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ICP Waters Report 130/2016

Biological intercalibration: Invertebrates 2016

The international Cooperative Programme on Assessment and Monitoring Effects of Air Pollution on Rivers and Lakes (ICP Waters)
Convention on Long-Range Transboundary Air Pollution

Norwegian Institute for Water Research
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REPORT

Main Office
Gaustadalléen 21
NO-0349 OSLO
N-0411 Oslo, Norway
Phone (47) 22 18 51 00
Telefax (47) 22 18 52 00
Internet: www.niva.no

Regional Office, Sørlandet
Jon Lilletuns vei 3 4879
Grimstad

Telefon (47) 22 18 51 00
Telefax (47) 37 04 45 13

Regional Office, Østlandet
Sandvikaveien 59 2312
Ottestad

Telefon (47) 22 18 51 00
Telefax (47) 62 57 66 53

Regional Office, Vestlandet
Thormøhlensgate 53 D 5006
Bergen

Telefon (47) 22 18 51 00
Telefax (47) 55 31 22 14

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Author(s) Godtfred Anker Halvorsen, Uni Research Environment AS, Bergen, Norway Arne Johannessen, Uni Research Environment AS, Bergen, Norway Torunn Svanevik Landås, Uni Research Environment AS, Bergen, Norway	Topic group Acidification	Distribution Open
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Abstract

Two European laboratories participated in the 20th ICP Waters biological intercalibration, which took place in 2016. The laboratories identified a high proportion of the individuals in the test samples, 93 % of the total number of species was correctly identified. On the genus level, few faults were recorded. The mean Quality assurance index ranged between 86.1 and 96.2, well above the value 80 - indicating acceptable taxonomic work.

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Heleen de Wit
Project manager

Øyvind Kaste
Research Manager

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CONVENTION ON LONG-RANGE TRANSBOUNDARY AIR POLLUTION

INTERNATIONAL COOPERATIVE PROGRAMME ON ASSESSMENT AND MONITORING OF ACIDIFICATION OF RIVERS AND LAKES

Biological intercalibration: Invertebrates 2016

ICP Waters Programme Subcentre
Uni Research AS, P.O.box 7810
N-5020 Bergen, Norway

Preface

The international cooperative programme on 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. Numerous assessments, workshops, reports and publications covering the effects of long-range transported air pollution have been published over the years.

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 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 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 bias between analyses carried out by the individual participants of the Programme has to be identified and controlled. The tests will also be a valuable tool in improving the taxonomic skill of the participating laboratories.

We here report the results from the 20th intercalibration on invertebrate fauna.

Bergen, November 2016

Godtfred Anker Halvorsen
ICP Waters Programme Subcentre

Contents

Summary

The 20th intercalibration of invertebrates in the ICP Waters programme had contribution from two laboratories. The biological intercalibration is important for harmonising biological material/databases and will be of high value in programmes where community analyses is in focus or where the ecological status should be stated, like EU Water Framework Directive. The biological intercalibration under the ICP Waters programme is a unique test, as it operates on a species level.

The laboratories identified a high proportion of the individuals in the test samples, 93 % of the total number of species was correctly identified. Few faults were recorded on genus level. The mean Quality assurance index ranged between 86.1 and 96.2 well above the value 80 - indicating acceptable taxonomic work.

1. Introduction

The purpose of the biological intercalibration is to evaluate the quality of the taxonomic work on the biological material delivered to the Programme centre. The quality can influence on the evaluation of the samples, which is based on the species and their tolerance (Raddum *et al.* 1988, Fjellheim and Raddum 1990, Raddum 1999). The control is therefore important for evaluation of the significance of trends in biotic indexes both for a specific site/watershed, as well as for comparisons of trends between different regions and countries. The material is also used in multivariate statistical analysis (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 of this type of data treatment 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 intercalibration of biological material were outlined in 1991 at the 7th ICP Waters Task Force meeting in Galway, Ireland. The different countries/laboratories have to know, first of all, their native fauna. Since the fauna in different geographical regions vary, it is necessary to prepare specific samples for each participating laboratory, based on their native fauna. It is a problem for the exercise of the intercalibration that it is not possible to use standardised samples for all participants. To solve this problem, each laboratory send identified samples of invertebrates from their own monitoring sites to the Programme centre. The Programme centre will additionally add 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, see Raddum (2005). This index evaluates the skill of identifying the species as well as the genus. 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 test-samples

Samples of identified invertebrates were received from all participating laboratories. These samples were used to compose test samples, with the addition of specimens from earlier exercises and from own stocks. The geographical distribution of species 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 country has first identified the source material for the test samples. Two of us have verified the identification of the species/taxa as far as possible without damaging the individuals.
- The content of the two test samples for each laboratory, with respect to species and numbers, is listed in a table. Two persons control that the correct number and species is placed in the test samples according to the list.

Damages of the material

The quality of the test material may be reduced during handling and shipping. Taxonomically important parts of the body, as gills, legs, cerci, mouthparts etc., can be lost or destroyed in actions connected with identification, sample composition and transportation. Contamination of larvae may also occur during these processes as well as during the identification work at the participating laboratories. All mentioned possibilities for faults could influence on the results of the identifications and disturb the results in a negative way.

Evaluation

The results of the tests are sent to the laboratories for eventual comments before publishing the report. In this way, we can remove taxonomical biases - for example misidentified or destroyed test material. In cases of disagreement, material may be sent back to the programme subcentre for control. This procedure may act educational for both parts.

For calculation of faults (in percent), we must take into account possible destructions of the material as mentioned above. Further, a wrong identification of a species is one fault even if the sample contains many individuals of the species. We encourage the participants to give comments on matters that may impede the identification. For example, misidentification of species in cases where important taxonomic characters have been destroyed may be neglected, if this is pointed out by the participants.

We have discriminated between "short coming" identification, probably due to damaged material, and virtual fault (wrong species – or genus name). Due to this, some subjective evaluations of the results have to be made. The percent of faults is therefore not always the exact calculated percent of faults, but can be a modified value where some "expert judgement" is taken into account.

It is also of interest to know how many individuals that have been identified of the total number in the sample. This is named *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 gets between 60 and 130 individual species in the two samples. Samples with low diversity will be 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 Europaean mayfly, stonefly and caddisfly species (per 2015) is 1814. However, the biodiversity differs between countries. Normally the freshwater fauna gets poorer moving from South Europe towards the Northern countries. This is also a fact to bear in mind when judging taxonomical capacity. As an example of this, the

freshwater fauna of Switerland is much richer than in Norway and Sweden – despite the fact that the area of Switerland is approximately 1/10 of the two Nordic countries (Figure 2).

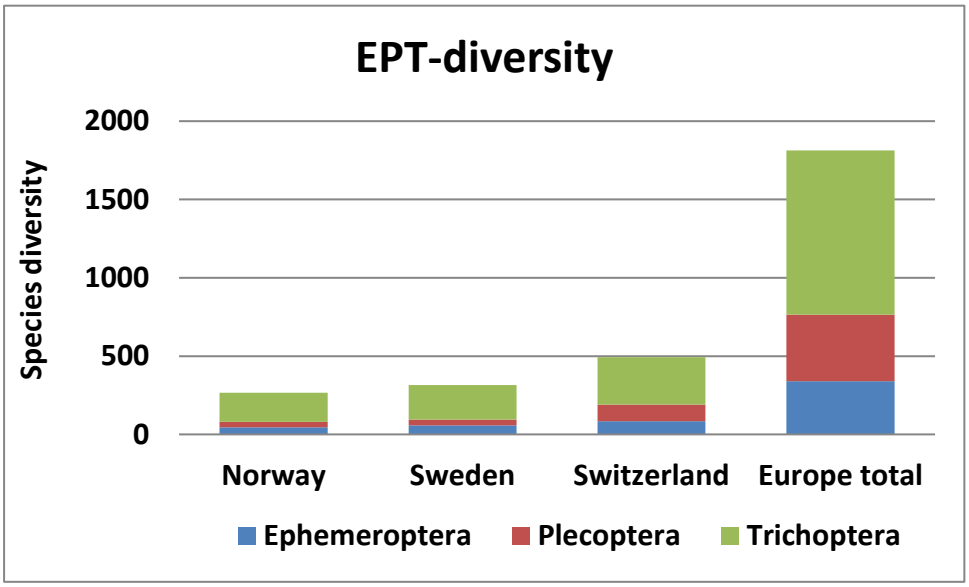

Figure 2. Species (EPT) diversity of mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddisflies (Trichoptera) in Norway, Sweden and Switzerland (after Fauna Europaea Web Service, http://www.faunaeur.org.

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 are the highest score that can be obtained. A score ≥80 is regarded as acceptable taxonomical work.

Test of the subcentre

The ICP waters subcentre in Bergen, Norway is tested with the help from Sweden each second year (not in 2015). 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

Two laboratories participated in the intercalibration of invertebrates in 2016 (Appendix A). The content of species in the test samples delivered – and the results of the identification by the different laboratories are shown in Appendix Tables 1 – 2.

Mayflies

The identification of mayflies (Ephemeroptera) was very good for Laboratory 1 (Figure 2, Appendix Table 1- 2). Laboratory 2 forgot to put on a lid on one of the petri-dishes, so parts of the sample dried out. The Qi was calculated to 94.4 and 80.8 for Laboratory 1 and 2 respectively. This indicates very high quality of work for Laboratory 1 and acceptable for Laboratory 2, taking in account the accident with the dried out sample.

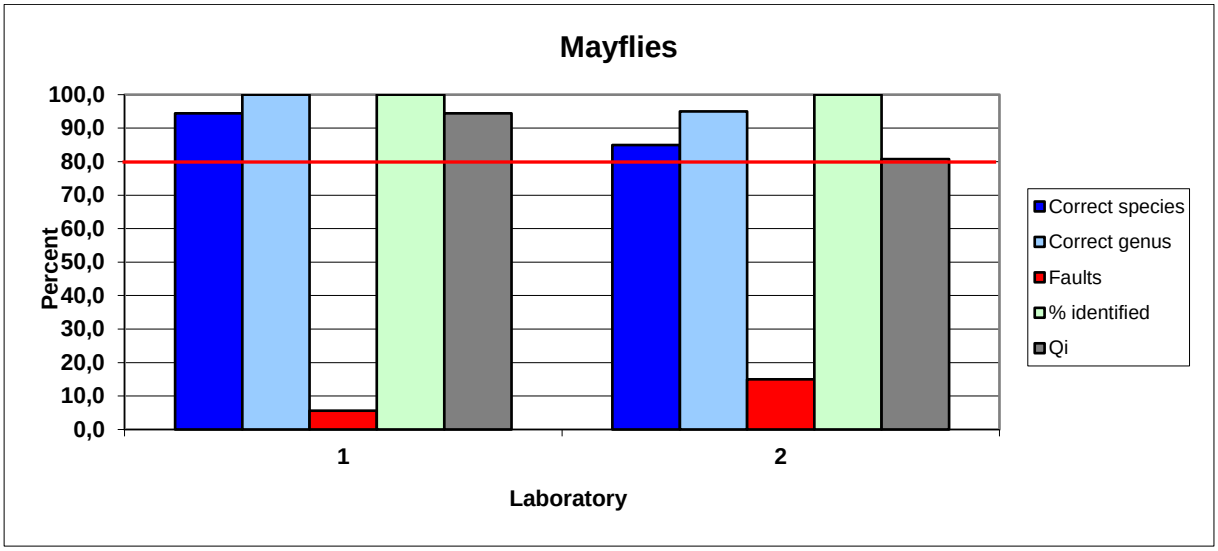

Figure 2. *Results of the identification of the mayflies. The red line indicates the level of acceptance.*

Stoneflies

The identification of the stoneflies is presented in Figure 3 and Appendix tables 1 – 2. The results show a good taxonomical knowledge of the group for both laboratories with only one error for each lab. The Qi was 84.1 and 82.6 for the labs respectively, all above the limit of acceptance.

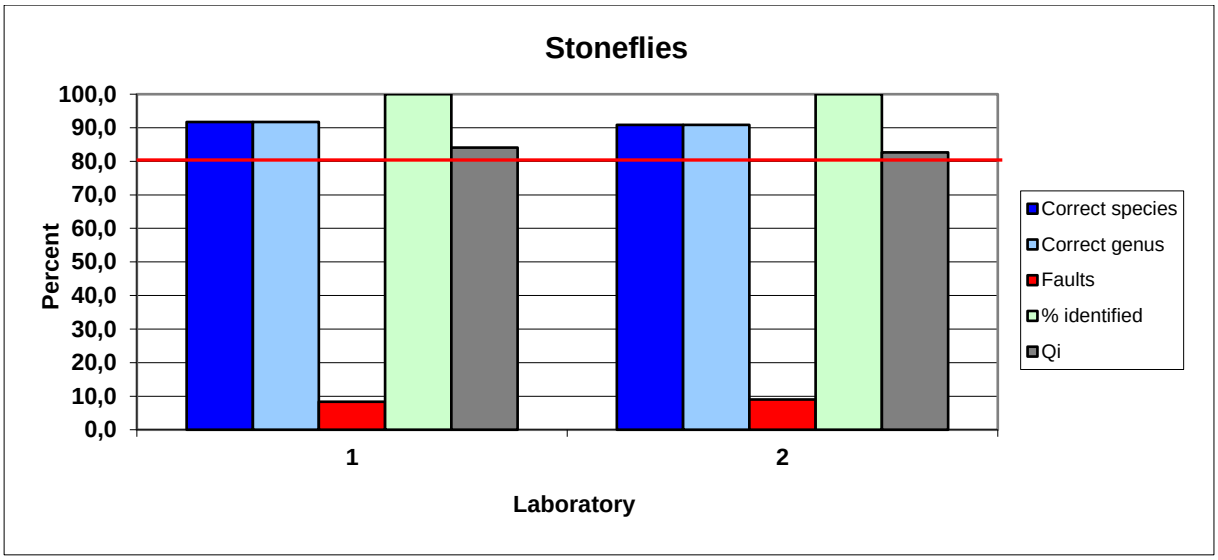

Figure 3. *Results of the identification of stoneflies. The red line indicates the level of acceptance.*

Caddisflies

The identification of caddisflies (Trichoptera) is presented in Figure 4 and Appendix tables 1 – 2. The quality of the identification was excellent for all laboratories, Qi values being 96.1 and 97.5 respectively.

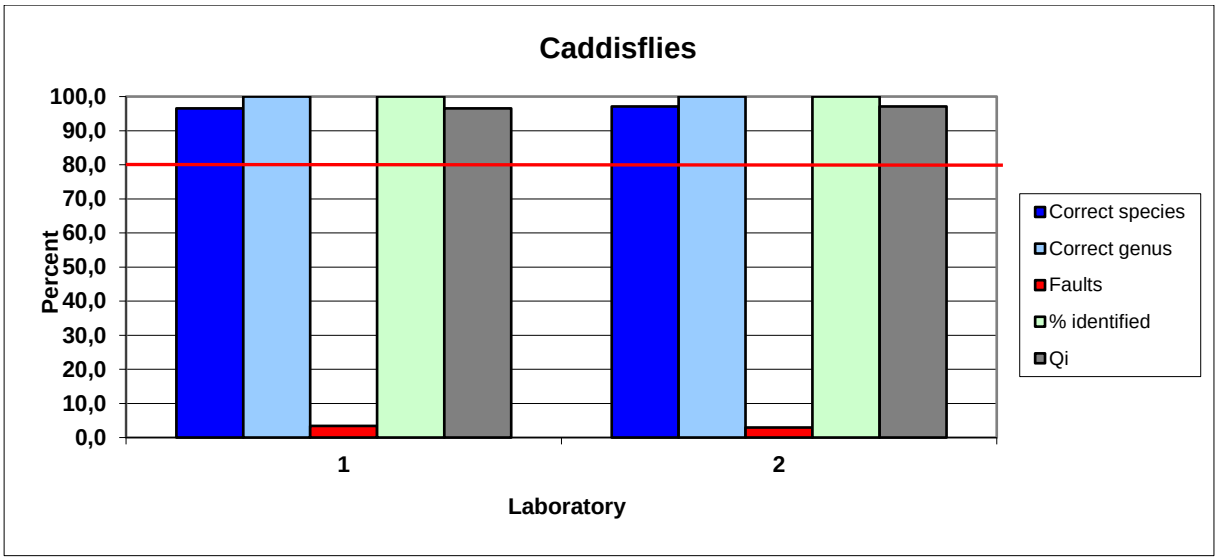

Figure 4. *Results of the identification of caddisflies. The red line indicates the level of acceptance.*

Other groups

In this intercalibration we have included water beetles (Coleoptera), larger crustaceans (Malacostraca), leeches (Hirudinea), molluscs (Gastropoda), alder-flies (Megaloptera), Diptera etc. Both larvae and imagines have been included for some of the groups. Leeches, molluscs and larger crustaceans are sensitive to acid water and important for the evaluation of acidification. The tolerance of the invertebrates among Coleoptera, Megaloptera, Diptera etc. is little known, but generally they are regarded as tolerant to acidic water and consequently have low importance for evaluation of acidity indices. However, all species will be important for invertebrate community analysis. Figure 5 and Appendix tables 1 – 2 shows the results of the identification of these groups. The identifications made by laboratory 1 were very good with only one error. The

result of laboratory 2 was acceptable. The Qi score was 96.0 and 84.0 for participants 1 and 2, respectively.

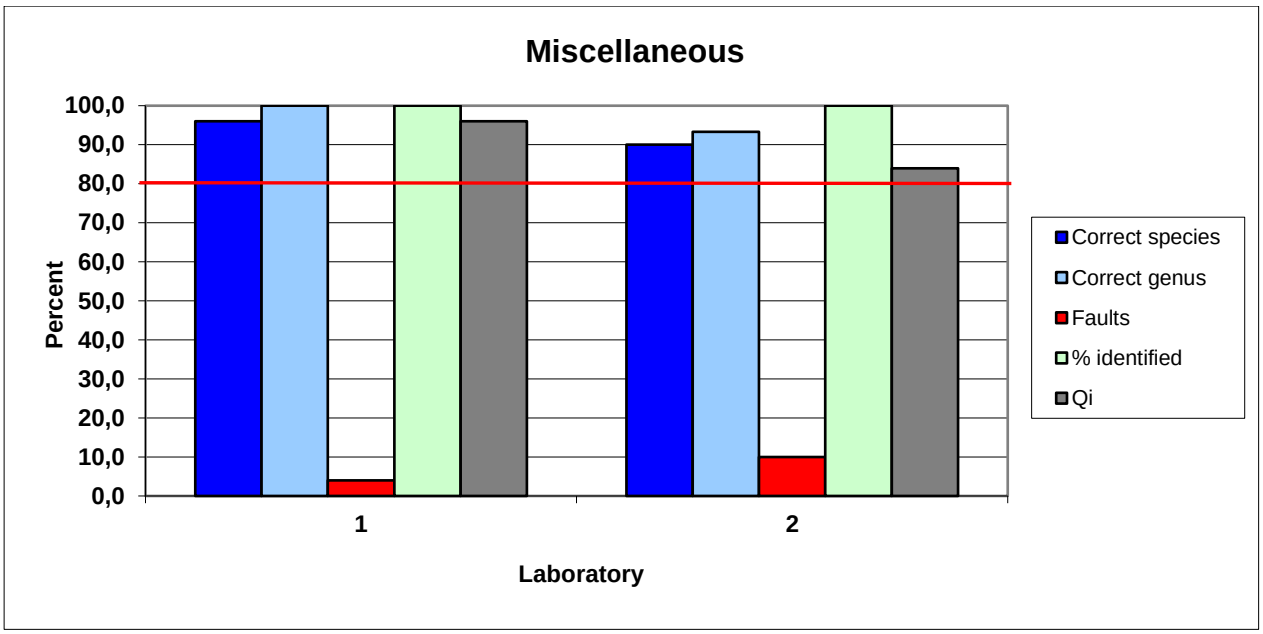

Figure 5. *Results of the identification of miscellaneous groups. The red line indicates the level of acceptance.*

Total number of species in the sample

There were no discrepancy between the number of individuals put into the samples and the reported number of specimens. A total of 181 individuals were sent to the two different laboratories. Of these, 100 percent were reported back to the programme sub-centre.

4. Evaluation/conclusion

The laboratories generally 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 6. Laboratory 1 and 2 got a mean Qi score of 92.7 and 86.1 respectively. This is characterized as very good taxonomic work, taken into account the drying of part of the material in Laboratory 2. The biological intercalibration is important for harmonising biological material/databases and will be of high value in programmes where community analyses is in focus or where the ecological status should be stated.

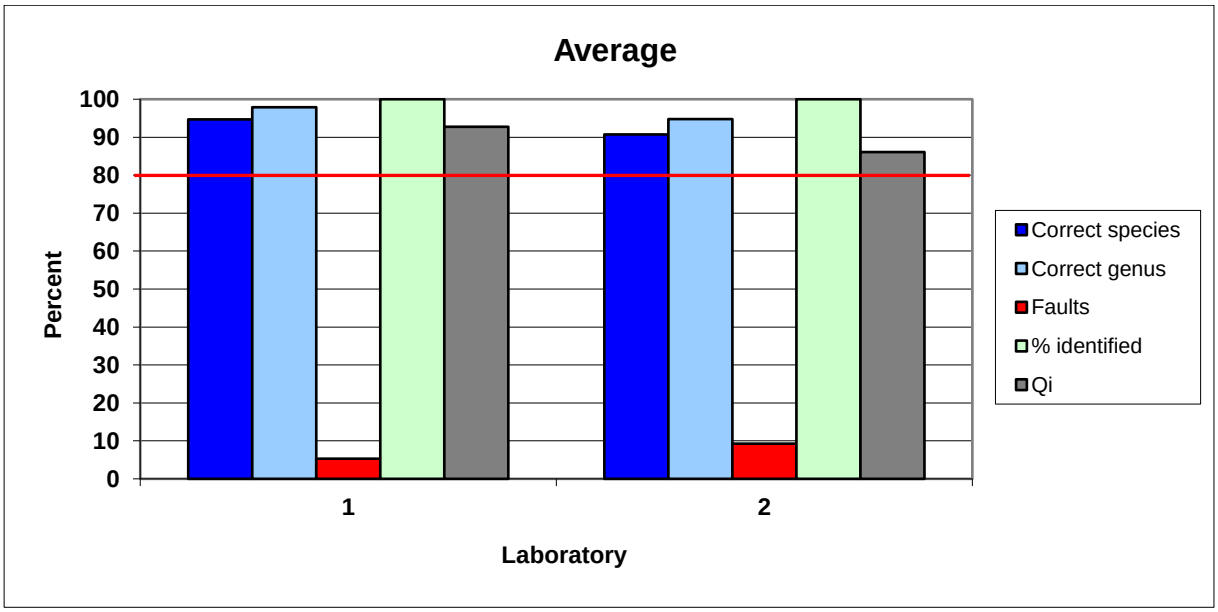

Figure 6. *Mean skill in percent of identifying species and genus and mean Qi for each laboratory. The red line indicates the level of acceptance.*

The biological intercalibration under the ICP Waters programme was the first regular test aiming to test taxonomic skills of identifying benthic invertebrates. Today, similar tests are run by the the North American Benthological Society (http://www.nabstcp.com) 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).

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Appendix A.

Responsible laboratories

Each participating laboratory is identified by a number, which is identical with the table number. Laboratories participating in the intercalibration of invertebrates in 2015 and their code numbers are:

1. Swedish University of Agricultural Sciences, Dept. of Environmental Assessment, P.O. Box 7050, S-75007 Uppsala, **Sweden**. Responsible taxonomist: Dr. Magda-Lena Wiklund.

2. Uni Research Environment AS, P.O. Box 7810, N-5020 Bergen, **Norway**. Responsible taxonomists: Torunn S. Landås and Arne Johannessen.

Appendix B.

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

	Sample 1			Sample 2	
Taxa:	**Delivered**	**Identified**		**Delivered**	**Identified**
Ephemeroptera					
Alainites muticus				1	1
Arthroplea congener				1	1
Baetis rhodani	1	1		1	1
Caenis horaria	1	1			
Caenis luctuosa	1	1		1	1
Caenis rivulorum				1	1
Cloeon inscriptum	1				
Cloeon dipterum		1			
Ephemera danica	1	1		1	1
Ephemera vulgata				1	1
Ephemerella aroni	1	1			
Ephemerella mucronata				1	1
Heptagenia sulphurea				1	1
Kageronia fuscogrisea	1	1			
Leptophlebia vespertina	1	1			
Nigrobaetis niger	1	1			
Plecoptera					
Amphinemura borealis	1	1		1	1
Brachyptera risi				1	1
Capnia bifrons	1	1			
Capnopsis shilleri				1	1
Diura nanseni	1	1			
Leuctra hippopus	1	1			
Leuctra nigra				1	
Capnopsis shilleri					1
Nemoura cinerea				1	1
Nemoura flexuosa	1	1			
Protonemura meyeri				1	1
Taeniopteryx nebulosa	1	1			
Trichoptera					
Agapetus ochripes	1	1		1	1
Brachycentrus subnubilus	1	1		1	1
Ceratopsyche silfvenii				1	1
Chematopsyche lepida	1	1			
Chimarra marginata	1	1		1	1
Cyrnus flavidus				1	1
Cyrnus trimaculatus				1	1
Ecclisopteryx dalecarlica	1	1			
Hydropsyche angustipennis				1	1
Hydropsyche siltalai	1	1			
Lepidostoma hirtum	1	1			
Micrasema setiferum	1	1		1	1
Molanna angustata				1	1
Molannodes tinctus				1	1
Neureclipsis bimaculata	1	1			
Oecetis testacea				1	1

Plectrocnemia conspersa	1				
Plectrocnemia sp.		1			
Polycentropus flavomaculatus				1	1
Rhyacophila fasciata				1	1
Rhyacophila nubila	1	1		1	1
Sericostoma personatum	1	1			
Setodes argentipuntellus	1	1			
Silo pallipes	1	1			
Miscellaneous					
Odonata					
Onychogompus forcipatus	1	1		1	1
Enallagma cyathigerum				1	1
Heteroptera					
Notonecta glauca	1	1			
Velia caprai				1	1
Hirudinea					
Erpobdella octoculata	1	1		1	1
Helobdella stagnalis	1	1			
Megaloptera					
Sialis lutaria	1	1			
Gastropoda					
Bathyomphalus contortus	1	1			
Acroloxus lacustris	1	1			
Gyraulus acronicus	1	1			
Gyraulus crista				1	1
Potamopyrgus antipodarum				1	1
Bithynia tentaculatum				1	1
Diptera					
Anthoca vitripennis				1	1
Oligochaeta					
Eiseniella octoculata	1				
Eiseniella tetraedra		1			
Malacostraca					
Asellus aquaticus	1	1		1	1
Gammarus pulex	1	1		1	1
Coleoptera					
Stenelmis canaliculata	1	1			
Hyphydrus ovatus				1	1
Orectochilus villosus				1	1
Hygrotus versicolor				1	1

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

	Sample 1			Sample 2	
Taxa:	**Delivered**	**Identified**		**Delivered**	**Identified**
Ephemeroptera					
Alainites muticus	2	2			
Baetis rhodani	1	1			
Caenis horaria				1	1
Caenis luctuosa	2	2		1	1
Centroptilum luteolum				1	1
Ephemera danica	1	1			
Ephemera vulgata				1	1
Ephemerella mucronata				1	1
Ephemerella aroni	1	1			
Heptagenia dalecarlica				1	1
Heptagenia sulphurea	1	1			
Kageronia fuscogrisea	1	1			
Leptophlebia marginata				1	
Leptophlebia sp.					1
Leptophlebia vespertina	1	1			
Nigrobaetis digitatus				2	
Nigrobaetis sp.					1
Baetidae indet.					1
Nigrobaetis niger				1	1
Plecoptera					
Amphinemura borealis	1	1		1	1
Amphinemura standfussi	1	1			
Brachyptera risi				1	1
Capnopsis schilleri	1	1			
Dinocras cephalotes	1	1			
Leuctra fusca				1	1
Leuctra nigra				1	1
Nemoura avicularis				1	1
Nemoura cinerea	1				
Nemurella pictetii		1			
Taeniopteryx nebulosa				1	1
Trichoptera					
Agapetus ochripes				1	1
Athripsodes aterrimus	2	2			
Athripsodes cinereus				1	1
Athripsodes commutatus				1	1
Chimarra marginata	1	1		1	1
Cyrnus trimaculatus	1	1			
Ecclisopteryx dalecarlica				1	1
Goera pilosa	1	1			
Holocentropus dubius	1	1			
Hydropsyche angustipennis				1	1
Hydropsyche pellucidula	1	1		1	1
Hydropsyche siltalai	2	2			
Lepidostoma hirtum				1	1

Micrasema gelidum				1	1
Molannodes tinctus	1	1			
Neureclipsis bimaculata				1	1
Oecetis testacea	1	1			
Philopotamus montanus	1	1			
Polycentropus flavomaculatus	1	1			
Polycentropus irroratus	1	1			
Potamophylax cingulatus				1	1
Rhyacophila fasciata				1	1
Rhyacophila nubila	2	2			
Sericostoma personatum				1	1
Setodes argentipunctellus	1	1		1	1
Silo pallipes	1	1			
Tinodes waeneri	1	1			
Wormaldia occipitalis				1	
Wormaldia subnigra					1
Miscellaneous					
Odonata					
Cordulegaster boltoni				1	1
Enallagma cyathigerum	1	1			
Erythromma najas				1	
Coenagrion hastulatum					1
Onychogompus forcipatus	1	1			
Malacostraca					
Asellus aquaticus	1	1		1	1
Gammarus lacustris	1	1		1	1
Pallasea quadrispinosa	1	1			
Heteroptera					
Aphelocheirus aestivalis	1	1			
Callicorixa praeusta				1	1
Hirudinea					
Erpobdella octoculata	1			1	1
Erpobdella testacea		1			
Glossophonia complanata	1	1			
Helobdella stagnalis				1	1
Diptera					
Elophila trimaculata	1	1			
Megaloptera					
Sialis fuliginosa	1	1		1	1
Sialis lutaria				1	1
Coleoptera					
Elmis aenea	1	1		1	1
Limnius volkmari	1	1			
Nebrioporus depressus				1	1
Orectochilus villosus				1	1
Stenelmis canaliculata				1	1
Gastropoda					
Bathyomphalus contortus				1	1

Gyraulus acronicus	1	1			
Radix balthica				1	
Myxas glutinosa					1
Valvata cristata	1	1			
Turbellaria					
Polycelis sp.				1	1

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NIVA
Norwegian Institute for Water Research

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