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ICP Waters Report 140/2019
Biological intercalibration: Invertebrates 2019

Photo: Gaute Velle

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

Convention on Long-Range Transboundary Air Pollution

LRTAP
Long-range Transboundary Air Pollution

Norwegian Institute for Water Research

REPORT

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Summary

Two laboratories participated in the 23rd biological intercalibration of invertebrates in the ICP Waters programme. The intercalibration is important for harmonising taxonomic work across countries and is of high value 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 a high proportion of the specimens in the test samples. In total, 97 % of the species and 98 % of the genera were correctly identified. With a mean Quality assurance index (Qi) of 92 and 98, the laboratories performed well above the threshold for acceptable taxonomic work (Qi 80). Trends in the biological intercalibration over time indicate that an average of five laboratories have participated. The results show that the mean Qi has remained above 80% for the full period, suggesting skilled taxonomists in the laboratories affiliated to ICP Waters. When the Qi is broken into invertebrate groups, the laboratories, on average, perform best for caddis flies and worst for stoneflies. The mean Qi decreased steeply in 2015-2017. According to the taxonomists that participate, this may be mostly due to an increase in difficulty. The quality has increased since 2017 while the difficulty remains high.

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CONVENTION OF LONG-RANGE
TRANSBOUNDARY AIR POLLUTION

INTERNATIONAL COOPERATIVE PROGRAMME ON
ASSESSMENT AND MONITORING EFFECTS OF AIR
POLLUTION ON RIVERS AND LAKES

Biological Intercalibration:
Invertebrates 2019

Prepared at the ICP Waters Programme Subcentre
NORCE Norwegian Research Centre AS
Bergen, November 2019

Preface

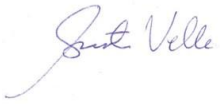
The International Cooperative Programme on Assessment and Monitoring of the Effects of Air Pollution on Rivers and Lakes (ICP Waters) was established under the Executive Body of the UNECE Convention on Long-range Transboundary Air Pollution (CLRTAP) in July 1985. Since then, ICP Waters has been an important contributor to document the effects of implementing the Protocols under the Convention. ICP Waters has prepared numerous assessments, reports and publications that address the effects of long-range transported air pollution.

ICP Waters and its Programme Centre is chaired and hosted by the Norwegian Institute for Water Research (NIVA), respectively. A programme subcentre is established at NORCE (previously known as Uni Research), Bergen. ICP Waters is supported financially by the Norwegian Environment Agency and the Trust Fund of the UNECE LRTAP Convention.

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.

An objective of the ICP Waters programme 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 are inter-laboratory quality assurance tests. Here 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.

Here we report the results from the 23rd intercalibration of invertebrate fauna. We also compare results from all 23 intercalibrations.



Gaute Velle

ICP Waters Programme Subcentre
Bergen, November 2019

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Summary

Two laboratories participated in the 23rd biological intercalibration of invertebrates in the ICP Waters programme. The intercalibration is important for harmonising taxonomic work across countries, and is of high value where the focus is on community analyses, e.g. for the classification of ecological status according to the EU Water Framework Directive. The ICP Waters intercalibration was the first regular test of species level identification in 1992.

The laboratories correctly identified a high proportion of the specimens in the test samples. In total, 97 % of the species and 98 % of the genera were correctly identified. With a mean Quality assurance index (Qi) of 92 and 98, the laboratories performed well above the threshold for acceptable taxonomic work (Qi 80). One laboratory identified all but two specimens correctly. The Quality index was lowest for stoneflies, where one laboratory performed below the acceptable limit.

Trends in the biological intercalibration over time indicate that an average of five laboratories have participated. The results show that the mean Qi has remained above 80% for the full period, suggesting 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, perform best for caddis flies and worst for stoneflies. The mean Qi decreased steeply during 2015-2017. According to the taxonomists that participate in the intercalibration, the drop in quality may mostly be due to an increase in difficulty. The quality has increased since 2017, while the difficulty remains high.

1 Introduction

The purpose of the biological intercalibration of invertebrates is to evaluate the quality of the biological data delivered to the Programme subcentre and to highlight the importance of taxonomic skills to the participants. The data are used nationally and by ICP Waters to indicate environmental conditions, with respect to acidification, using 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 more general analyses of environmental conditions (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 participating 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 one standardised sample for all participants since the fauna is region-specific and since the specimens are easily damaged when identified by several 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 monitoring region.

The taxonomic skill for each participant 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 number of specimens that are not identified from 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).

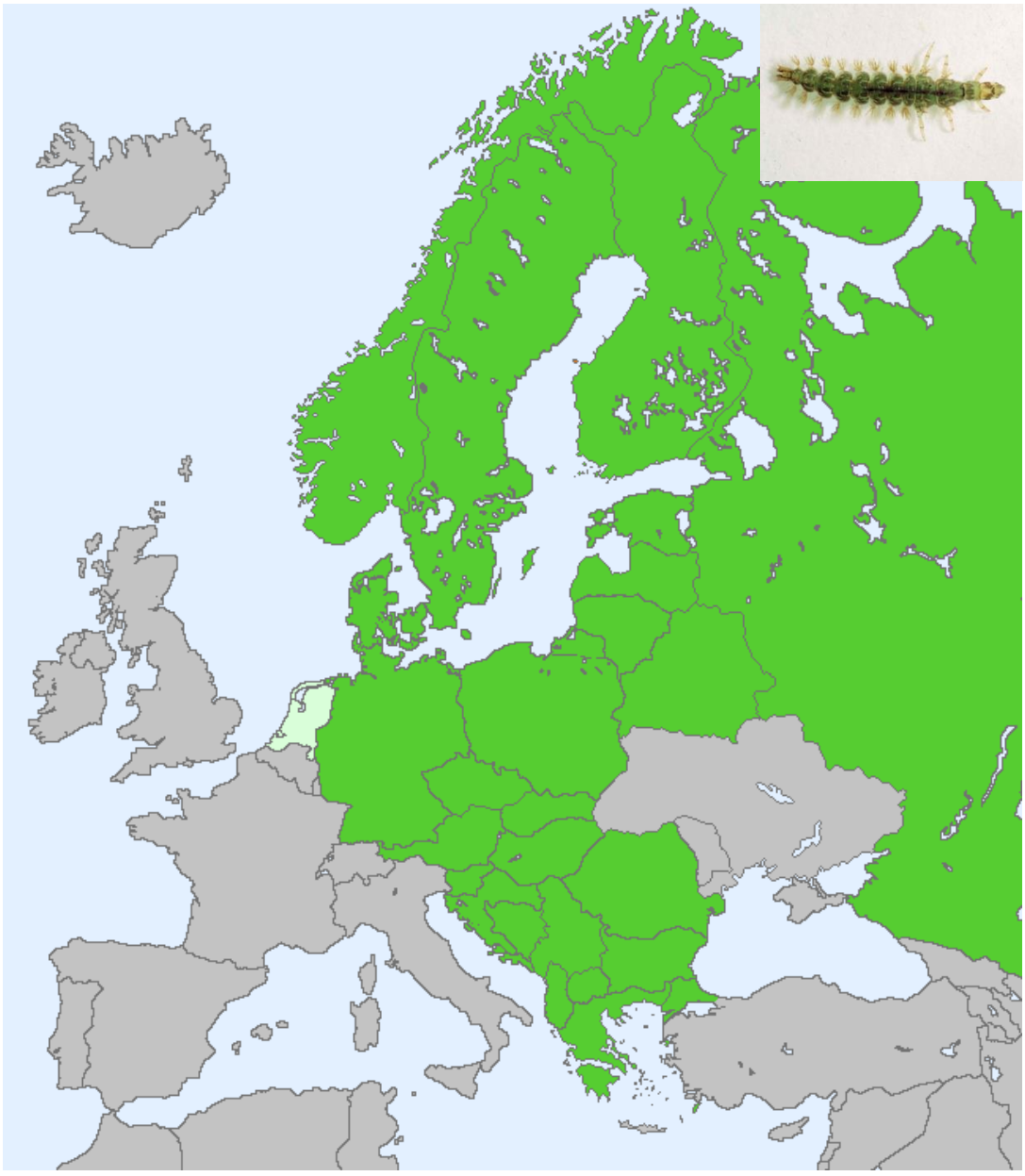

Figure 1. *Geographical distribution of the caddisfly* Rhyacophila nubila *in Europe. This species is widely distributed, but is absent from several West-European countries. Map after Fauna Europaea Web Service, http://www.faunaeur.org, Photo: Arne Fjellheim*

Identification

To minimise possible faults, the following procedure is used to prepare 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 above-mentioned 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 be educational for both parts.

For calculation of errors, we 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, denoted as *percent identified*. A low percent identified implies that many specimens were not identified and will consequently reduce the value of the taxonomic work.

In cases where more specimens are identified than sent to the laboratories, each excess specimen will count as one error.

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).

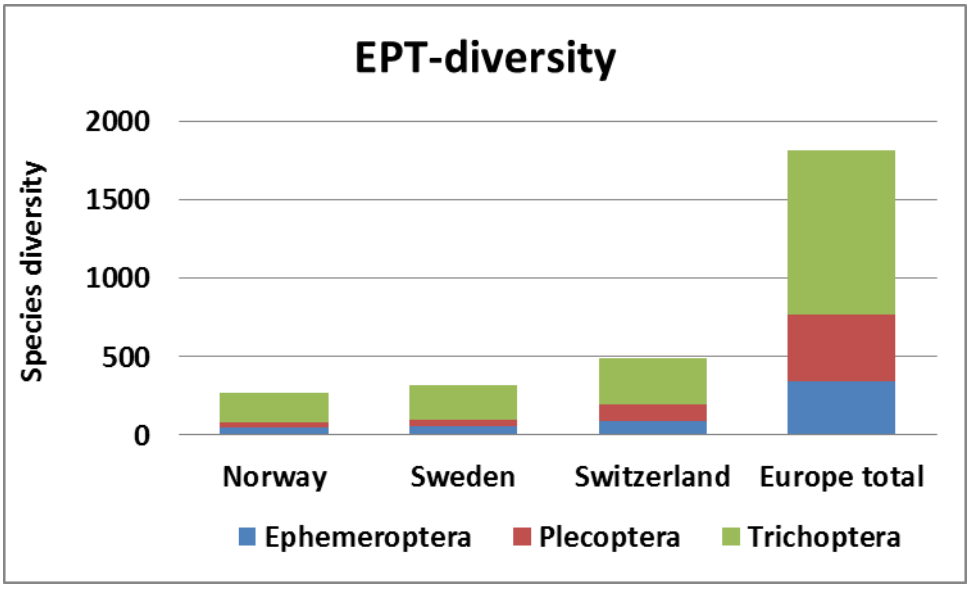

Figure 2. *Species diversity of mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddisflies (Trichoptera) in Norway, Sweden and Switzerland (after Fauna Europaea Web Service, http://www.faunaeur.org.*

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 ≥ 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

Two laboratories participated in the intercalibration of invertebrates in 2019 (Appendix A). The species lists and the identification results are shown in Appendix B, Tables 1 – 2.

Mayflies

The identification of mayflies (Figure 3) was excellent for both laboratories with no misidentifications.

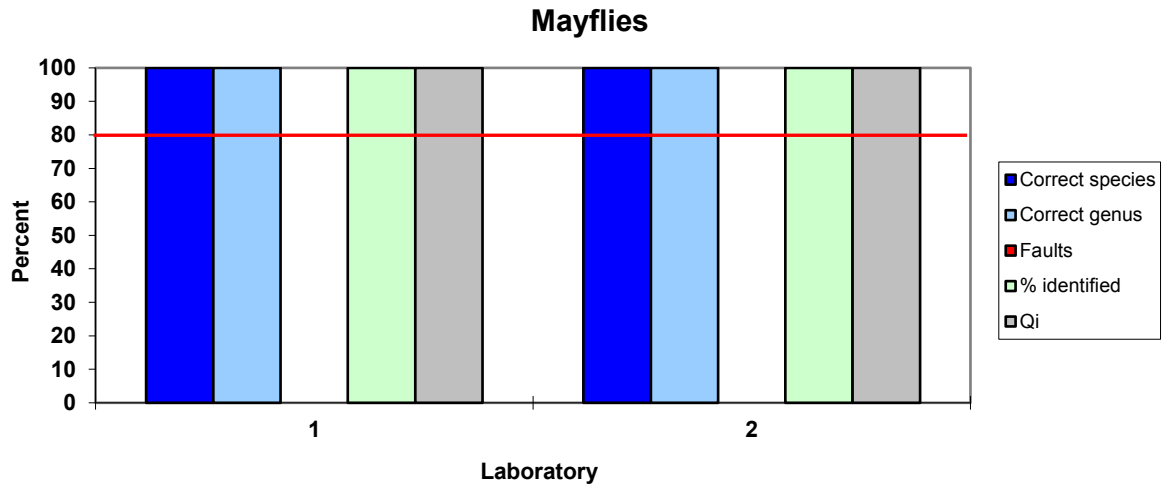

Figure 3. *Results from the identification of mayflies. The red line indicates the acceptable limit.*

Stoneflies

The identification of stoneflies is shown in Figure 4. The results were above the acceptable limit for Laboratory 2 and below for Laboratory 1. Here, two specimens were assigned to the wrong species and also to the wrong genus. Laboratory 2 assigned one specimen to the right genus and made a comment with the right specie, but specified that some characteristics were presents and others not. One half error was given to the laboratory for this error.

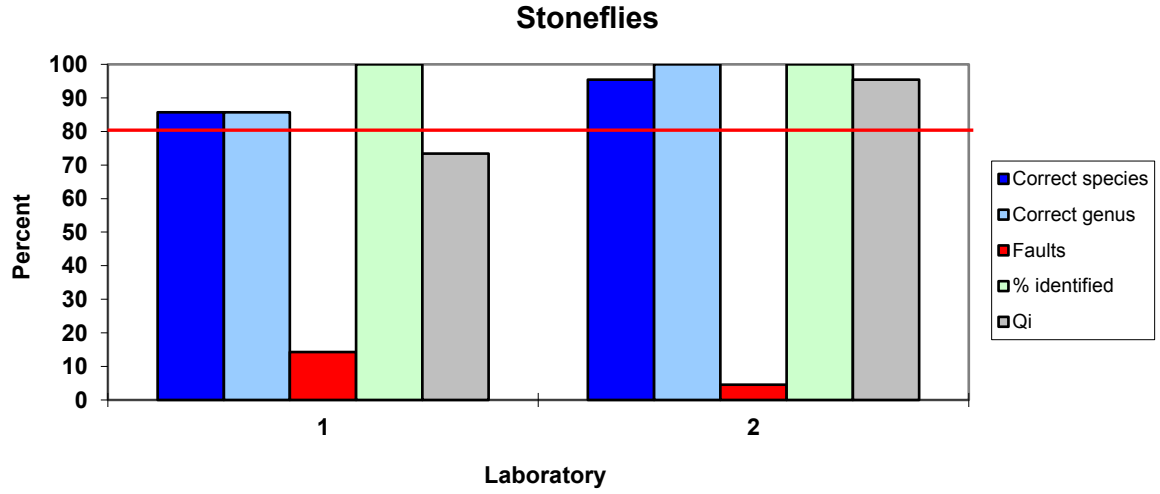

Figure 4. *Results from the identification of stoneflies. The red line indicates the acceptable limit.*

Caddisflies

The identification of caddisflies was above the acceptable limit for both laboratories (Figure 5). Laboratory 1 had assigned one specimen to the wrong species and also to the wrong genus, while Laboratory 2 had assigned one specimen to the wrong species.

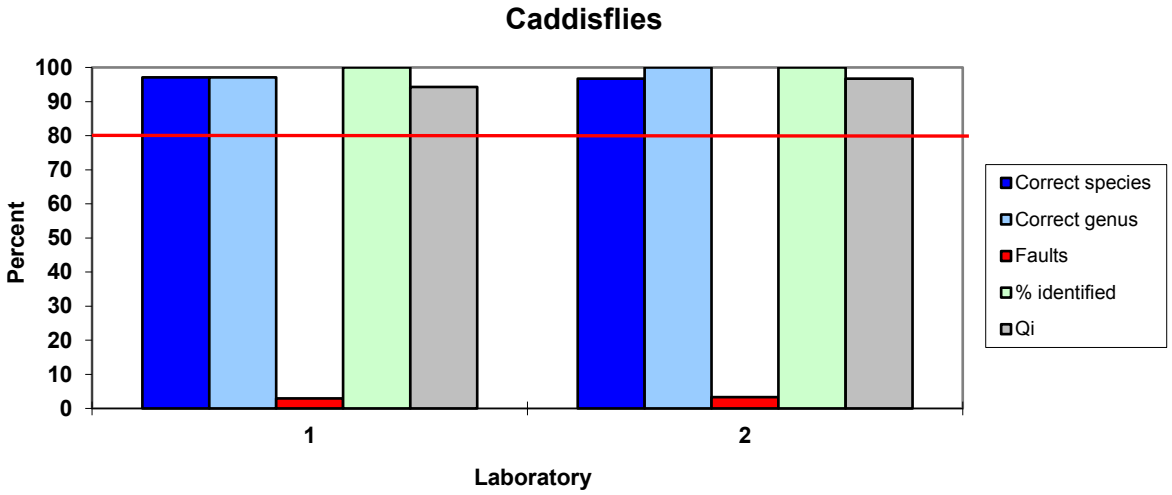

Figure 5. *Results from the identification of caddisflies. The red line indicates the acceptable limit.*

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 spiders (Araneae). Both larvae and imagines were included. Leeches, molluscs and larger crustaceans are sensitive to acid water and important for the evaluation of acidification. The environmental tolerances of some species of Coleoptera, Megaloptera and Diptera are poorly known, but they are often regarded as tolerant to acidic water and of low importance for the evaluation of acidification indices. However, all species are important for invertebrate community analyses.

The identifications made by both laboratories were excellent with no misidentifications (Figure 6).

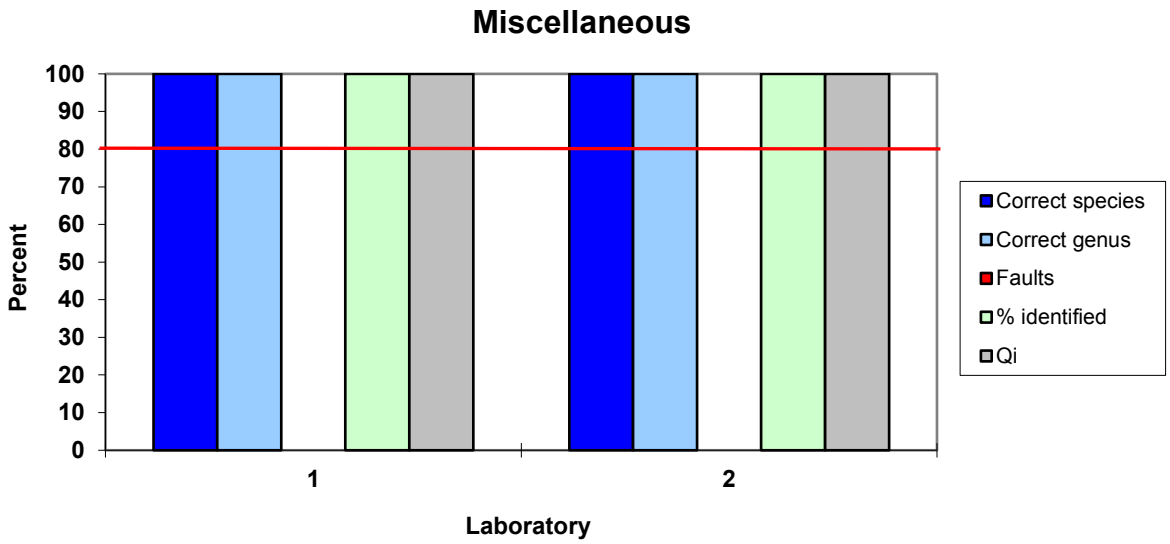

Figure 6. *Results from the identification of miscellaneous groups of invertebrates. The red line indicates the acceptable limit.*

Total number of species in the sample
A total of 176 individuals were sent to the two laboratories. Of these, all specimens were reported back to the program subcentre.

4 Evaluation

The laboratories correctly identified a very 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 was 92.0 for Laboratory 1 and 98.0 for Laboratory 2. This means that Laboratory 1 identified all but three specimens correctly and Laboratory 2 identified all but two specimens 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.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). In 2017-2018, NORCE (ICP Waters subcenter) also organized an extensive test similar to the ICP waters intercalibration for Norwegian Laboratories (Velle *et al.* 2018). Tests of the Norwegian laboratories will continue in 2019-2020 as the Norwegian Environment Agency will use participation in intercalibration tests as part of the evaluation when assigning companies to new projects.

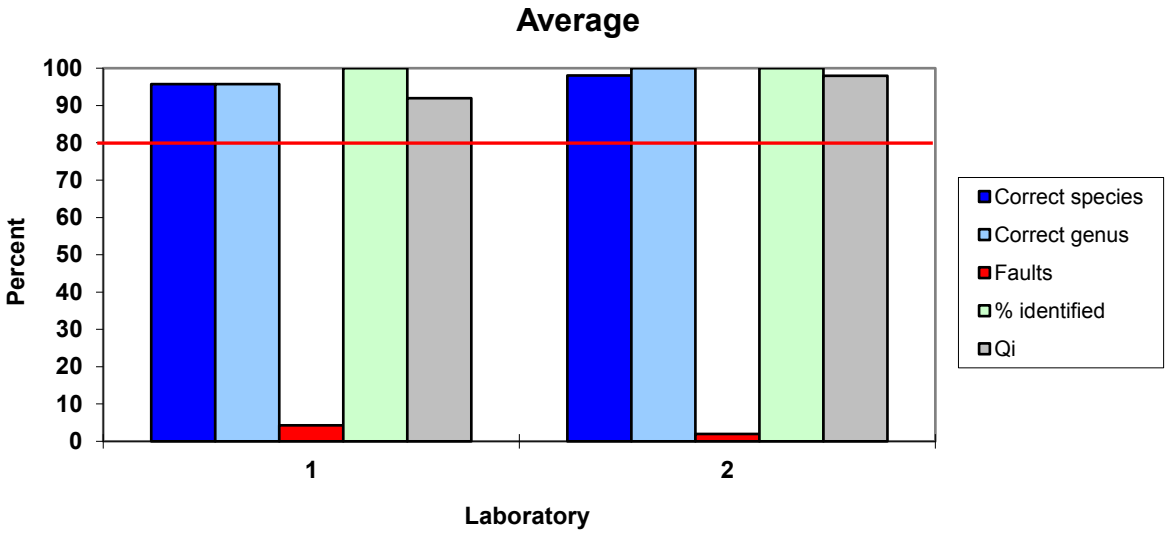

Figure 7. *Mean skill in percent of identifying species and genus, and mean Qi for each laboratory. The red line indicates the acceptable limit.*

5 Trends over time

The invertebrate intercalibration in ICP Waters started in 1992. An 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.

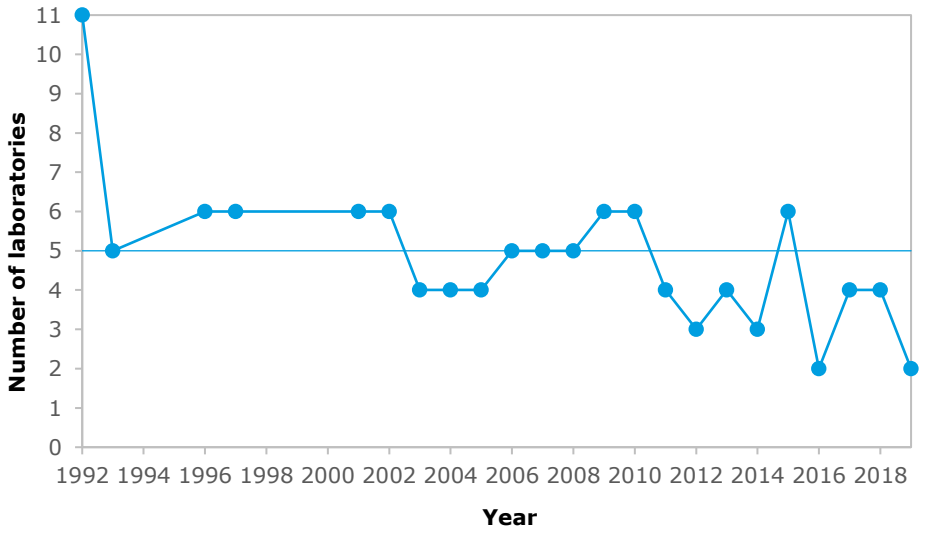

Figure 8. *The number of participating laboratories in the ICP Waters invertebrate intercalibration since the first intercalibration in 1992.*

The intercalibration protocol is unchanged since 1992, while the quality assurance index (Qi) has been used since 2005 (Raddum, 2005). After calculating the Qi for the period prior to 2005, trends in the Qi-score show that the mean quality has remained above 80% (Figure 9), 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.

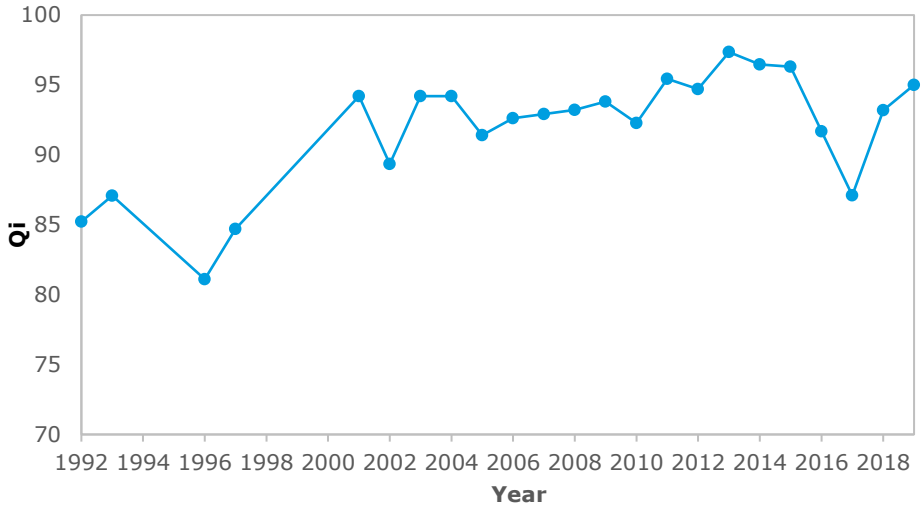

Figure 9. *The mean quality assurance index for all invertebrate groups in the intercalibration over time.*

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 and Figure 10). 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 one Laboratory in 2018, which resulted in a lower Qi (Figure 10).
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 includes specimen from other regions to the test. It is therefore important that the participants send an abundance of specimens.

The mean Qi has decreased from 2016. According to the taxonomists, the difficulty increased from this year, and especially for stoneflies. In addition, it seems some other factors apply; there was a new participant, one key taxonomist retired, one sample dried out and one laboratory sent too few specimens. Despite a higher difficulty, the quality has improved over the two last years.

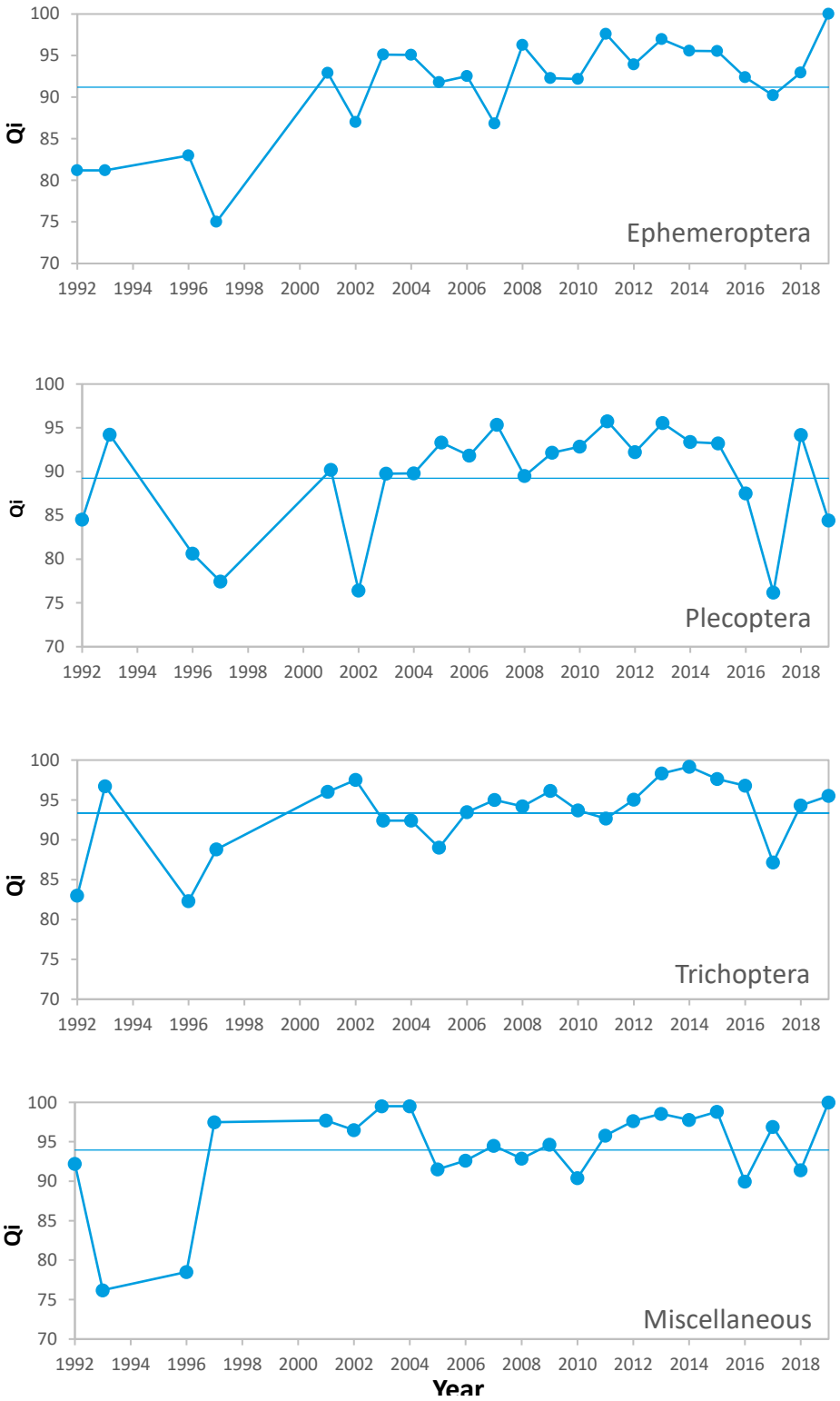

Figure 10. *The mean quality assurance index (Qi) of the intercalibrations through time for mayflies (Ephemeroptera), stoneflies (Plecoptera), caddisflies (Trichoptera) and miscellaneous groups of invertebrates. The straight line represents the overall mean Qi for each invertebrate group. Qi above 80 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 2019 were:

1. Latvian Environment, Geologi and Meterology Centre, Latvian Environmantal Laboratory, Ošu iela 5, Jūrmala, LV-2015, **Latvia**. Responsible taxonomist: Natalja Grudule.

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

8 Appendix B. Species lists

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

	Sample 1		Sample 2	
	Delivered	**Identified**	**Delivered**	**Identified**
Ephemeroptera				
Baetis rhodani	1	1	1	1
Caenis horaria	1	1	1	1
Caenis macrura	1	1	1	1
Caenis robusta			1	1
Cloeon dipterum			1	1
Ephemera danica	1	1		
Ephemera lineata	1	1		
Ephemera vulgata			1	1
Ephemerella karelica	1	1	1	1
Ephemerella notata			1	1
Heptagenia sulphurea	1	1		
Potamanthus luteus	1	1		
Plecoptera				
Amphinemura borealis	1	1	1	1
Chloroperla apicalis	1		1	
Isoperla grammatica	1	1	1	1
Leuctra nigra	1	1	1	1
Nemoura avicularis	1	1	1	1
Protonemura meyeri	1	1	1	1
Siphonoperla burmeisteri		1		1
Taeniopteryx nebulosa	1	1	1	1
Trichoptera				
Athripsodes aterrimus			1	1
Athripsodes cinereus	1	1	1	1
Beraeodes minutus	1	1	1	1
Brachycentrus subnubilus			1	1
Cyrnus flavidus			1	2
Holocentropus dubius	1	1	1	1
Hydropsyche pellucidula	1	1	1	1
Hydropsyche siltalai	1	1	1	1
Lepidostoma hirtum	1	1	1	1
Leptocerus tineiformis	1	1	1	1
Micrasema setiferum	1	1		
Molanna angustata	1	1	1	1
Mystacides azurea			1	1
Mystacides longicornis	1	1	1	1
Neureclipsis bimaculata			1	1
Notodobia ciliaris	1	1	1	1
Plectrocnemia conspersa			1	
Polycentropus flavomaculatus	1	1	1	1
Rhyacophila nubila	1	1	1	1
Sericostoma personatum	1	1	1	1
Trianodes bicolor	1	1	1	1

Miscellanous				
Coleoptera				
Brychius elevatus			1	1
Elmis aenea	1	1	1	1
Limnius volckmari	2	2	1	1
Corixidae				
Aphelocheirus aestivalis			1	1
Cymatia bonsdorffi	1	1		
Plea leachi	1	1	1	1
Malacostraca				
Gammarus lacustris			1	1
Gammarus pulex	1	1		
Gastropoda				
Ancylus fluviatilis	1	1		
Bathyomphalus contortus	1	1		
Bithynia tentaculata	1	1		
Physa fontinalis	1	1		
Valvata pulchella	1	1		
Viviparus contectus			1	1
Viviparus viviparus			1	1
Megaloptera				
Sialis lutaria	1	1	1	1
Hirudinea				
Erpobdella octoculata	1	1	1	1
Diptera				
Atherix ibis	1	1		
Dicranota bimaculata			1	1

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

	Sample 1		Sample 2	
	Delivered	**Identified**	**Delivered**	**Identified**
Ephemeroptera				
Ameletus inopinatus	1	1		
Arthroplea congener			1	1
Baetis digitatus			1	1
Baetis niger			1	1
Baetis rhodani	1	1		
Caenis horaria	1	1	1	1
Caenis luctuosa			1	1
Caenis rivulorum	1	1		
Ephemera danica	1	1	1	1
Ephemera vulgata	1	1	1	1
Ephemerella aurivilli	1	1		
Ephemerella mucronata			1	1
Heptagenia dalecarlica			1	1
Heptagenia fuscogrisea	1	1		
Heptagenia sulphurea			1	1
Leptophlebia marginata	1	1	1	1
Rhithrogena germanica	1	1		
Plecoptera				
Amphinemura borealis	1	1		
Capnopsis schilleri			1	1
Diura nanseni	1	1	1	1
Leuctra nigra	1	1	1	1
Nemoura flexuosa			1*	
Nemoura sp.				1*
Protonemura meyeri	1	1	1	1
Taeniopteryx nebulosa	1	1	1	1
Trichoptera				
Apatania sp.				1
Apatania wallengreni			1	
Arctopsyche ladogensis			1	1
Cheumatopsyche lepida	1	1		
Chimarra marginata			1	1
Cyrnus insolutus	1	1	1	1
Cyrnus trimaculatus	1	1	1	1
Ecnomus tenellus	1	1	1	1
Holocentropus picicornis	1	1	1	1
Hydropsyche pellucidula	1	1	1	1
Hydropsyche siltalai	1	1	1	1
Ironoquia dubia	1	1	1	1
Lepidostoma hirtum	1	1	1	1
Mystacides azurea	1	1		
Neureclipsis bimaculata			1	1
Oecetis testacea	1	1		
Philopotamus montanus	1	1	1	1
Polycentropus flavomaculatus	1	1	1	1
Sericostoma personatum	1	1	1	1

Tinodes waeneri			1	1
Miscellanous				
Aranea				
Argyroneta aquatica	1	1		
Gastropoda				
Bathyomphalus contortus			1	1
Bithynia tentaculata			1	1
Gyraulus acronicus			1	1
Gyraulus albus	1	1		
Radix bathica	1	1		
Spaerium sp.	1	1		
Theodoxys fluviatilis	1	1		
Malacostraca				
Asellus aquaticus	1	1	1	1
Diptera				
Eloeophila sp.	1		1	1
Dicranota sp.	1			
Tipula sp.			1	1
Coleoptera				
Elmis aenea	1	1	1	1
Limnius volckmari	1	1	1	1
Nebrioporus depressus	1	1	1	1
Normandia nitens	1	1		
Hirudinea				
Erpobdella octoculata	1	1		
Odonata				
Erythromma najas	1	1		
Phyrrosoma nymphula			1	1

* Laboratory 2 assigned this specimen to the right genus and made a comment with the right specie, but specified that some characteristics were presents and other not. For this the laboratory got one half error.

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