

ICP Waters Report 138/2018 Biological intercalibration: Invertebrates 2018









International Cooperative Programme on Assessment and Monitoring Effects of Air Pollution on Rivers and Lakes

LRTAP

Long-range Transboundary Air Pollution

Convention on Long-Range Transboundary Air Pollution

Norwegian Institute for Water Research

REPORT

Main Office

Gaustadalléen 21 NO-0349 Oslo, Norway Phone (47) 22 18 51 00 Telefax (47) 22 18 52 00

Internet: www.niva.no

NIVA Region South

Jon Lilletuns vei 3 NO-4879 Grimstad, Norway Phone (47) 22 18 51 00 Telefax (47) 37 04 45 13 **NIVA Region East**

Sandvikaveien 59 NO-2312 Ottestad, Norway Phone (47) 22 18 51 00 Telefax (47) 62 57 66 53 **NIVA Region West**

Thormøhlensgate 53 D NO-5006 Bergen Norway Phone (47) 22 18 51 00 Telefax (47) 55 31 22 14 NIVA Denmark

Ørestads Boulevard 73 DK-2300 Copenhagen Phone (45) 8896 9670

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Summary

The 22nd biological intercalibration of invertebrates in the ICP Waters programme included four laboratories. The intercalibration is important for harmonising taxonomic work across countries, and is of high value in programmes where the focus is on community analyses, e.g. for the classification of ecological status according to the EU Water Framework Directive. The laboratories correctly identified 96 % of the species and 97% of the genera. The mean Quality assurance index ranged between 85 and 99. No laboratories had a mean value below 80 – the limit for acceptable taxonomic work. Trends in biological intercalibration of invertebrates from the initial intercalibration in 1992 to 2018 are presented, showing that the Qi has remained above 80% for the full period, suggesting skilled taxonomists in the laboratories affiliated to ICP Waters. For individual invertebrate groups, the laboratories perform best for caddis flies and worst for stoneflies. The Quality assurance index increased steadily during the first 22 years and has seemingly decreased during the last three years, possibly because of an increase in difficulty.

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Heleen de Wit Project Manager Therese Fosholt Moe
Research Manager

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Biological Intercalibration: Invertebrates 2018

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Preface

The international cooperative programme on the assessment and monitoring of air pollution on rivers and lakes (ICP Waters) was established under the Executive Body of the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP) in July 1985. Since then, ICP Waters has been an important contributor to document the effects of implementing the Protocols under the Convention. ICP Waters has prepared numerous assessments, workshops, reports and publications covering the effects of long-range transported air pollution.

The ICP Waters Programme Centre is hosted by the Norwegian Institute for Water Research (NIVA), while the Norwegian Environment Agency manages the programme. A programme subcentre is established at NORCE (previously known as Uni Research), Bergen. The Programme Centre's work is supported financially by the Norwegian Environment Agency and from the UNECE LRTAP Trust Fund.

The main aim of the ICP Waters Programme is to assess, on a regional basis, the degree and geographical extent of the impact of atmospheric pollution, in particular acidification, on surface waters. More than 20 countries in Europe and North America participate in the programme on a regular basis.

The Programme's objective is to establish and maintain an international network of surface water monitoring sites and promote international harmonisation of monitoring practices. A tool in this work is the inter-laboratory quality assurance tests. The biases between analyses carried out by individual participants of the Programme are identified and controlled. The tests are also a valuable tool for taxonomic discussions and the exchange of identification keys among the participating laboratories, thereby improving the taxonomic skill.

We here report results from the 22nd intercalibration of the invertebrate fauna. We also compare results from all 22 intercalibrations.

Sut Velle

Gaute Velle

ICP Waters Programme Subcentre Bergen, November 2018

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Summary

The 22nd biological intercalibration of invertebrates in the ICP Waters programme included four laboratories. The intercalibration is important for harmonising taxonomic work across countries, and is of high value in programmes where the focus is on community analyses, e.g. for the classification of ecological status according to the EU Water Framework Directive. The intercalibration under the ICP Waters programme was the first regular test of species level identification.

The laboratories correctly identified a high proportion of the specimens in the test samples. In total, 96 % of the species and 97% of the genera were correctly identified. The mean Quality assurance index ranged between 85 and 99. No laboratories had a mean value below 80 – the limit for acceptable taxonomic work, and one laboratory identified all but one specimen correctly.

For the first time, we present trends in biological intercalibration of invertebrates from the initial intercalibration in 1992 up to the present. The average number of laboratories that took part on each occasion was five. The results show that the Qi has remained above 80% for the full period, suggesting skilled taxonomists of the laboratories affiliated to ICP Waters. When the Qi is broken into individual invertebrate groups, it is clear that the laboratories, on average over the years, perform best for caddis flies and worst for stoneflies. The Quality assurance index increased steadily during the first 22 years and has seemingly decreased during the last three years. According to the taxonomists that participate in the intercalibration, the drop in quality may mostly be due to an increase in difficulty.

1 Introduction

The purpose of the biological intercalibration of invertebrates is to evaluate the quality of the biological data delivered to the Programme centre. The data are used nationally and by ICP Waters to indicate environmental conditions from the species and their tolerances (Raddum et al. 1988, Fjellheim and Raddum 1990, Raddum 1999). The significance of potential trends in biotic indexes, both for a specific site/watershed and for comparisons of trends among regions or among countries, can be evaluated once the data quality is known. The data are also used in numerical analyses (Larsen *et al.* 1996, Skjelkvåle *et al.* 2000, Halvorsen *et al.* 2002, Halvorsen *et al.* 2003), and in analyses of biodiversity (Velle *et al.*, 2013, Velle *et al.* 2016). The results from such data analyses are especially sensitive to the quality of the species identification. The biological intercalibration focuses on the taxonomic skills of the participants and is a tool for improving the quality of work at the different laboratories, as well as harmonisation of the biological database.

The methods for the intercalibration of biological material were outlined in 1991 at the seventh ICP Waters Task Force meeting in Galway, Ireland. The countries/laboratories have to know, first, their native fauna. Since the fauna vary according to geographical regions, it is necessary to prepare specific samples for each participating laboratory, based on their native fauna. We cannot use standardised samples for all participants. Therefore, each laboratory sends identified samples of invertebrates from their own monitoring sites to the Programme subcentre. The Programme subcentre adds species known to be present in the region of the specific laboratory. Based on this, each laboratory receives individual test samples composed of species representing their own monitoring region.

The taxonomic skill of the different participants is measured by using a quality assurance index (Raddum 2005). This index evaluates the skill of participants when identifying species and genera. It also takes into account the effort of identifying all specimens in the sample. The highest index score is 100, while a value of 80 is set as the limit of good taxonomic work.

2 Methods

Preparation of the test-samples

Samples of identified invertebrates were sent from all participating laboratories to the organiser at the ICP Waters subcentre. These samples were used to compose test samples, with the addition of specimens from earlier exercises and from collections at the subcentre. The geographical distribution of the taxa was checked by the use of the Fauna Europaea Web Service 2013 (http://www.faunaeur.org). This is a database of the scientific names and distribution of multicellular European land and fresh-water animals (see example in Figure 1).

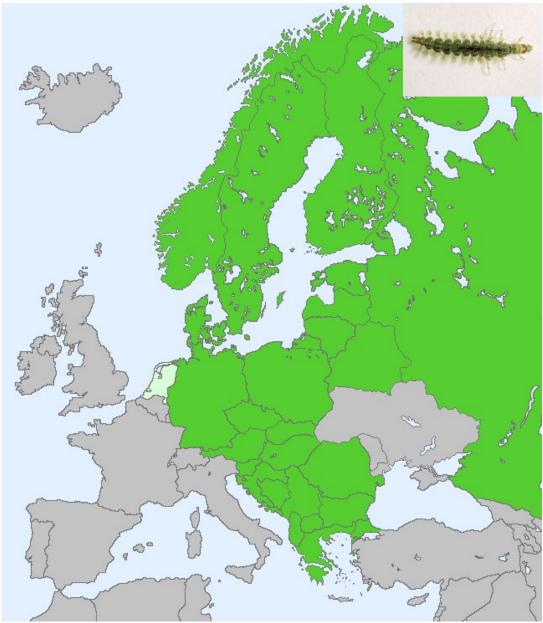


Figure 1. Geographical distribution of the caddisfly Rhyacophila nubila in Europe. This species is widely distributed, but is absent from several West-European countries. Map after Fauna Europaea Web Service, http://www.faunaeur.org, Photo: Arne Fjellheim

Identification

To minimise possible faults, the following procedure is used in preparing the test samples:

- The participating laboratory first identifies the source material for the test samples and ships the specimens to the organiser.
- Two persons from the organising institution verify the identification of the specimen as far as
 possible without damaging the individuals.
- The content of two test samples per participant is listed in a table. Two persons control that the correct numbers and species are placed in the test samples according to the table.

Damage to the material

The quality of the test material may be reduced during handling and shipping. Taxonomically important parts of the body, such as gills, legs, cerci and mouthparts can be lost or damaged during identification, handling and transportation. Contamination of larvae from other samples may also occur during these processes, as well as during the identification work at the participating laboratories. All abovementioned possibilities for faults could influence on the results of the identifications and influence the results negatively.

Evaluation

The participants are invited to comment on the results before the report is published. In this way, we can remove taxonomical biases - for example misidentification caused by damaged test material. In cases of disagreement between the participant and the organiser, the material may be checked again by the organiser. This procedure may act educational for both parts.

For calculation of errors, we must take into account possible degradation of the material. Further, a misidentified species counts as only one fault, even if the sample includes many individuals of the species. We encourage participants to give comments on matters that may impede the identification. For example, a misidentification will not count as a fault if a specimen lacks important taxonomic characters. Such comments must be made before the results are sent to the organiser.

We have discriminated between short-comings in identification, probably due to damaged material, and true errors (wrong species – or genus). Due to this, some subjective evaluation of the results has to be made. The number of errors is therefore subject to some degree of expert judgement.

The organiser also notes how many specimens a participant has identified per sample. This is called *percent identified*. A low percent means that many individuals were not identified and will consequently reduce the value of the taxonomic work.

Available material for making test samples varies. Normally, each laboratory receives between 60 and 130 species in the two samples. Samples with low diversity are easier to handle than samples with high diversity (see Appendix tables). This should also be kept in mind when the results are evaluated. Small samples should be avoided, as only a few misidentifications could result in a low score.

According to Fauna Europaea, the total number of European mayfly-, stonefly- and caddisfly species (in 2015) is 1814. However, the biodiversity differs between countries. Generally, the number of species decreases along a gradient from Southern to Northern Europe. This is also a fact to bear in mind when judging taxonomical capacity. As an example of this, the freshwater fauna of Switzerland is much richer than in Norway and Sweden — despite the fact that the area of Switzerland is approximately 1/10 of the two Nordic countries (Figure 2).

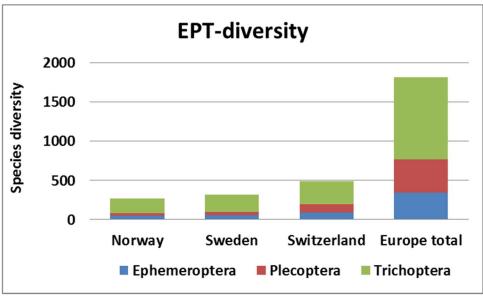


Figure 2. Species diversity of mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddisflies (Trichoptera) in Norway, Sweden and Switzerland (after Fauna Europaea Web Service, http://www.faunaeur.org

Quality assurance index

We have calculated the Quality assurance index, Qi, for important groups of invertebrates as well as the mean index for each participant. The Qi integrates the separate levels of the identifications as follows:

Qi = (% correct species/10) * (% correct genus/10) * (% identified individuals/100)

Qi will be a number between 0 and 100. 100 is the highest score that can be obtained. A score \geq 80 is regarded as good and thus acceptable taxonomical work.

Test of the subcentre

The ICP Waters subcentre in Bergen is tested with the help from the Swedish participant every second year. The Swedish University of Agricultural Sciences in Uppsala prepares and evaluates the test of the subcentre. Methodology and implementation is otherwise identical to the other tests.

3 Results and discussion

Four laboratories participated in the intercalibration of invertebrates in 2018 (Appendix A). The species lists and the identification results are shown in Appendix B, Tables 1-4.

Mayflies

The identification of the mayflies (Figure 3) ranged from below acceptable to excellent. The low Qi for Laboratory 1 is a result of two of 15 specimens identified to wrong genus and wrong species. As often occurs, the two specimens lacked some legs and one specimen also lacked a cerci.

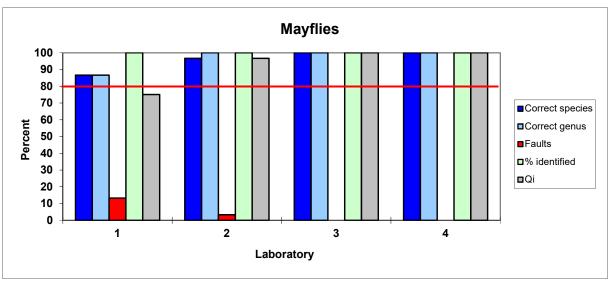


Figure 3. Results from the identification of mayflies. The red line indicates the acceptable limit.

Stoneflies

The identification of the stoneflies is shown in Figure 4. The results were above the acceptable limit and very good for all laboratories. All specimens were assigned to the correct genus, and only three specimens assigned to the wrong species.

Caddisflies

The identification of the caddisflies was excellent for three of the laboratories with no misidentifications, and below the acceptable limit for Laboratory 1 (Figure 5). Here, three specimens were assigned to the wrong species, of which two also were assigned to the wrong genus.

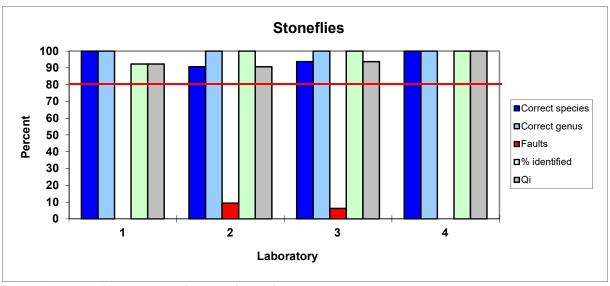


Figure 4. Results from the identification of stoneflies. The red line indicates the acceptable limit.

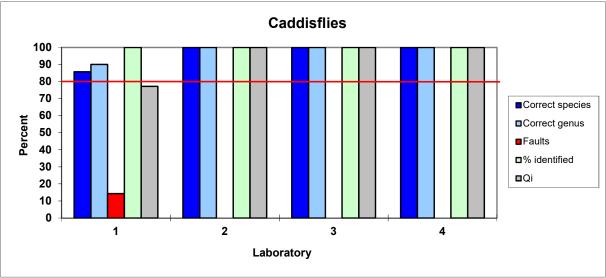


Figure 5. Results from the identification of caddisflies. The red line indicates the acceptable limit.

Other groups

The miscellaneous group included water beetles (Coleoptera), larger crustaceans (Malacostraca), leeches (Hirudinea), molluscs (Gastropoda), alderflies (Megaloptera), dragonflies (Odonata), water boatmen (Corixidea), midges and flies (Diptera) and flatworms (Turbellaria). Both larvae and imagines were included. Leeches, molluscs and larger crustaceans are sensitive to acid water and important for the evaluation of acidification. The tolerance of some species of Coleoptera, Megaloptera and Diptera is poorly known, but they are often regarded as tolerant to acidic water and of low importance for the evaluation of acidity indices. However, all species are important for invertebrate community analysis.

The identifications made by laboratories 1, 3 and 4 were very good with only one specimen each not assigned to the correct species or genus (Figure 6 and Appendix tables 1-4). Laboratory 2 did not reach the acceptable limit. According to laboratory 4, they were not able to identify two of the species since the specimen were very small and one species because they lacked taxonomic literature.

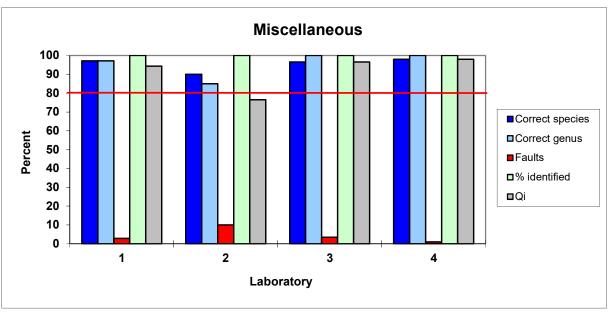


Figure 6. Results from the identification of miscellaneous groups of invertebrates. The red line indicates the acceptable limit.

In addition, two species of beetles were assigned to the wrong species. Laboratory 4 wrongly identified on species of Odonata before the specimen was sent to the organizer to arrange the test samples. The specimen was correctly identified during the intercalibration. We have chosen to indicate this as one half error (Figure 6).

Total number of species in the sample

A total of 355 individuals were sent to the four laboratories. Of these, all but two specimens were reported back to the programme subcentre.

4 Evaluation

The laboratories correctly identified a high portion of the total number of species in the test samples. The mean skill of identifying species, genus and Qi- score per laboratory is shown in Figure 7. The mean Qi ranged from good (Qi = 85) for laboratory one to excellent (Qi = 99) for laboratory four, which means that laboratory four identified all but one specimen correctly.

The biological intercalibration is important for harmonising biological material/databases and will be of high value in projects which focus on community analyses, or where the ecological status of waterbodies should be determined. The biological intercalibration under the ICP Waters programme was the first regular test aiming to test taxonomic skills in identifying benthic invertebrates. Today, similar tests are run by the North American Benthological Society (http://www.faunaeur.org) and by the Natural History museum, London (Identification Qualifications – IdQ test). The invertebrate groups covered in the latter test are those used in the BMWP water quality score system (Armitage et al., 1983) and include groups used for monitoring freshwater environments under the EU water framework directive (Schartau et al. 2008). In 2017-2018, Uni Research Environment also organized an extensive test similar to the ICP waters intercalibration for Norwegian Laboratories (Velle et al. 2018).

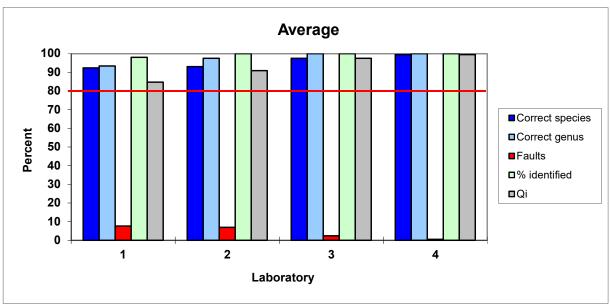


Figure 7. Mean skill in percent of identifying species and genus, and mean Qi for each laboratory. The red line indicates the acceptable limit.

5 Trends over time

The invertebrate intercalibration in ICP Waters started in 1992. An overall high of 11 laboratories participated in the first intercalibration (Figure 8). Since then, the average has been five participants per year. Twenty different laboratories from 17 countries have participated over the years, including Austria, Belgium, Canada, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Ireland, Latvia, Norway, Russia, Sweden, Switzerland and UK.

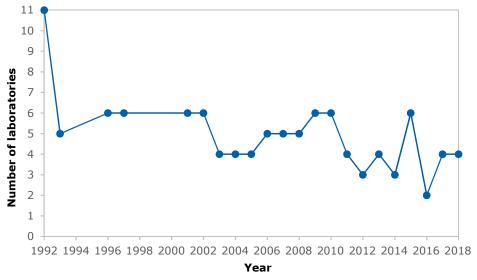


Figure 8. The number of participating laboratories in the ICP Waters invertebrate intercalibration since the first intercalibration in 1992.

The intercalibration laboratory protocol is unchanged since 1992, while the quality assurance index (Qi) has been used since it was introduced in 2005 (Raddum, 2005). In the current report, we have back- calculated the Qi for the period prior to 2005 so that the Qi now is available from 1992 and up to the present (Figure 9). Trends in the Qi-score show that the mean has remained above 80%, suggesting good taxonomic work and skilled taxonomists in the laboratories affiliated to ICP Waters. When the Qi is broken into individual invertebrate groups, it is clear that the laboratories, on average over the years, perform best for caddisflies and worst for stoneflies (Figure 10). This suggests that many laboratories may benefit from focusing their future efforts on the identification of stoneflies.

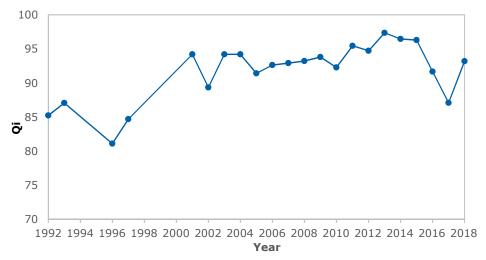


Figure 9. The mean quality assurance index for the invertebrate intercalibration through time.

One of the aims of the intercalibration is to improve the taxonomic skill of the participating laboratories. The mean Qi has increased since the intercalibration started, suggesting that the skills have indeed improved (Figure 9). Still, at least four issues influence the Qi:

- 1) The Qi varies according to the skills of the participants. A consequence is that the Qi often decreases when new labs participate or if a skilled taxonomist retires. As an example, the expert on the miscellaneous group retired from Laboratory 2 in 2018, which resulted in a low Qi (Figure 6).
- 2) The Qi varies according to the difficulty of the test, which mostly depends on the size of the specimen and the rarity of the species. For example, more species in the miscellaneous group were included in the intercalibration around 2005 since new acidification indices demanded a higher taxonomic resolution for this group. Hence, the Qi subsequently dropped for some years before it gradually increased (Figure 10). The increase likely reflects improved taxonomic skill.
- 3) There is inevitably some chance involved. For example, samples have occasionally dried out, a taxonomist may have overlooked a specimen or forgotten to make comments on a damaged specimen. 4) Some years, the participants send too few specimens from their home region to the intercalibration organiser. This may influence the results since the organiser then needs to include specimen from other regions to the test of that specific participant. It is therefore important that the participants send an abundance of specimens to the organiser.

The mean Qi has decreased during the last three years. According to the taxonomists, the difficulty has increased during the last three years, and especially for stoneflies. In addition, it seems some other abovementioned factors apply; there was a new participant, one key taxonomist retired, one sample dried out and one laboratory sent too few specimen from their home region. Hopefully, the abundance of such events will decline during forthcoming intercalibrations.

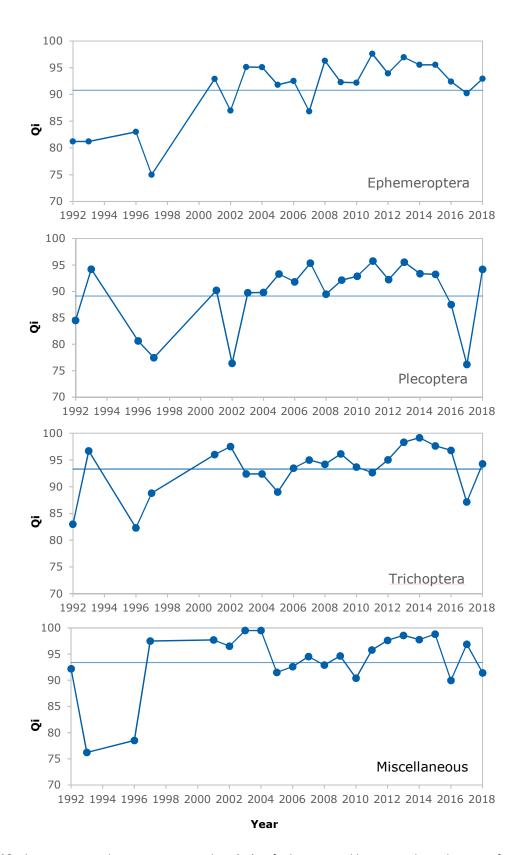


Figure 10. The mean quality assurance index (Qi) of the intercalibrations through time for mayflies (Ephemeroptera), stoneflies (Plecoptera), caddisflies (Trichoptera) and miscellaneous groups of invertebrates. The straight line represents the overall mean Qi for each invertebrate group. Qi above 80 is regarded as good and thus acceptable taxonomical work.

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7 Appendix A. Responsible laboratories

Each participating laboratory is identified by a number, which is identical with the table number in the Appendix and laboratory numbers in the report. Laboratories participating in the intercalibration of invertebrates in 2018 are:

- 1. Environmental Protection Agency, John Moore Road, Castlebar, Co. Mayo, **Ireland**. Responsible taxonomist: Ruth Little.
- 2. NORCE AS, P.O. box 7810 N-5020 Bergen, **Norway**. Responsible taxonomists: Torunn S. Landås and Arne Johannessen.
- 3. Swedish University of Agricultural Sciences, Dept. of Environmental Assessment, P.O. Box 7050, S-75007 Uppsala, **Sweden**. Responsible taxonomist: Dr. Magda-Lena Wiklund.
- 4. Estonian Environmental Research Centre, Tartu Department, Vaksali 17a, 50410 Tartu, **Estonia.** Responsible taxonomist: Dr. Lilian Varblane

8 Appendix B. Species lists

Table 1. Identified species/genus in sample 1 and 2 by Laboratory 1

| | Sam | Sample 1 | | Sample 2 | |
|------------------------------|-----------|--------------|-----------|------------|--|
| Taxa: | Delivered | Identified | Delivered | Identified | |
| Ephemeroptera | | | | | |
| Baetis rhodani | 1 | 1 | | | |
| Caenis horaria | 1 | 1 | | | |
| Caenis luctuosa | | | 1 | 1 | |
| Centroptilum luteolum | 1 | 1 | 1 | 1 | |
| Cloeon simile | 1 | _ | 1 | 1 | |
| Electrogena lateralis | 1 | | 1 | 1 | |
| Ephemera danica | 1 | 1 | | | |
| Heptagenia fuscogrisea | 1 | 1 | 1 | 1 | |
| Heptagenia sulphurea | | 1 | 1 | 1 | |
| Leptophlebia vespertina | 1 | 1 | 1 | 1 | |
| Procloeon bifidum | _ | 1 | _ | | |
| Plecoptera | | _ | | | |
| Brachyptera risi | 1 | 1 | 1 | 1 | |
| Dinocras cephalotes | <u> </u> | | 1 | 1 | |
| Isoperla grammatica | 1 | 1 | 1 | 1 | |
| Nemoura cinerea | 1 | 1 | 1 | 1 | |
| Protonemura meyeri | 1 | 1 | 1 | 1 | |
| Siphonoperla torrentium | 1 | 1 | 1 | 1 | |
| Taeniopteryx nebulosa | 1 | _ | 1 | 1 | |
| Trichoptera | | | - | | |
| Agapetus fuscipes | 1 | 1 | 1 | 1 | |
| Anabolia nervosa | 1 | 1 | 1 | 1 | |
| Cyrnus trimaculatus | 1 | - | 1 | 1 | |
| Halesus radiatus | 1 | 1 | 1 | 1 | |
| Hydroptila sp. | 1 | 1 | 1 | 1 | |
| Lepidostoma hirtum | 1 | 1 | 1 | 1 | |
| Limnephilus lunatus | - | | 1 | 1 | |
| Limnephilus marmoratus | 1 | 1 | 1 | 1 | |
| Plectrocnemia conspersa | - | 1 | - | _ | |
| Polycentropus flavomaculatus | 1 | - | | 1 | |
| Polycentropus kingi | | 1 | 1 | | |
| Sericostoma personatum | 1 | 1 | 1 | 1 | |
| Tinodes waeneri | 1 | 1 | 1 | 1 | |
| Miscellanous | - | - | - | _ | |
| Gastropoda | | | | | |
| Acroloxus lacustris | 1 | 1 | | | |
| Bathymophalus contortus | | - | 1 | 1 | |
| Bithynia tentaculata | 1 | 1 | <u> </u> | | |
| Gyraulus crista | | - | 1 | 1 | |
| Physa fontinalis | | | 1 | 1 | |
| Planorbis planorbis | | | 1 | 1 | |
| Potamopyrgus antipodarum | 1 | 1 | - | | |
| Radix balthica | 1 | - | | | |
| Theodoxus fluviatilis | 1 | 1 | 1 | 1 | |
| Valvata piscinalis | 1 | 1 | 1 | 1 | |
| Hirudinea | 1 | 1 | | | |

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Table 2. Identified species/genus in sample 1 and 2 by Laboratory 2

| | Sam | Sample 1 | | Sample 2 | | |
|----------------------------------|-----------|------------|-----------|------------|--|--|
| Таха: | Delivered | Identified | Delivered | Identified | | |
| Ephemeroptera | | | | | | |
| Ameletus inopinatus | 1 | 1 | 1 | 1 | | |
| Arthroplea congener | 1 | 1 | 1 | 1 | | |
| Baetis muticus | 2 | 3 | | | | |
| Baetis niger | 1 | 1 | 1 | 1 | | |
| Baetis rhodani | 1 | 1 | 1 | 1 | | |
| Caenis horaria | 1 | 1 | 1 | 1 | | |
| Caenis luctuosa | 1 | 1 | 1 | 1 | | |
| Ephemerella aurivilli | 1 | 1 | _ | | | |
| Ephemerella mucronata | 1 | 1 | 1 | 1 | | |
| Heptagenia dalecarlica | 1 | 1 | 2 | 2 | | |
| Heptagenia fuscogrisea | 1 | 1 | 1 | 1 | | |
| Heptagenia sulphurea | 2 | 2 | 1 | 1 | | |
| Leptophlebia marginata | 2 | 1 | 1 | 1 | | |
| | | 1 | | | | |
| Leptophlebia verspertina | | 1 | 1 | 1 1 | | |
| Siphlonurus lacustris Plecoptera | | | 1 | 1 | | |
| - | 1 | 1 | 1 | 1 | | |
| Amphinemura borealis | 1 | 1 | 1 | 1 | | |
| Amphinemura strandfussi | 1 | 1 | | 2 | | |
| Amphinemura sulcicollis | 1 | 1 | 2 | | | |
| Arcynopteryx compacta | 1 | 1 | | | | |
| Capnopsis schilleri | 2 | 2 | 1 | 1 | | |
| Diura nanseni | 1 | 1 | 1 | 1 | | |
| Isoperla grammatica | | | 2 | 2 | | |
| Leuctra fusca | | | 1 | 1 | | |
| Leuctra nigra | 1 | 1 | 2 | 2 | | |
| Nemoura avicularis | 1 | 1 | | | | |
| Nemoura cinerea | 1 | | 2 | 2 | | |
| Nemoura flexuosa | 1 | 2 | 2 | 2 | | |
| Nemurella pictetii | 2 | 2 | 1 | 1 | | |
| Siphonoperla burmeisteri | 1 | 1 | 1 | 1 | | |
| Taeniopteryx nebulosa | 1 | 1 | 1 | 1 | | |
| Trichoptera | | | | | | |
| Agapetus ochripes | 1 | 1 | | | | |
| Athripsodes aterrimus | 1 | 1 | | | | |
| Athripsodes commutatus | | | 1 | 1 | | |
| Ceraclea annulicornis | 1 | 1 | | | | |
| Ceratopsyche silfvenii | 1 | 1 | 1 | 1 | | |
| Chimarra marginata | | | 1 | 1 | | |
| Cyrnus insolutus | 1 | 1 | | | | |
| Glossosoma intermedium | | | 1 | 1 | | |
| Halesus radiatus | 1 | 1 | | | | |
| Holocentropus dubius | 1 | 1 | 1 | 1 | | |
| Hydropshyche siltalai | 1 | 1 | 1 | 1 | | |
| Hydropshyche angustipennis | | | 1 | 1 | | |
| Hydropsyche pellucidula | | | 1 | 1 | | |
| Hydropsyche saxonica | 1 | 1 | _ | | | |
| Molanna angustata | | _ | 1 | 1 | | |
| Molannodes tinctus | 1 | 1 | <u> </u> | <u> </u> | | |
| Nemotalius punctolineatus | <u>_</u> | 1 | 1 | 1 | | |
| Neureclipsis bimaculata | 1 | 1 | 1 | 1 | | |
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Table 3. Identified species/genus in sample 1 and 2 by Laboratory 3

| | Sam | Sample 1 | | Sample 2 | | |
|-------------------------|-----------|------------|-----------|------------|--|--|
| Taxa: | Delivered | Identified | Delivered | Identified | | |
| Ephemeroptera | | | | | | |
| Arthroplea congener | 1 | 1 | | | | |
| Baetis digitatus | | | 1 | 1 | | |
| Caenis horaria | | | 1 | 1 | | |
| Caenis luctuosa | | | 1 | 1 | | |
| Caenis rivulorum | 1 | 1 | | | | |
| Centroptilum luteolum | 1 | 1 | | | | |
| Ephemera danica | 1 | 1 | | | | |
| Ephemera vulgata | 1 | 1 | 1 | 1 | | |
| Ephemerella aurivilli | 1 | 1 | _ | | | |
| Ephemerella mucronata | | | 1 | 1 | | |
| Heptagenia sulphurea | | | 1 | 1 | | |
| Leptophlebia marginata | | | 1 | 1 | | |
| Leptophlebia vespertina | 1 | 1 | <u> </u> | | | |
| | 1 | 1 | | | | |
| Nigrobaetis niger | 1 | 1 | | | | |
| Plecoptera | 1 | 1 | 1 | 1 | | |
| Amphinemura borealis | 1 | <u> </u> | 1 | 1 | | |
| Capnopsis schilleri | 1 | 4 | 1 | 1 | | |
| Dinocras cephalotes | 1 | 1 | | 4 | | |
| Diura nanseni | 1 | 1 | 1 | 1 | | |
| Leuctra nigra | 1 | 1 | 1 | 1 | | |
| Nemoura sp. | | 1 | | | | |
| Nemoura avicularis | 1 | 1 | | | | |
| Nemoura cinerea | 1 | 1 | | | | |
| Nemoura flexuosa | 1 | | 1 | 1 | | |
| Protonemura meyeri | 1 | 1 | 1 | 1 | | |
| Taeniopteryx nebulosa | 1 | 1 | 1 | 1 | | |
| Trichoptera | | | | | | |
| Apatania wallengreni | 1 | 1 | 1 | 1 | | |
| Cyrnus flavidus | 1 | 1 | | | | |
| Ecnomus tenellus | 1 | 1 | 1 | 1 | | |
| Holocentropus dubius | 1 | 1 | 1 | 1 | | |
| Lepidostoma hirtum | | | 1 | 1 | | |
| Micrasema setiferum | 1 | 1 | 1 | 1 | | |
| Molanna angustata | 1 | 1 | | | | |
| Neureclipsis bimaculata | 1 | 1 | 1 | 1 | | |
| Notodobia ciliaris | 1 | 1 | | | | |
| Oecetis testacea | 1 | 1 | 1 | 1 | | |
| Oligotricha striata | 1 | 1 | 1 | 1 | | |
| Rhyacophila nubila | 1 | 1 | 1 | 1 | | |
| Miscellanous | | | | | | |
| Gastropoda | | | | | | |
| Bithynia tentaculata | 1 | 1 | 1 | 1 | | |
| Gyraulus albus | 1 | 1 | 1 | 1 | | |
| Hippeutis complanatus | 1 | 1 | | | | |
| Radix balthica | | | 1 | 1 | | |
| Theodoxus fluviatilis | | | 1 | 1 | | |
| Malacostraca | | | _ | | | |
| Asellus aquaticus | 1 | 1 | 1 | 1 | | |
| Gammarus pulex | 1 | 1 | 1 | 1 | | |
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| Erythromma najas | 1 | 1 |
|--------------------------|---|---|
| Onychogomphus forcipatus | | |
| Phyrrosoma nymphula | | |
| Coleoptera | | |
| Elmis aenea | 1 | 1 |
| Elodes sp. | | |
| Elodes minuta | | |
| Haliplus sp. | | |
| Hydraena gracilis | | |
| Hygrotus versicolor | 1 | 1 |
| Hyphydrus ovatus | 1 | 1 |
| Limnius volckmari | 1 | 1 |
| Nebrioporus depressus | 1 | 1 |
| Diptera | | |
| Dicranota sp. | 1 | 1 |
| Limnophora sp. | 1 | 1 |
| Tipula sp. | 1 | 1 |

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Table 4. Identified species/genus in sample 1 and 2 by Laboratory 4

| | Sam | Sample 1 | | Sample 2 | | |
|--|-----------|------------|-----------|------------|--|--|
| Таха: | Delivered | Identified | Delivered | Identified | | |
| Ephemeroptera | | | | | | |
| Alainites muticus | 1 | 1 | | | | |
| Baetis digitatus | | | 1 | 1 | | |
| Baetis rhodani | 1 | 1 | | | | |
| Caenis horaria | | _ | 1 | 1 | | |
| Caenis luctuosa | 1 | 1 | | | | |
| Caenis rivulorum | | | 1 | 1 | | |
| Centroptilum luteolum | 1 | 1 | | | | |
| Ephemera lineata | 1 | 1 | | | | |
| Ephemera vulgata | | _ | 1 | 1 | | |
| Ephemerella mucronata | 1 | 1 | - | | | |
| Heptagenia sulphurea | 1 | 1 | | | | |
| Kageronia fuscogrisea | | - | 1 | 1 | | |
| Leptophlebia marginata | 1 | 1 | | | | |
| | | 1 | 1 | 1 | | |
| Nigrobaetis niger | | | 1 | 1 | | |
| Paraleptophlebia submarginata Potamanthus luteus | 1 | 1 | 1 | 1 | | |
| | 1 | 1 | | | | |
| Plecoptera | | 1 | | 4 | | |
| Amphinemura borealis | 1 | 1 | 1 | 1 | | |
| Brachyptera risi | 1 | 1 | 1 | 1 | | |
| Capnia bifrons | 1 | 1 | 1 | 1 | | |
| Capnopsis schilleri | 1 | 1 | 1 | 1 | | |
| Isoptena serricornis | 1 | 1 | 1 | 1 | | |
| Nemurella pictetii | 1 | 1 | 1 | 1 | | |
| Perlodes dispar | 1 | 1 | 1 | 1 | | |
| Rhabdiopteryx acuminata | 1 | 1 | 1 | 1 | | |
| Siphonoperla burmeisteri | 1 | 1 | 1 | 1 | | |
| Trichoptera | | | | | | |
| Athripsodes cinereus | 1 | 1 | 1 | 1 | | |
| Brachycentrus subnubilus | 1 | 1 | | | | |
| Chimarra marginata | | | 1 | 1 | | |
| Cyrnus flavidus | 1 | 1 | | | | |
| Glyphotaelius pellucidus | 1 | 1 | | | | |
| Halesus radiatus | 1 | 1 | | | | |
| Ithytrichia lamellaris | 1 | 1 | 1 | 1 | | |
| Lasiocephala basalis | | | 1 | 1 | | |
| Lepidostoma hirtum | 1 | 1 | 1 | 1 | | |
| Limnephilus rhombicus | | | 1 | 1 | | |
| Micrasema setiferum | | | 1 | 1 | | |
| Molanna angustata | 1 | 1 | | | | |
| Mystacides azurea | | | 1 | 1 | | |
| Mystacides niger | 1 | 1 | | | | |
| Notidobia ciliaris | 1 | 1 | 1 | 1 | | |
| Oecetis testacea | | | 1 | 1 | | |
| Polycentropus flavomaculatus | | | 1 | 1 | | |
| Psychomyia pusilla | | | 1 | 1 | | |
| Tinodes waeneri | 1 | 1 | | | | |
| Miscellanous | | - | | | | |
| Gastropoda | | | | | | |
| Acroloxus lacustris | 1 | 1 | 1 | 1 | | |
| Ancylus fluviatilis | 1 | 1 | 1 | 1 | | |
| rancyrus jiuviutins | | | | 1 + | | |

| Bithynia tentaculata | 1 | 1 | 1 | 1 |
|-----------------------|--------------|---|----------|----|
| Theodoxus fluviatilis | 1 | 1 | 1 | 1 |
| Hirudinea | _ | 1 | <u>+</u> | ± |
| Erpobdella octoculata | 1 | 1 | 1 | 1 |
| | 1 | 1 | | |
| Haemopis sanguisuga | | | 1 | 1 |
| Helobdella stagnalis | 1 | 1 | 1 | 1 |
| Malacostraca | | | | |
| Asellus aquaticus | 1 | 1 | | |
| Odonata | | | | |
| Aeshna cyaena | 1 | 1 | | |
| Calyopteryx splendens | | | | 1* |
| Calyopteryx virgo | | | 1* | |
| Gomphus vulgatissimus | 1 | 1 | | |
| Platycnemis pennipes | | | 1 | 1 |
| Coleoptera | | | | |
| Elmis maugetii | | | 1 | 1 |
| Nebrioporus depressus | 1 | 1 | | |
| Normandia nitens | 1 | 1 | 1 | 1 |
| Olimnius tuberculatus | 1 | 1 | 1 | 1 |
| Orectochilus villosus | 1 | 1 | | |
| Megaloptera | | | | |
| Sialis fuliginosa | 1 | 1 | | |
| Sialis sordida | | | 1 | 1 |
| Diptera | | | | |
| Atherix ibis | 1 | 1 | | |

^{*} Laboratory 4 wrongly identified the specimen to *Calyopteryx virgo* before the specimen was sent to the organizer to arrange the test samples. The specimen was correctly identified to *Calyopteryx splendens* during the intercalibration.

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Gaustadalléen 21 • NO-0349 Oslo, Norway Telephone: +47 22 18 51 00 • Fax: 22 18 52 00 www.niva.no • post@niva.no