovakia	CHEL	CLBE	CLBE										1	
Slovakia	DMON		-								1			
Slovakia	-		DVUL									1		
Slovakia	FCVA	FCAP	FCAP			1								
Slovakia	GMIN	GPUM	GPUM						1					
Slovakia	CYMB	RSIN	RSIN				1							
Greece	CPLI	CPLA	CPLI	CPLA	1	1								
Greece	CPPE	CPLA	CPPL	CPLA	1	1								
Greece	DMON		-								1			
Greece	-		DVUL									1		
Greece	FSAP		-								1			
Greece	DITE	DVUL	DVUL						1					
Greece	FCVA	FCAP	FCCP	FCAP	1	1								
Greece	RSIN		-								1			
Greece	-		UULN									1		
Greece	UBIC	UULN							1					
Greece	NIFR	NFON							1					
Greece	NIPM	NFON							1					
Poland	-		ALFF									1		
Poland	AEXG		-								1			
Poland	AAMB	AULA			1									
Poland	AUGR	AULA			1									
Poland	AUIS	AULA			1									
Poland	AUIT	AULA			1									
Poland	CNDI	CPLA	CPLI	CPLA	1									
Poland	CNTH	CPLA	CPPL	CPLA	1									
Poland	COPS	CPLA			1									
Poland	CDIS	CPLA			1									

	taxon partner	taxon adjus- ted	taxon audit	taxon adjus- ted	taxonomic level too low by		taxonomic level too high by		identification error by		taxon only identified by			differe nce caused by taxono- mic confusi on
					part -ner	audi -tor	part- ner	audi -tor	part- ner	audit -or	part- ner	audi -tor	syno- nym	
Poland	GYAC		-								1			
Poland	-		GYAT									1		
Poland	NCRY		-								1			
Poland	-		NCTE									1		
Poland	NNOV		-								1			
Poland			NIAR									1		
Poland			PLFR	PTLA		1								
Poland			SCBI	SCON		1								
Poland			SRPI	SCON		1								
					34	37	5	2	9	0	24	20	2	3

APPENDIX K STAR DIATOM TAXON CODE ADJUSTMENT FOR RAW AND ADJUSTED OMNIDIA INPUT

Original code	STAR code	STAR code Omidia	STAR code adjusted	genus	species	authority
ABIA1	ABIA1	ABI1	ADBI	Achnanthes	biasolettiana	
ACLE1	ACLE1	ACL1	KCLE	Achnanthes	clevei	
ACOF1	ACOF1	ACO1	ACOF	Amphora	coffeaeformis	
ADAU1	ADAU1	ADA1	PDAU	Achnanthes	daui	
ADEL1	ADEL1	ADE1	PTDE	Achnanthes	delicatula	
AEXG1	AEXG1	AEX1	AEXG	Achnanthes	exigua	
AFLE1	AFLE1	AFL1	EUFL	Achnanthes	flexella	
ALAP1	ALAP1	ALA2	NULA	Achnanthes	lapidosa	
ALVS1	ALVS1	ALV1	EULA	Achnanthes	laevis	
AMIN1	AMIN1	AMI1	ADMI	Achnanthes	minutissima	
ANMN1	ANMN1	ANM1	ANMN	Actinocyclus	normanii	
APLO1	APLO1	APL1	KPLO	Achnanthes	ploenensis	
CAEX1	CAEX1	CAE1	CAEX	Cymbella	excisa	
CAFF1	CAFF1	CAF1	CAFF	Cymbella	affinis	
CAMP1	CAMP1	CAM1	CAMP	Caloneis	amphisbaena	
CDTG1	CDTG1	CDT1	CDTG	Cyclotella	distinguenda	
CELL1	CELL1	CEL1	CELL	Cymatopleura	elliptica	
CHUS1	CHUS1	CHU1	CHUS	Cymbella	hustedtii	
CLAN1	CLAN1	CLA1	CLAN	Cymbella	lanceolata	
CPLA1	CPLA1	CPL1	CPLA	Cocconeis	placentula	
CPRX1	CPRX1	CPR1	CPRX	Cymbella	proxima	
CSOL1	CSOL1	CSO1	CSOL	Cymatopleura	solea	
CTGL1	CTGL1	CTG1	CTGL	Cymbella	turgidula	
DGEM1	DGEM1	DGE1	DGEM	Didymosphenia	geminata	
DHIE1	DHIE1	DHI1	DHIE	Diatoma	hyemalis	
EARC1	EARC1	EAR1	EARC	Eunotia	arcus	
EBIL1	EBIL1	EBI1	EBIL	Eunotia	bilunaris	
EINC1	EINC1	EIN1	EINC	Eunotia	incisa	
EPAR1	EPAR1	EPA1	EPAR	Eunotia	parallela	
EPEC1	EPEC1	EPE1	EPEC	Eunotia	pectinalis	
EPRA1	EPRA1	EPR2	EPRA	Eunotia	praerupta	
ETUR1	ETUR1	ETU1	ETUR	Epithemia	turgida	
EUPA1	EUPA1	EUP1	EUPA	Eunotia	paludosa	
FARC1	FARC1	FAR1	FARC	Fragilaria	arcus	
FCAP1	FCAP1	FCA1	FCAP	Fragilaria	capucina	
FCON1	FCON1	FCO1	SCON	Fragilaria	construens	
FFAM1	FFAM1	FFA1	FFAM	Fragilaria	famelica	
FLEP1	FLEP1	FLE1	SSLE	Fragilaria	leptostauron	
FPAR1	FPAR1	FPA1	SDPA	Fragilaria	parasitica	
FPIN1	FPIN1	FPI1	SRPI	Fragilaria	pinnata	
FULN1	FULN1	FUL1	UULN	Fragilaria	ulna	
GBOH1	GBOH1	GBO1	GBOH	Gomphonema	bohemicum	
GMIC1	GMIC1	GMI1	GMIC	Gomphonema	micropus	
GMIN1	GMIN1	GMI2	GMIN	Gomphonema	minutum	
GOLI1	GOLI1	GOL1	GOLI	Gomphonema	olivaceum	
GPAR1	GPAR1	GPA1	GPAR	Gomphonema	parvulum	
MCIR1	MCIR1	MCI1	MCIR	Meridion	circulare	

90

Original code	STAR code	STAR code Omidia	STAR code adjusted	genus	species	authority
NAMP1	NAMP1	NAM1	NAMP	Nitzschia	amphibia	
NATO1	NATO1	NAT1	MAAT	Navicula	atomus	
NDIS1	NDIS1	NDI1	NDIS	Nitzschia	dissipata	
NIFR1	NIFR1	NIF1	NIFR	Nitzschia	frustulum	
NLIN1	NLIN1	NLI1	NLIN	Nitzschia	linearis	
NMEN1	NMEN1	NME1	NMEN	Navicula	menisculus	
NRCH1	NRCH1	NRC1	NRCH	Navicula	reichardtiana	
NSAL1	NSAL1	NSA1	NSAL	Navicula	salinarum	
NSHR1	NSHR1	NSH1	NSES	Navicula	schroeteri	
NSIN1	NSIN1	NSI1	NSIN	Nitzschia	sinuata	
NTRV1	NTRV1	NTR1	NTRV	Navicula	trivialis	
NULA1	NULA1	NUL1	NULA	Nupela	lapidosa	
PAPP1	PAPP1	PAP1	PAPP	Pinnularia	appendiculata	
PBOR1	PBOR1	PBO1	PBOR	Pinnularia	borealis	
PBRE1	PBRE1	PBR1	PBRE	Pinnularia	brebissonii	
PDIV1	PDIV1	PDI1	PDIV	Pinnularia	divergens	
PLTA1	PLTA1	PLT1	PTLA	Pinnularia	latarea	
PMES1	PMES1	PME1	PMES	Pinnularia	mesolepta	
PMIC1	PMIC1	PMI1	PMIC	Pinnularia	microstauron	
PNOD1	PNOD1	PNO1	PNOD	Pinnularia	nodosa	
PSCA1	PSCA1	PSC1	PSCA	Pinnularia	subcapitata	
PVIR1	PVIR1	PVI1	PVIR	Pinnularia	viridis	
	S137	S137	NPSB	Navicula	protracta	(Grunow) Cleve
SBRE1	SBRE1	SBR1	SBRE	Surirella	brebissonii	

APPENDIX L THE OMNIDIA DIATOM METRICS

OMNIDIA calculates 14 diatom metrics indicating the water quality:

√   IDAP (Artois Picardie: Prygiel et al. 1995),
√   IBD (biologique diatomique Lenoir & Coste 1996),
√   SHE (Steinberg et Shiefele 1988-91),
√   TDI (Kelly & Whitton 1995),
√   %PT (Kelly & Whitton 1995),
√   WAT (Watanabe 1982-90),
√   IPS (Coste in Cemagref 1982),
√   SLA (Sládeček 1986),
√   DES (Descy 1979-80),
√   L&M (Leclercq et Maquet 1987),
√   IDG (Rumeau & Coste 1988),
√   CEE (Descy & Coste 1990),
√   ROTT (Rott et al. 1999),
√   EPI-D (Dell'Uomo 1999).

Descy's Pollution Metric (DESCY), Specific Pollution Sensitivity Metric (IPS), Generic Diatom Metric (IDG), Sládeček's Pollution Metric (SLA), Leclercq & Marquet's Pollution Metric (LMI), Steinberg & Schiefele's Trophic Metric (SHE)

The saprobic conditions are calculated based on six separate metrics (DESC, IPS, IDG, SLA, ILM, SHE):

$$ID = \sum_{j=1}^{n} A_j I_j V_j / \sum_{j=1}^{n} A_j V_j \qquad \text{Zelinka \& Marvan 1961}$$

with: A = taxon j abundance
         I = saprobic value (1-5) of taxon j
         V = saprobic valence of taxon j (varying between 1-3, 1-4 or 1-5, see Table 1)
         j = taxon j

Table 1. Saprobic valence range.

saprobic metric	saprobic valence range
DESC	1 – 5
IPS	1 - 5
IDG	1 - 5
SLA	4 - 0
ILM	1 - 5
SHE	7 - 1

The resulting quality class ranges from good to bad:
ID > 4.5   no pollution
ID = 4.5 – 4.0 slight pollution
ID = 4.0 – 3.5 moderate pollution
ID = 3.5 – 3.0 reasonable pollution
ID = 3.0-2.0   strong pollution
ID = 2.0 – 1.0 very strong pollution
The final score is transformed to a scale between 1 – 20.

Pollution Metric based on diatoms (EPI-D)

The EPI-D metric is calculated with the Zelinka & Marvan formula using relative abundance. The interpretation of the EPI-D resulting for each site sampled (scale 0 – 4) is the following:

0.0 < EPI-D 1.0	excellent water quality
1.0 < EPI-D 1.5	good water quality
1.5 < EPI-D 1.8	water quality good enough
1.8 < EPI-D 2.0	slightly polluted environment
2.0 < EPI-D 2.2	moderately polluted environment
2.2 < EPI-D 2.5	heavily polluted environment
2.5 < EPI-D 3.0	very heavily polluted environment
3.0 < EPI-D 4.0	completely altered environment

Trophic conditions (SHE metric) according to Steinberg & Schiefe (1987) and modified by Schiefele & Schreiner (1991) are listed in Table 2.

Table 2. trophic conditions according to the SHE metric.

trophic level	sensitivity
1 = mt	most tolerant taxa (very resistant)
2 = ht	highly tolerant
3 = tt	tolerant
4 = ls or wa	less sensitive
5 = eu	eutrophic
6 = ss	sensitive

IBD metric

The IBD metric is calculated according to the following formula:

$$P(i) = \sum\nolimits^{TPSN}{}_{AAMB} \text{abondxxxx P'xxxx}(i) \text{vxxxx} / \sum\nolimits^{TPSN}{}_{AAMB} \text{abondxxx vxxxx}$$

with:
xxxx = paired taxa between AAMB and TPSN
I = water quality classes from 1 to 7
abundxxxx = abundances of paired taxa expressed in ‰
P'xxxx(i) = presence probability of paired taxon xxxx for quality class i
Vxxxx = ecological value of paired taxon
Seven values are obtained which correspond to a presence probability of fictitious taxon representing the studied population. The barycenter of these 7 values corresponds to the BDI value of 7 and is calculated as follows:
This value is transformed into a note on 20.

TDI metric (trophic diatom index)

The TDI metric (trophic diatom index) is calculated as follows:
s = the general tolerance or resistance of the taxon to the pollution level (including organic load affinity) i.e. an integrated specific metric (from 0.0 to 4.0): the sensitivity of the taxon
v = the reliability of the taxon (the French 'value indicatrice'): the indicator value of the taxon is 5, 3 or 1
%PTV = % pollution tolerant taxa
TDI = +asv / + sv

The interpretation of the proportion of count composed of taxa tolerant to organic pollution (%PTV):

Proportion of count	Interpretation
< 20 % of total valves belonging to tolerant taxa	free of significant organic pollution some evidence of organic pollution
> 21 % and < 40 % of total valves belonging to tolerant taxa	organic pollution likely to contribute significant site is heavily contaminated with organic pollution

Rott metric (ROTT)

The formula for the Rott Metric follows the calculation of the Zelinka & Marvan formula where:

s = saprobic value

The final result is divided by 20 and is obtained by the linear transformation as follows:

ROTT=(rott x -6,786)+26,786

Watanabe et al Pollution Metric (WAT index)

The WAT metric is based on three groups (1=saprophilous taxa, 2=saproxenous taxa and 0=indifferent taxa) and calculated as follows:

$$DAIpo = 50 + \tfrac{1}{2} \left(\sum_{i=1}^{p} X_i - \left(\sum_{j=1}^{q} S_j \right) \right)$$

with:　$\sum_{i=1}^{p} X_i$　　　= sum of relative abundance (%) of saproxenous taxa
　　　　$\sum_{j=1}^{q} S_j$　　　= sum of relative abundance (%) of saprophilous taxa

APPENDIX M T-TEST OF DIATOM METRICS RESULTS BASED ON COUNTS (A) AND ON PERCENTAGES (B).

A.

	count comparison	IPS	SLAD.	DESCY	L&M	SHE	WAT	TDI	%PT	EPI-D	ROTT	IDG	CEE	IBD	IDAP
Austria	valid	0.47	0.34	0.21	0.01	0.14	0.10	0.07	0.24	0.15	0.33	0.89	0.11	0.23	0.71
Austria	adjusted	0.28	0.05	0.19	0.00	0.69	0.34	0.16	0.24	0.82	0.37	0.48	0.00	0.92	0.28
Czech Republic	valid	0.02	0.22	0.93	0.77	0.42	0.04	0.03	0.62	0.25	0.31	0.06	0.08	0.07	0.76
Czech Republic	adjusted	0.58	0.33	0.43	0.17	0.28	0.91	0.79	0.80	0.41	0.39	0.77	0.06	0.55	0.19
Denmark	valid	0.07	0.43	0.77	0.45	0.10	0.76	0.57	0.97	0.14	0.53	0.08	0.08	0.07	0.46
Denmark	adjusted	0.40	0.06	0.94	0.91	0.47	0.20	0.66	0.97	0.40	0.86	0.05	0.88	0.57	0.99
France	valid	0.26	0.92	0.33	0.20	0.66	0.69	0.08	0.61	0.34	0.18	0.12	0.65	0.09	0.07
France	adjusted	0.26	0.10	0.50	0.13	0.79	0.35	0.07	0.54	0.08	0.08	0.40	0.32	0.13	0.22
Germany	valid	0.27	0.05	0.65	0.73	0.74	0.37	0.16	0.20	0.08	0.01	0.41	0.07	0.25	0.12
Germany	adjusted	0.67	0.15	0.16	0.87	0.89	0.99	0.69	0.45	0.17	0.06	0.58	0.66	0.54	0.49
Greece	valid	0.89	0.27	0.14	0.63	0.33	0.18	0.44	0.36	0.08	0.24	0.47	0.03	0.33	0.16
Greece	adjusted	0.43	0.18	0.42	0.52	0.46	0.56	0.63	0.38	0.26	0.48	0.43	0.43	0.32	0.15
Italy	valid	0.01	0.07	0.92	0.29	0.87	0.03	0.09	0.37	0.34	0.63	0.05	0.12	0.05	0.44
Italy	adjusted	0.22	0.94	0.65	0.08	0.28	0.73	0.41	0.15	0.11	0.67	0.10	0.20	0.36	0.57
Poland	valid	0.00	0.00	0.47	0.08	0.03	0.00	0.00	0.23	0.13	0.09	0.00	0.00	0.00	0.40
Poland	adjusted	0.90	0.02	0.82	0.95	0.17	0.54	0.51	0.44	0.49	0.43	0.14	0.69	0.02	0.88
Portugal	valid	0.22	0.60	0.02	0.52	0.27	0.10	0.10	0.01	0.24	0.57	0.95	0.37	0.55	0.36
Portugal	adjusted	0.14	0.24	0.02	0.24	0.26	0.24	0.48	0.01	0.19	0.35	0.26	0.03	0.69	0.22
Slovak Republic	valid	0.00	0.17	0.01	0.29	0.03	0.00	0.00	0.10	0.51	0.58	0.01	0.00	0.02	0.06
Slovak Republic	adjusted	0.15	0.10	0.54	0.59	0.00	0.01	0.09	0.10	0.74	0.01	0.30	0.09	0.49	0.80
Sweden	valid	0.01	0.52	0.19	0.19	0.17	0.00	0.75	0.39	0.56	0.10	0.00	0.00	0.05	0.05
Sweden	adjusted	0.05	0.05	1.00	0.02	0.02	0.00	0.30	0.39	0.02	0.41	0.01	0.48	0.08	0.01
United Kingdom	valid	0.58	0.87	1.00	0.03	0.55	0.41	0.25	0.13	0.16	0.45	0.65	0.32	0.63	0.87
United Kingdom	adjusted	0.03	0.81	0.51	0.91	0.03	0.10	0.79	0.74	0.42	0.05	0.26	0.49	0.54	0.64

B.

percentage	comparison	IPS	SLAD.	DESCY	L&M	SHE	WAT	TDI	%PT	EPI-D	ROTT	IDG	CEE	IBD	IDAP
Austria	valid	0.49	0.29	0.68	0.02	0.12	0.10	0.04	0.27	0.10	0.62	0.81	0.33	0.18	0.86
Austria	adjusted	0.23	0.08	0.04	0.00	0.69	0.42	0.29	0.27	0.69	0.67	0.43	0.52	0.75	0.28
Czech Republic	valid	0.02	0.22	0.93	0.70	0.36	0.04	0.02	0.70	0.22	0.23	0.06	0.04	0.08	0.14
Czech Republic	adjusted	0.51	0.33	0.41	0.20	0.33	0.91	0.93	0.87	0.63	0.34	0.85	0.47	0.88	0.21
Denmark	valid	0.07	0.78	0.70	0.42	0.12	0.78	0.87	0.89	0.10	0.63	0.04	0.40	0.02	0.39
Denmark	adjusted	0.32	0.08	0.83	0.83	0.42	0.14	0.38	0.88	0.34	0.94	0.04	0.48	0.58	0.96
France	valid	0.38	1.00	0.38	0.16	0.68	0.83	0.06	0.42	0.29	0.23	0.15	0.25	0.10	0.34
France	adjusted	0.14	0.09	0.51	0.18	1.00	0.29	0.05	0.39	0.60	0.11	0.52	0.13	0.16	0.24
Germany	valid	0.28	0.06	0.60	0.66	0.64	0.35	0.26	0.25	0.07	0.03	0.40	0.19	0.30	0.71
Germany	adjusted	0.69	0.13	0.08	0.96	0.90	0.96	0.89	0.54	0.16	0.16	0.62	0.67	0.52	0.62
Greece	valid	0.86	0.25	0.14	0.58	0.33	0.18	0.45	0.36	0.06	0.29	0.52	0.04	0.27	0.59
Greece	adjusted	0.42	0.18	0.40	0.54	0.48	0.57	0.68	0.36	0.23	0.49	0.40	0.46	0.39	0.30
Italy	valid	0.02	0.08	0.71	0.37	0.88	0.03	0.11	0.92	0.79	0.62	0.07	0.05	0.05	0.45
Italy	adjusted	0.25	0.94	0.55	0.11	0.61	0.89	0.45	0.26	0.24	0.63	0.10	0.32	0.34	0.52
Poland	valid	0.00	0.00	0.70	0.13	0.04	0.00	0.00	0.18	0.11	0.07	0.00	0.00	0.00	0.24
Poland	adjusted	0.90	0.06	0.55	0.76	0.15	0.41	0.47	0.49	0.82	0.36	0.05	0.10	0.04	0.70
Portugal	valid	0.12	0.71	0.04	0.62	0.21	0.09	0.11	0.02	0.23	0.67	0.92	0.22	0.60	0.93
Portugal	adjusted	0.06	0.26	0.04	0.36	0.18	0.18	0.48	0.02	0.19	0.34	0.36	0.44	0.61	0.10
Slovak Republic	valid	0.00	0.19	0.01	0.20	0.07	0.00	0.00	0.15	0.38	0.44	0.01	0.00	0.03	0.07
Slovak Republic	adjusted	0.29	0.10	0.83	0.80	0.01	0.01	0.13	0.13	1.00	0.00	0.47	0.14	0.57	0.92
Sweden	valid	0.01	0.49	0.30	0.18	0.17	0.00	0.67	0.18	0.56	0.09	0.01	0.03	0.06	0.02
Sweden	adjusted	0.03	0.05	0.67	0.04	0.02	0.00	0.37	0.20	0.02	0.35	0.02	0.68	0.08	0.05
United Kingdom	valid	0.54	0.73	0.90	0.07	0.51	0.43	0.28	0.20	0.18	0.45	0.69	0.78	0.61	0.80
United Kingdom	adjusted	0.07	0.92	0.48	0.89	0.04	0.21	0.82	0.75	0.45	0.04	0.27	0.40	0.58	0.64

APPENDIX N R^2 OF DIATOM METRICS RESULTS BASED ON COUNTS (A) AND ON PERCENTAGES (B).

A.

count	comparison	IPS	SLAD	DESCY	L&M	SHE	WAT	TDI	%PT	EPI-D	ROTT	IDG	CEE	IBD	IDAP
Austria	valid	0.76	0.88	0.89	0.96	0.93	0.77	0.45	0.83	0.92	0.94	0.83	0.88	0.12	0.00
Austria	adjusted	0.88	0.90	0.99	0.98	0.88	0.91	0.84	0.83	0.93	0.91	0.89	0.99	0.96	0.53
Czech Republic	valid	0.36	0.58	0.82	0.68	0.49	0.02	0.77	0.96	0.88	0.43	0.75	0.12	0.04	0.75
Czech Republic	adjusted	0.84	0.81	0.57	0.84	0.60	0.93	0.87	0.96	0.98	0.44	0.76	0.96	0.94	0.44
Denmark	valid	0.97	0.68	0.09	0.16	0.49	0.77	0.45	0.64	0.33	0.73	0.97	0.83	0.99	0.20
Denmark	adjusted	0.77	0.86	0.22	0.86	0.25	0.77	0.47	0.64	0.06	0.98	0.99	0.40	0.78	0.05
France	valid	0.97	0.94	0.83	0.99	0.92	0.81	0.69	0.97	0.98	0.84	0.95	0.98	0.99	0.95
France	adjusted	0.97	0.88	0.98	0.99	0.95	0.82	0.78	0.97	1.00	0.82	0.96	0.98	0.97	0.93
Germany	valid	0.38	0.72	0.31	0.16	0.33	0.09	0.00	0.33	0.17	0.93	0.56	0.35	0.62	0.46
Germany	adjusted	0.33	0.67	0.48	0.16	0.38	0.09	0.18	0.39	0.12	0.92	0.58	0.46	0.72	0.34
Greece	valid	0.01	0.09	0.12	0.26	0.22	0.02	0.08	0.98	0.16	0.01	0.56	0.29	0.01	0.04
Greece	adjusted	0.05	0.58	0.22	0.01	0.41	0.15	0.19	0.99	0.24	0.11	0.56	0.86	0.10	0.03
Italy	valid	0.75	0.32	0.50	0.28	0.80	0.35	0.32	0.72	0.86	0.17	0.75	0.07	0.57	0.36
Italy	adjusted	0.49	0.27	0.69	0.50	0.97	0.17	0.79	0.60	0.92	0.88	0.82	0.80	0.18	0.22
Poland	valid	0.51	0.61	0.55	0.81	0.37	0.38	0.26	0.84	0.14	0.45	0.57	0.00	0.47	0.30
Poland	adjusted	0.80	0.82	0.85	0.95	0.60	0.68	0.85	0.87	0.51	0.71	0.75	0.58	0.86	0.65
Portugal	valid	0.99	0.69	0.90	0.86	0.97	0.19	0.55	0.95	0.63	0.30	0.85	0.87	0.37	0.01
Portugal	adjusted	0.81	0.84	0.93	0.80	0.60	0.63	0.90	0.95	0.44	0.67	0.91	0.91	0.68	0.28
Slovak Republic	valid	0.74	0.27	0.41	0.72	0.79	0.23	0.30	0.99	0.72	0.73	0.71	0.86	0.21	0.20
Slovak Republic	adjusted	0.91	0.91	0.37	0.95	0.98	0.43	0.20	0.99	0.95	0.98	0.96	0.87	0.89	0.29
Sweden	valid	0.71	0.87	0.46	0.63	0.85	0.00	0.40	0.99	0.38	0.12	0.88	0.00	0.27	0.00
Sweden	adjusted	0.94	0.96	0.82	0.99	1.00	0.87	0.37	0.99	0.85	0.73	0.98	0.00	0.97	0.20
United Kingdom	valid	0.67	0.81	0.80	0.88	0.62	0.55	0.84	0.99	0.92	0.04	0.90	0.70	0.18	0.07
United Kingdom	adjusted	0.98	0.95	0.52	0.86	0.94	0.98	0.89	0.99	0.87	0.93	0.94	0.95	0.67	0.30

EVKI-CT 2001-00089

B.

percentage	comparison	IPS	SLAD.	DESCY	L&M	SHE	WAT	TDI	%PT	EPI-D	ROTT	IDG	CEE	IBD	IDAP
Austria	valid	0.80	0.87	0.80	0.97	0.96	0.77	0.83	0.85	0.91	0.89	0.85	0.69	0.23	0.03
Austria	adjusted	0.89	0.87	0.98	0.98	0.88	0.89	0.77	0.85	0.87	0.83	0.93	0.12	0.95	0.69
Czech Republic	valid	0.37	0.53	0.81	0.65	0.52	0.03	0.78	0.96	0.89	0.54	0.67	0.06	0.03	0.89
Czech Republic	adjusted	0.85	0.81	0.55	0.84	0.57	0.93	0.87	0.96	0.97	0.49	0.77	0.11	0.95	0.24
Denmark	valid	0.97	0.63	0.00	0.02	0.41	0.78	0.52	0.55	0.50	0.70	0.99	0.32	0.99	0.21
Denmark	adjusted	0.76	0.94	0.03	0.99	0.24	0.80	0.55	0.55	0.11	0.98	0.99	0.23	0.78	0.06
France	valid	0.96	0.93	0.75	0.98	0.91	0.83	0.55	0.98	0.99	0.79	0.94	0.95	1.00	0.96
France	adjusted	0.97	0.86	0.98	0.97	0.97	0.83	0.74	0.98	0.98	0.80	0.96	0.97	0.96	0.98
Germany	valid	0.38	0.73	0.23	0.15	0.32	0.10	0.00	0.32	0.19	0.83	0.54	0.38	0.58	0.00
Germany	adjusted	0.33	0.69	0.47	0.11	0.36	0.10	0.21	0.36	0.11	0.79	0.59	0.45	0.70	0.01
Greece	valid	0.00	0.11	0.02	0.31	0.17	0.02	0.09	1.00	0.22	0.01	0.54	0.23	0.01	0.32
Greece	adjusted	0.04	0.68	0.25	0.02	0.32	0.15	0.25	1.00	0.24	0.12	0.55	0.87	0.11	0.15
Italy	valid	0.71	0.32	0.60	0.26	0.83	0.39	0.37	0.70	0.83	0.10	0.71	0.10	0.57	0.64
Italy	adjusted	0.50	0.26	0.68	0.50	0.98	0.18	0.85	0.67	0.89	0.91	0.83	0.17	0.22	0.50
Poland	valid	0.47	0.57	0.50	0.72	0.41	0.44	0.24	0.83	0.11	0.41	0.57	-	0.50	0.21
Poland	adjusted	0.82	0.81	0.86	0.92	0.66	0.69	0.82	0.86	0.44	0.67	0.79	0.02	0.85	0.62
Portugal	valid	0.98	0.68	0.81	0.87	0.96	0.17	0.53	0.91	0.66	0.25	0.76	0.26	0.33	0.03
Portugal	adjusted	0.86	0.81	0.84	0.80	0.60	0.67	0.84	0.91	0.46	0.70	0.84	0.49	0.65	0.23
Slovak Republic	valid	0.70	0.26	0.43	0.73	0.77	0.23	0.28	0.98	0.71	0.77	0.69	-	0.22	0.08
Slovak Republic	adjusted	0.90	0.90	0.36	0.94	0.96	0.43	0.15	0.98	0.94	0.98	0.94	0.83	0.90	0.01
Sweden	valid	0.65	0.85	0.46	0.60	0.81	0.01	0.15	0.98	0.46	0.10	0.87	0.07	0.18	0.25
Sweden	adjusted	0.95	0.94	0.80	0.99	0.99	0.85	0.12	0.98	0.88	0.69	0.97	0.15	0.95	0.08
UK-CEH	valid	0.72	0.75	0.79	0.85	0.62	0.56	0.81	0.99	0.92	0.03	0.89	0.00	0.21	0.12
UK-CEH	adjusted	0.97	0.93	0.51	0.82	0.95	0.98	0.82	0.99	0.88	0.94	0.93	0.45	0.70	0.28

APPENDIX O DIFFERENCE (PRIMARY ANALYST MINUS AUDITOR RESULTS) BETWEEN DIATOM METRICS RESULTS BASED ON COUNTS (A) AND ON PERCENTAGES (B)

A.

country		IPS	SLAD	DESCY	L&M	SHE	WAT	TDI	%PT	EPI-D	ROTT	IDG	CEE	IBD	IDAP
Austria	valid	-0.500	-0.250	0.300	0.717	0.583	-1.150	6.933	-2.750	-0.350	0.500	-0.083	-1.067	-0.950	0.317
Austria	adjusted	0.417	0.567	-0.083	0.667	0.133	0.500	-2.700	-2.750	-0.033	0.267	0.233	0.550	-0.017	0.433
Czech Republic	valid	-1.788	-0.637	0.025	-0.125	-0.425	-2.825	-13.625	-1.225	-0.300	-0.313	-0.900	-5.300	-1.913	-0.137
Czech Republic	adjusted	-0.175	-0.263	-0.188	-0.425	-0.450	0.037	0.775	-0.625	-0.088	-0.350	-0.100	-0.475	-0.100	-0.813
Denmark	valid	-0.675	0.175	-0.325	0.900	2.100	-0.275	-3.150	-0.200	0.925	-0.450	-0.975	-1.300	-0.675	1.175
Denmark	adjusted	-0.675	-0.625	-0.075	-0.075	-0.775	-0.850	2.525	-0.200	-0.750	0.050	-0.875	-0.200	0.525	-0.025
France	valid	-0.417	-0.033	0.333	0.167	-0.100	-0.300	5.867	0.717	-0.417	-0.350	-0.833	-0.150	-0.400	-0.583
France	adjusted	0.233	0.933	0.117	0.167	-0.050	0.583	5.667	0.833	-0.117	-0.500	-0.250	0.250	1.117	-0.200
Germany	valid	-0.970	-0.870	-0.320	-0.220	-0.270	-1.210	7.600	-3.910	-1.390	-0.640	-0.480	-1.960	-0.730	0.900
Germany	adjusted	-0.330	-0.470	-0.620	0.100	-0.090	-0.010	-1.420	-2.220	-1.010	-0.440	0.270	-0.310	0.280	0.410
Greece	valid	-0.217	-0.650	2.750	0.633	0.850	-2.583	10.400	-1.717	-1.417	0.950	-1.000	-1.800	-1.783	-1.350
Greece	adjusted	1.000	0.400	1.317	0.583	0.417	0.900	5.717	-1.233	-0.550	0.433	0.600	-0.233	1.050	-1.417
Italy	valid	-1.313	-0.913	0.037	-0.538	-0.063	-2.313	11.675	0.425	-0.250	0.363	-0.850	-3.638	-1.388	1.725
Italy	adjusted	-0.525	-0.025	0.113	-0.525	-0.125	-0.212	3.013	1.388	-0.313	0.087	-0.563	-0.300	0.637	1.300
Poland	valid	-2.115	-1.377	0.462	-0.331	1.269	-3.369	12.808	-1.623	-0.662	-0.708	-1.238	-6.762	-5.023	0.538
Poland	adjusted	-0.038	-0.423	-0.054	-0.008	0.454	0.169	1.215	0.800	-0.177	-0.192	0.338	-0.185	-0.500	-0.062
Portugal	valid	-0.500	0.260	-0.840	0.220	-0.300	-2.040	17.940	2.380	-0.620	0.420	-0.020	0.420	-0.420	-1.460
Portugal	adjusted	-0.800	0.380	-0.520	0.460	-0.960	-1.000	4.080	2.380	-0.940	0.420	-0.280	0.880	-0.200	-0.960
Slovak Republic	valid	-1.680	-0.740	0.760	0.290	-0.540	-5.400	-20.220	-1.730	0.200	-0.100	-1.170	-1.990	-2.200	1.240
Slovak Republic	adjusted	-0.190	0.280	-0.140	-0.060	-0.300	-1.380	-8.820	-1.730	-0.030	-0.190	-0.130	-0.460	-0.170	0.140
Sweden	valid	-1.690	-0.230	0.650	-0.510	-0.640	-7.390	-2.430	-0.280	-0.490	1.410	-1.090	-9.410	-2.230	-4.210
Sweden	adjusted	-0.540	-0.240	0.000	-0.090	-0.280	-1.210	-7.570	-0.280	-0.440	0.220	-0.380	1.510	-0.290	-2.630
United Kingdom	valid	-0.363	-0.037	0.000	0.538	0.438	0.875	2.800	-0.975	0.337	0.675	-0.125	-0.713	-0.475	-0.100
United Kingdom	adjusted	-0.300	0.025	-0.163	-0.025	-0.575	0.337	0.475	-0.225	-0.238	-0.275	-0.275	-0.163	0.262	-0.275

B.

country	Type	IPS	SLAD	DESCY	L&M	SHE	WAT	TDI	%PT	EPI-D	ROTT	IDG	CEE	IBD	IDAP
Austria	valid	-0.467	-0.283	0.133	0.600	0.483	-1.183	7.583	-2.567	-0.400	0.283	-0.133	-2.267	-0.883	0.150
Austria	adjusted	0.450	0.517	-0.233	0.617	0.133	0.450	-2.183	-2.583	-0.083	0.150	0.217	-1.750	0.050	0.350
Czech Republic	valid	-1.763	-0.675	0.025	-0.175	-0.475	-2.813	-14.213	-1.000	-0.313	-0.350	-0.913	-6.725	-1.850	-0.488
Czech Republic	adjusted	-0.200	-0.262	-0.200	-0.400	-0.413	0.037	0.250	-0.438	-0.063	-0.375	-0.063	1.913	-0.025	-1.025
Denmark	valid	-0.900	0.075	-0.425	0.900	2.100	-0.275	-1.000	0.725	0.925	-0.400	-1.125	3.575	-0.725	1.350
Denmark	adjusted	-0.925	-0.800	-0.250	-0.125	-0.925	-0.975	5.300	0.750	-0.900	0.025	-1.000	-3.200	0.500	0.075
France	valid	-0.333	0.000	0.367	0.250	-0.117	-0.167	6.733	1.050	-0.550	-0.350	-0.850	-3.583	-0.383	-0.333
France	adjusted	0.350	1.050	0.133	0.250	0.000	0.683	6.350	1.117	-0.100	-0.467	-0.200	0.667	1.083	0.117
Germany	valid	-0.950	-0.850	-0.430	-0.290	-0.410	-1.290	7.680	-3.710	-1.420	-0.630	-0.520	-2.990	-0.680	-0.560
Germany	adjusted	-0.320	-0.530	-0.830	0.030	-0.090	-0.060	0.540	-1.960	-1.050	-0.390	0.240	0.880	0.300	0.430
Greece	valid	-0.283	-0.667	2.950	0.750	0.917	-2.567	10.167	-1.750	-1.500	0.883	-0.933	-8.533	-2.000	1.150
Greece	adjusted	1.067	0.383	1.333	0.533	0.417	0.883	4.800	-1.233	-0.567	0.433	0.667	-0.233	0.900	-0.917
Italy	valid	-1.263	-0.900	0.125	-0.463	0.050	-2.238	11.100	0.050	-0.063	0.400	-0.863	-7.613	-1.375	1.975
Italy	adjusted	-0.475	-0.025	0.150	-0.450	-0.050	-0.087	2.400	1.075	-0.188	0.087	-0.550	-2.563	0.638	1.713
Poland	valid	-2.331	-1.477	0.292	-0.354	1.162	-3.392	13.608	-1.785	-0.746	-0.854	-1.285	-8.731	-4.646	0.854
Poland	adjusted	-0.038	-0.354	-0.154	0.046	0.454	0.215	1.308	0.777	-0.069	-0.262	0.415	-4.131	-0.462	0.177
Portugal	valid	-0.660	0.180	-0.860	0.180	-0.360	-2.120	19.120	2.860	-0.640	0.340	-0.040	-6.040	-0.380	0.240
Portugal	adjusted	-1.020	0.400	-0.600	0.380	-1.160	-1.100	4.520	2.860	-0.940	0.420	-0.300	-2.640	-0.260	-1.520
Slovak Republic	valid	-1.630	-0.720	0.800	0.350	-0.470	-5.350	-20.230	-1.680	0.300	-0.130	-1.160	-15.290	-2.050	1.430
Slovak Republic	adjusted	-0.140	0.290	-0.050	-0.030	-0.300	-1.300	-8.260	-1.570	0.000	-0.210	-0.100	-0.430	-0.130	0.100
Sweden	valid	-1.800	-0.250	0.530	-0.560	-0.700	-7.470	-4.480	-0.460	-0.440	1.480	-1.080	-7.110	-2.320	-6.300
Sweden	adjusted	-0.600	-0.280	-0.070	-0.120	-0.400	-1.250	-8.900	-0.430	-0.410	0.270	-0.400	-1.280	-0.370	-4.080
United Kingdom	valid	-0.363	-0.088	-0.025	0.500	0.475	0.825	2.750	-0.912	0.325	0.688	-0.113	-0.888	-0.475	-0.150
United Kingdom	adjusted	-0.300	0.013	-0.188	-0.037	-0.500	0.250	0.500	-0.250	-0.213	-0.288	-0.288	1.450	0.225	-0.275

PART 2

Results of the La Bresse sampling and analysis workshop
Deliverable 5b

Compiled by Johan van der Molen and Piet Verdonschot
Partner no 4 (Alterra, The Netherlands)

Results of the La Bresse Diatom sampling and analysis workshop

Contents

Introduction

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 was 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 different levels of experience in diatom research, ranging from almost none to several years of professional experience. Experience was, 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 were collected from various parts of the stream. A possible patchy distribution of diatoms could introduce differences between replicate samples collected from one sampling site.

Sample treatment in the laboratory and microscope slide preparation

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

Microscope slide analysis

The analysis of the slides was done by identifying and counting diatom valves until 300 valves had been counted. In most cases more than 300 valves were present on the slide. A subsection of the slide was counted, which introduced a sub-sampling error.

Total error

The variation measured between replicate samples collected by one partner was the result of the combined errors introduced in the steps of sample collection, treatment and analysis. The total variation observed when comparing samples between partners comprised the 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 was performed for quality control of benthic diatom samples. The methods of performing the audit are introduced in this report by applying these methods to the results of the La Bresse workshop. The outcome of these analyses however, is of a different order than those were generated as part of the audit.

This report is focused on the difference between replicate samples, microhabitats and partners. This variation was studied based on two sampling sites where samples were collected from three different microhabitats. This set-up differed from the design of the audit. During the audit each microscope slide was 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 were only the result of difference in identification and counting (see Figure 1 for an illustration of the different levels at which variation between analyses was investigated during the workshop and audit).
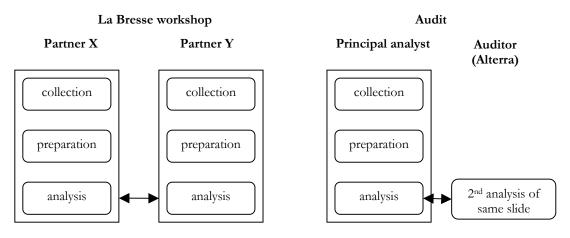
Figure 1Diagram illustrating the different levels at which results were compared between partners during the La Bresse workshop and the audit. Each square represents a stage in the analysis at which error was introduced. In the La Bresse workshop the overall variation based on collection, preparation and analysis was assessed. During the audit comparison was only made on the analysis (taxonomic 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 provided information on the variation at the level of diatom identification and counting only.

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 at 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 diatom nomenclature used for identification followed the taxon list used in the OMNIDIA programme (Lecointe *et al.*, 2003). This list was widely used throughout Europe and was kept up to date to allow for taxonomic revisions and new autecological information. The taxon list generally used the nomenclature following Krammer and Lange-Bertalot (1986-1998) and included 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 kth 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 provided a graphical means of summarising a variable in raw data, and illustrated the spread of values about the median. Visually each variable was 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	Sládeček's pollution index	1986	(Sládeček, 1986)
DESCY	Descy's pollution index	1979	(Descy, 1979)
LMI	Leclercq & Maquet's pollution index	1987	(Leclercq & Maquet, 1987)
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	1999	(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 were to 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 were 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 was indirect as it involved 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). A panel of experts decided on the decisions to be made within the comparison primary and audit identification results; this is called the executive action.

Results

General composition of the data matrix

Diatom 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.

Part-ner	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 number 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) for counts, logarithmic transformed data before and after the executive action.

Counts

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

Counts log transformed

	PL0H	PL0M	PL0S	PL5H	PL5M	PL5S
Number of taxa	22.8	33.0	33.7	27.0	41.9	39.5
	(4.8)	(13.3)	(16.5)	(7.6)	(16.9)	(18.7)
Shannon diversity	1.3	1.4	1.4	1.3	1.5	1.4
	(0.1)	(0.1)	(0.2)	(0.1)	(0.2)	(0.2)
Evenness	1.0	0.9	1.0	0.9	0.9	0.9
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)

Counts after executive action

	PL0H	PL0M	PL0S	PL5H	PL5M	PL5S
Number of taxa	21.7	31.6	32.0	25.4	39.1	37.2
	(4.5)	(12.9)	(15.9)	(7.1)	(16.1)	(17.3)
Shannon diversity	1.0	1.1	1.0	1.0	1.0	1.0
	(0.1)	(0.1)	(0.2)	(0.1)	(0.2)	(0.2)
Evenness	0.8	0.8	0.7	0.7	0.7	0.6
	(0.1)	(0.1)	(0.1)	(0.0)	(0.1)	(0.1)

Counts after executive action log transformed

	PL0H	PL0M	PL0S	PL5H	PL5M	PL5S
Number of taxa	21.7	31.6	32.0	25.4	39.1	37.2
	(4.5)	(12.9)	(15.9)	(7.1)	(16.1)	(17.3)
Shannon diversity	1.3	1.4	1.4	1.3	1.5	1.4
	(0.1)	(0.1)	(0.2)	(0.1)	(0.2)	(0.2)
Evenness	1.0	0.9	0.9	0.9	0.9	0.9
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)

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 was determined between average samples, based on all three replicates. 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 where similarities between partners were around 40% for all habitats.
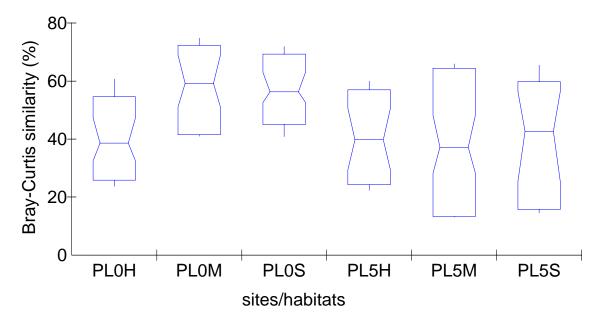
Figure 2 Spread about the median of the Bray-Curtis similarity values between samples from different partners at sites PL0 and PL5 from habitats stone (H), macrophyte (M) and sand/sediment (S).

A logarithmic transformation increased the similarity by a few percent, the standard deviation decreased also. In most cases the similarity was just about 50%. The logarithmic transformation added more weight to the lower abundances. Therefore, samples with a number of taxa in low abundances became more different using transformed data.

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

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

Table 4 Mean index values (standard deviation) of samples at sites PL0 and PL5 from habitats H (stone), M (macrophyte) and S (sand). First table is for the raw data and the second table after the executive action (taxonomic adjustment between partners based on a group of experts' judgement, second table). For explanation of the codes used for the index systems see Table 1.

Raw Data	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)

Executive Action	PL0H	PL0M	PL0S	PL5H	PL5M	PL5S
IPS	16.9	14.8	14.4	15.8	14.2	13.9
	(1.3)	(0.9)	(1.0)	(1.0)	(0.7)	(0.7)
SLAD	13.2	12.4	10.7	12.9	12.6	11.4
	(1.2)	(0.3)	(0.9)	(0.8)	(0.3)	(1.0)
DESCY	16.8	15.3	15.3	14.6	14.2	14.3
	(1.0)	(0.8)	(1.6)	(0.9)	(0.6)	(1.1)
L&M	11.2	11.4	9.7	11.3	11.0	9.9
	(1.3)	(0.5)	(0.7)	(1.0)	(0.7)	(0.7)
SHE	13.7	13.2	11.6	15.0	15.6	13.8
	(2.2)	(1.0)	(1.2)	(0.9)	(1.4)	(2.0)
WAT	16.6	15.4	13.4	16.6	14.8	13.6
	(1.7)	(1.3)	(1.1)	(1.0)	(1.2)	(1.1)
TDI	62.0	54.4	66.9	59.1	49.6	58.4
	(7.0)	(3.4)	(9.5)	(5.2)	(9.1)	(10.0)
EPI-D	8.8	9.1	7.7	10.1	9.4	8.1
	(2.3)	(1.6)	(1.0)	(1.0)	(0.7)	(1.0)
ROTT	13.8	13.2	12.6	14.8	15.5	14.5
	(1.3)	(0.8)	(0.8)	(0.9)	(1.2)	(1.2)
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	14.5	12.5	12.3	13.6	12.3	11.9
	(1.7)	(0.5)	(0.9)	(1.3)	(0.3)	(0.4)
IBD	16.9	14.4	14.2	14.1	11.2	12.0
	(1.7)	(0.5)	(1.5)	(1.8)	(1.8)	(1.9)
IDAP	11.6	10.9	11.1	11.5	11.4	11.4
	(0.9)	(0.5)	(0.4)	(0.7)	(0.7)	(0.5)

The estimated variance components between partners (Figure 3) showed that the variation between index results differed, depending on the habitat that was used to compute the index. The IPS results, for example, showed relatively 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 used autecological information at generic 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 were much higher, and suggested 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 indicated 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.
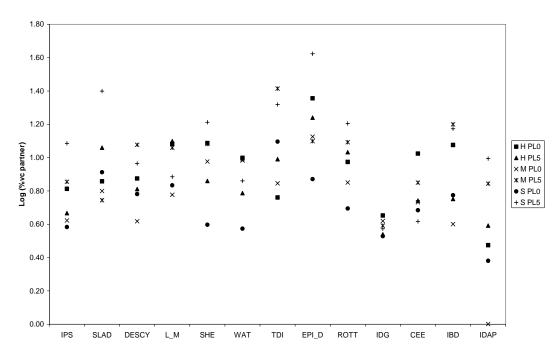

Figure 3 Estimated variance components between partners based on index values of Stone (H), Macrophyte (M) and Sand (S) samples from sites PL0 and PL5 in the Plaine River. For explanation of the codes used for the index systems see Table 1.
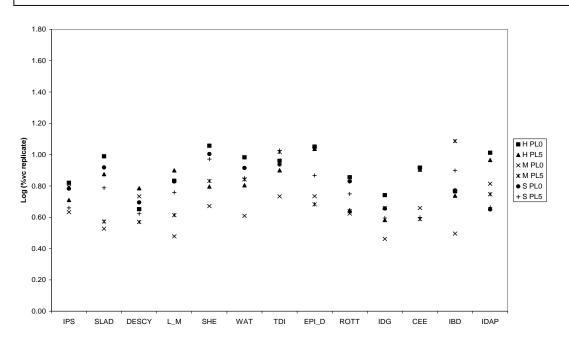
Figure 4 Estimated variance components between replicate samples based on index values of Stone (H), Macrophyte (M) and Sand (S) samples from sites PL0 and PL5 in the Plaine River. For explanation of the codes used for the index systems see Table 1.

A chi-squared test was done to determine whether 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

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.
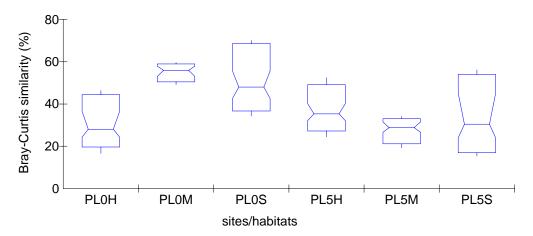

Figure 5 Bray-Curtis similarity between audit and primary samples at sites PL0 and PL5 from habitats H (stone), M (macrophyte) and S (sand).

Correlation of diatom indices between audit and primary samples

The correlation between the index values computed from primary and audit counts was shown for a selection of the indices (Figure 7). The IPS, TDI and IBD were among the most widely used index systems. The IDG and EPI-D were chosen because they showed a respectively low and a high variation between partners (see Figure 3). The correlation for the IBD after the executive action is shown in Figure 6. The results did not improve.

Figure 7 shows that the correlation between audit and primary samples was weak for most indices. The IDG index performed relatively well, but this index is based on genera and is therefore unlikely to provide enough separation between ecological classes to be useful The IBD, IPS and TDI showed a large spread and no significant correlation. This indicated 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 for the magnitude of variation that is acceptable.

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:

- taxa were selected that had a relative abundance ≥ 2% and were found in at least two samples.
- taxa were selected that had a similar count in samples from the same habitat and site but were named differently.
- these taxa were checked and knowledge of their appearance was used to determine whether a misidentification could have been the cause of the similar count.

These taxa were discussed during a diatom identification workshop held on 22 and 23 May 2003 in Wageningen, The Netherlands. During the workshop, participants were able to clarify some identification problems. It became clear that, in future, feedback would

remain important for dealing with problematic taxa. Quality control of identifications could be done by distributing diatom slides between laboratories (ring tests). Presenting the results of these ring tests and providing feedback on the identifications would further raise the level accuracy of diatom identification.

Figure 6 Correlation of the diatom index IBD after the executive action 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 the figure.

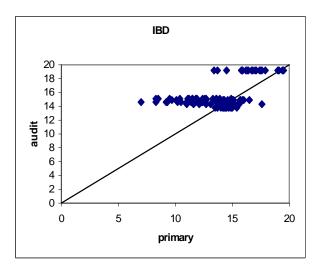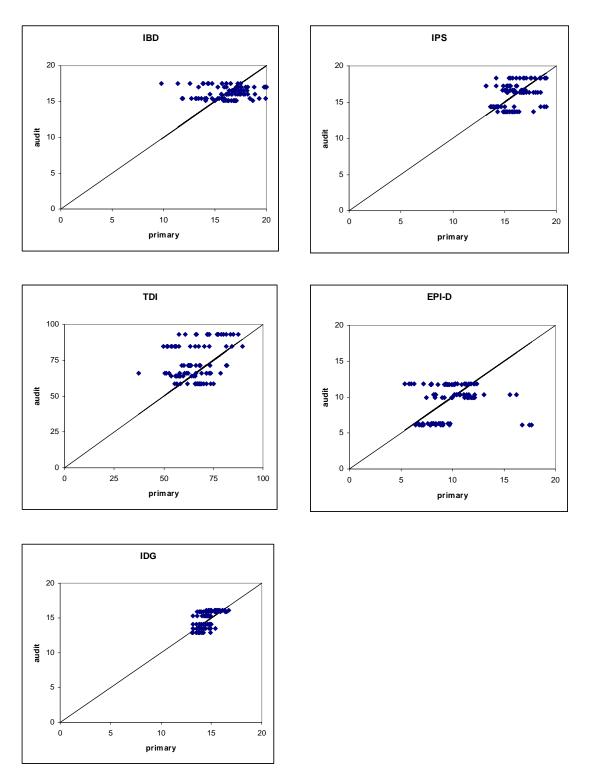*Figure 7 Correlation of diatom indices between primary and audit analyses for 110 samples from sites PL0 and PL5 from the La Plaine River. In the theoretical case that both primary and audit values are the same, the points would be plotted on the 1:1 line shown in each figure.*

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 observed between partners was made up of the combined errors that were introduced in the steps of sample collection, treatment and analysis plus the variation caused by different identifications and possible different interpretation of the sampling and analysis protocol. The design of this project did not allow for the contribution to the variation of each of these sources to be estimated.

The list of possible misidentified taxa consisted of taxa that were 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 noted 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 indicated 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, 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 at 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 regarded 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 meant that taxa with known indicator values were an important factor in determining the differences. We would expect this relationship to improve after misidentifications have been clarified and special emphasis should be placed on the identification of indicative taxa.

During the audit of STAR samples, both methods of comparing primary and audit samples were used. The variation that was found during the audit, however, was of a different nature than that shown by the La Bresse samples, because the audit only considered 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. The variation between primary and audit samples during the audit (with less sources of error) was less than what was 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 minimise the error in identification, periodic ring tests followed by coherent and frequent feedback.

Acknowledgements

References

Coste, M. (1987). Etude des méthodes biologique quantitatives d'appréciation de la qualité des eaux, Rapport Division Qualité des Eaux Lyon. Agence de l'Eau Rhône**:** 28.

Dell'Uomo, A. (1996). Assessment of water quality of an Apennine river as a pilot study for diatom-based monitoring of Italian water courses. In: B. A. Whitton & E. Rott. Use of Algae for Monitoring Rivers II. Innsbruck, Institut für Botanik, Universität Innsbruck**:** 64-72.

Descy, J-P. (1979). "A new approach to water quality estimation using diatoms." Nova Hedwigia **64**: 305-323.

Descy, J-P. & M. Coste (1991). "A test of methods for assessing water quality based on diatoms." Verh.int.Verein.Limnol. **24**: 2112-2116.

Kelly, M. G. (1997). "Sources of counting error in estimations of the Trophic Diatom Index." Diatom Research **12**(2): 255-262.

Kelly, M.G. & B.A. Whitton (1995). "The Trophic Diatom Index: a new index for monitoring eutrophication in rivers." Journal of Applied Phycology **7**: 433-444.

Kovach Computing Services (2002). Multi-Variate Statistics Package. v. 3.13b.

Krammer, K., H. Lange-Bertalot, N. Bate, A. Podzorski & J. Bukowska (1986-1998). Bacillariophyceae. Stuttgart, Fischer Verlag. 5 Bd; p.

Leclercq, L. & B. Maquet (1987). "Deux noveaux indices diatomique et the qualite chimique des eaux courantes. Comparison avec differents indices existants." Cah. Biol. Mar. **28**: 303-310.

Lecointe, C., M. Coste & J. Prygiel (2003). Omidia 3.2. Diatom index software including diatom database with taxonomic names, references and codes of 11645 diatom taxa. v.

Van der Molen, J.S. & P.F.M. Verdonschot (2002). Sampling protocol and audit benthic diatoms. STAR sampling protocol. Wageningen**:** 15.

Patterson, H.D. & R. Thompson (1971). "Recovery of inter-block information when block sizes are unequal." Biometrika **58**: 545-554.

Prygiel, J., P. Carpentier, S. Almeida, M. Coste, J. C. Druart, L. Ector, D. Guillard, M. A. Honore, R. Iserentant, P. Ledeganck, C. Lalanne-Cassou, C. Lesniak, I. Mercier, P. Moncaut, M. Nazart, N. Nouchet, F. Peres, V. Peeters, F. Rimet, A. Rumeau, S. Sabater, F. Straub, M. Torrisi, L. Tudesque, B. Van de Vijver, H. Vidal, J. Vizinet & N. Zydek (2002). "Determination of the biological diatom index (IBD NF T 90-354): results of an intercomparison exercise." Journal of Applied Phycology **14**(1): 27-39.

Prygiel, J. & M. Coste (1999). Progress in the use of diatoms for monitoring rivers in France. In: J. Prygiel, B. A. Whitton & J. Bukowska. Use of algae for monitoring rivers III. Douai, Agence de l'Eau Artois-Picardie**:** 165-179.

Rott, E., P. Pfister, H. Van Dam, E. Pipp, K. Pall, N. Binder & K. Ortler (1999). Indikationslisten für aufwuchsalgen. Wien, Bundesministerium für Land - und Forstwirtschaft**: 248.

Sládeček, V. (1986). "Diatoms as indicators of organic pollution." <u>Acta Hydrochimica et Hydrobiologica</u> **14**(5): 555-566.

Steinberg, C. & S. Schiefele (1988). "Biological indication of trophy and pollution of running waters." <u>Z. Wasser-Abwasser-Forsch.</u> **21**: 227-234

Van der Molen J. & Verdonschot P.F.M., 2002 Sampling protocol and audit benthic diatoms. Alterra note, STAR report, Wageningen. 1-17.

VSN International Ltd (2002). GenStat. v. 6.1. Oxford, UK.

Zar, J.H. (1996). <u>Biostatistical analysis</u>. Upper Saddle River, Prentice-Hall. 662 p.

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 occurrences	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*	84	9.5	8.5	There are taxonomists that

Code	Name	Number of occurren-ces	Mean	std	Comment
	var. *vaucheriae*				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	
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

Code	Name	Number of occurren-ces	Mean	std	Comment
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	