

Proficiency Test 04/2016

**Taxonomic identification of boreal freshwater lotic,
lentic, profundal and North-Eastern Baltic benthic
macroinvertebrates**

**Kristian Meissner, Henrik Nygård, Katarina Björklöf,
Marko Jaale, Miikka Hasari, Lauri Laitila,
Jouko Rissanen and Mirja Leivuori**

Proficiency Test 04/2016

**Taxonomic identification of boreal freshwater lotic,
lentic, profundal and North-Eastern Baltic benthic
macroinvertebrates**

**Kristian Meissner, Henrik Nygård, Katarina Björklöf,
Marko Jaale, Miikka Hasari, Lauri Laitila,
Jouko Rissanen and Mirja Leivuori**



REPORTS OF THE FINNISH ENVIRONMENT INSTITUTE 2|2017
Finnish Environment Institute SYKE
Profest SYKE

Layout: Markku Ilmakunnas

The publication is also available in the Internet: www.syke.fi/publication | helda.helsinki.fi/syke

ISBN 978-952-11-4658-9(PDF)
ISSN 1796-1726 (Online)

Author(s): Kristian Meissner, Henrik Nygård, Katarina Björklöf, Marko Jaale, Miikka Hasari, Lauri Laitila, Jouko Rissanen and Mirja Leivuori

Publisher and financier of publication: Finnish Environment Institute (SYKE)
P.O. Box 140, FI-00251 Helsinki, Finland, Phone +358 295 251 000, syke.fi.

Year of issue: 2017



ABSTRACT

The Finnish Environment Institute (SYKE) conducted a benthic macroinvertebrate taxa identification proficiency test in which 22 participants took part. In the lake littoral subtest, one participant out of seven scored lower than 95% correct and three out of seven achieved perfect results in this test. Half of the ten participants in the lake profundal subtest achieved a score higher than 95%, with four participants scoring a perfect result. In the lotic subtest, two participants out of ten scored 100% and half of the participants scored more than 95% correct. In the Baltic test the average result was 94% correctly identified taxa. Even if the general performance of the participants were good, the results clearly demonstrates the need for recurring taxonomic identification tests for lotic, lentic profundal, littoral and Baltic coastal macroinvertebrates as well as regularly arranged taxonomic workshops.

Warm thanks to all the participants!

Keywords: biological identification, macroinvertebrates, fresh water, Baltic, lake, costal, proficiency test

TIIVISTELMÄ

Proftest SYKE järjesti pohjaeläintunnistuksen vertailukokeen, johon osallistui 22 määrittäjää. Järvien litoraalityössä yksi osallistujaa seitsemästä alitti 95 % oikein ja kolme tunnistivat 100 % oikein. Puolet syväntaksonien tunnistustesteihin osallistuneista ylittivät 95 % oikein, joista neljällä oli kaikki oikein. Virtavesien tunnistustestissä kaksi osallistujaa kymmenestä saivat 100 % oikein ja puolet yli 95 % oikein. Itämeren osiossa tunnistettiin keskimäärin 94 % oikein. Vaikka osallistujien suoritukset olivat yleisesti hyviä, osoittavat tulokset selvästi tarpeen toistuville pohjaeläinvertailukokeille sekä säännöllisesti järjestettäville taksonomisille koulutustilaisuuksille.

Kiitos vertailukokeen osallistujille!

Avainsanat: biologiset määritykset, pohjaeläimet, sisävedet, rannikko, järvi, litoraali, pätevyyskoe

SAMMANDRAG

I denna Proftest SYKEs provningsjämförelse för artbestämning av botteninvertebrater deltog totalt 22 experter. I sjöars litoraltestet underskred en deltagare av sju 95 % rätt och tre deltagare hade alla taxa rätt. Hälften av deltagarna för testet av djupbottenzoner överskred 95 % rätt, varav fyra deltagare hade alla rätt. I testet för flödande vattens arter hade två av tio alla rätt och hälften hade 95 % rätt. I Östersjödelen identifierades i genomsnitt 94 % rätt. Trots att deltagarnas resultat överlag var goda, visar resultaten att provningsjämförelser för artbestämning av botteninvertebrater och taxonomiska skolningstillfällen bör ordnas regelbundet.

Ett varmt tack till alla deltagarna i testet!

Nyckelord: biologiska bestämningar, makroinvertebrater, bottenfauna, kust, sjö, litoral, djupbottenzon, kompetensprovning

CONTENTS

	Abstract • Tiivistelmä • Sammandrag.....	3
1	Correction page.....	6
2	Introduction	7
	2.1 Aim and scope the proficiency test.....	7
3	Organizing the proficiency test	8
	3.1 Responsibilities	8
	3.2 Participants and timetable	8
	3.3 Test material and evaluation criteria	10
4	Results.....	11
5	Evaluation of performance and discussion.....	16
	5.1 Feedback from the proficiency test	17
6	Summary and conclusion	18
7	Summary and conclusion in Finnish	19
	References.....	20

1 Correction page

In the final report published on the 11th of January 2017, an error was observed in Table 3. This error has been corrected in this version. *Lymnaea peregran* is an old name which has been replaced by its current name *Radix balthica*.

2 Introduction

The Finnish Environment Institute (SYKE) is an appointed National Reference Laboratory in the environmental sector in Finland. The duties of the reference laboratory include providing interlaboratory proficiency tests and other comparisons for analytical laboratories and other producers of environmental information. Profest SYKE carried out this international proficiency test (PT) for taxonomic identification of boreal freshwater lotic, lentic, profundal and North-Eastern (NE) Baltic benthic macroinvertebrates in March 2016 (ZOO 04/2016). The majority of Profest SYKE proficiency testing services conform to the requirements of ISO/IEC 17043 [1], ISO 13528 [2], and IUPAC technical report [3]. Profest SYKE is accredited by the Finnish Accreditation Service as a proficiency testing provider (PT01, ISO/IEC 17043, www.finas.fi/Documents/PT01_M08_2016.pdf). While organizing of macroinvertebrate proficiency test does not belong to the accredited scope, the macroinvertebrate proficiency test ZOO 04/2016 is the fourth macroinvertebrate proficiency test organized by SYKE since 2003. The previous macroinvertebrate proficiency test was organized in 2011 [4].

2.1 Aim and scope the proficiency test

The ZOO 04/2016 test was held to assess the proficiency and reliability of professional and semi-professional identification of macroinvertebrate taxa routinely encountered during North-Eastern Baltic coastal or boreal lake and river monitoring. The test material included mainly taxa used in Finnish application of the EU Water Framework Directive's (WFD) and Marine Strategy Framework Directive (MSFD) ecological status assessment. Participants could choose to take part in any number of subtests on lentic, lotic, freshwater profundal and North-Eastern Baltic coastal macroinvertebrates.

This proficiency test is in accordance with the WFD's demand for quality assurance of biological data. As taxonomic identification of macroinvertebrates is routinely done only by a single analyst, Profest SYKE conducted the macroinvertebrate proficiency test for individual taxonomists rather than the organization they represent. Therefore participants received personal participation diplomas indicating the percentage of correctly identified taxa for the test they participated in, while organizations were not evaluated.

In addition to identification by keying for traits by single experts, the freshwater samples were intended to be identified by genetic traits after the participants had keyed them. Initially reported differences in the keying results between participants and organizers were intended to be resolved based on the genetic identifications. Unfortunately results of the genetic identifications were not reliable due to cross-contamination and lack of genetic material in some of the specimens so that DNA results could not be used in the intended way.

3 Organizing the proficiency test

3.1 Responsibilities

Organizing laboratory

Profstest SYKE, Finnish Environment Institute (SYKE), Laboratory Centre
Hakuninmaantie 6, FI-00430 Helsinki, Finland
Phone: +358 295 251 000, Fax. +358 9 448 320
E-mail: profstest@environment.fi

The responsibilities in organizing the proficiency test

Contact persons: Kristian Meissner, person in charge for freshwater, Fresh Water Centre (kristian.meissner@environment.fi)
Henrik Nygård, person in charge for marine, Marine Research Centre (henrik.nygard@environment.fi)
Katarina Björklöf, proficiency test coordinator, Profstest SYKE, Laboratory Centre (katarina.bjorklof@environment.fi)

Expert panel: Kristian Meissner, SYKE, Freshwater Centre
Marko Jaale, SYKE, Marine Research Centre
Mikko Tolonen, SYKE, Natural Environment Centre
Miikka Hasari, SYKE, Freshwater Centre

Assisting experts: Jouko Rissanen, SYKE, Marine Research Centre, Baltic macroinvertebrate identification
Lauri Laitila, SYKE, Marine Research Centre, Baltic macroinvertebrate identification
Vasco Erlbrecht, DNA analysis, University of Duisburg Essen, Germany

The test was coordinated by the contact persons. The members of the expert panel and the assisting expert were responsible for the sample preparation and also the validity assessment of complaints. In addition, freshwater samples were sent to Dr. Vasco Elbrecht at the University of Duisburg-Essen for genetic identification after the participants had keyed them.

3.2 Participants and timetable

In total 22 participants took part in this proficiency test (Table 1). The majority of participants were employed or self-employed.

Table 1. List of the names, nationality and institutions of participants in the different macroinvertebrate tests.

Nationality	Name	Institution	
Estonia	Kolesova, Natalja	TTU Marine Systems Institute	
	Reisalu, Greta	Estonian Marine Institute, University of Tartu	
Finland	Anttila-Huhtinen, Marja	Kymijoen vesi ja ympäristö ry	
	Haapala, Antti	Etelä-Savon ELY-keskus	
	Iso-Tuisku, Jussi	Kokemäenjoen vesistön vesiensuojeluyhdistys ry.	
	Koivunen, Jonna	Nab Labs Oy	
	Lensu, Terhi	Self-employed	
	Leppä, Markus	Probenthos Oy	
	Lindell-Jokinen, Annette	Lounais-Suomen vesi- ja ympäristötutkimus Oy	
	Majuri, Pekka	Ramboll Finland Oy	
	Mettinen, Aki	Länsi-Uudenmaan vesi ja ympäristö ry	
	Saarikari, Vesa	Lounais- Suomen vesi- ja ympäristötutkimus oy	
	Suonpää, Anu	Länsi-Uudenmaan vesi ja ympäristö ry	
	Suurkuukka, Heli	University of Oulu	
	Väisänen, Anna	Kokemäenjoen vesistön vesiensuojeluyhdistys ry	
	Lithuania	Solovjova, Sabina	Marine Research Department Environment Protection
	Sweden	Ericsson, Ulf	Medins Havs och Vattenkonsulter AB
Fredriksson, Susanna		Linnaeus University	
Konradsson, Katarina		Umeå Marine Sciences Centre, Umeå University	
Liungman, Annika		Medins Havs och Vattenkonsulter AB	
Rådman, Petra		Umeå Marine Sciences Centre, Umeå University	
Svensson, Ola		Stockholm University, Benthos Group	

Taxonomic requirements and other information on the test were available since December 2015 on SYKE's Profest website. The first circular was sent to all former participants of tests as well as to Finnish universities that offer training in macroinvertebrate taxonomy in December 2015. In addition, information on the test was distributed at international meetings. The deadline for binding registrations was set in January 2016 and the test material was to be distributed in February 2016. In all, around 40 preliminary contacts were received of which 22 participated. During registration the use of microscopes at the SYKE field offices was offered to participants lacking own equipment. With this offer, Profest SYKE wanted to encourage and enable semi-professionals and student participants to take part in this test. However, despite the offer, all participants used their own equipment and taxonomic keys to identify the sample specimens.

After the registration deadline the test material was posted to participants on March 1st 2016. The Northern Baltic Sea macroinvertebrate picture sets were compressed into a zip-file which was uploaded to the file transfer service WeTransfer (www.wetransfer.com). The download link, valid for 7 days, was then distributed to the test participants via e-mail. Participants were requested to return the test results via e-mail by March 14th 2016. The first results were received on March 9th. Upon arrival, results were checked against the master lists and participants were given an initial estimate of the percentage of correctly identified individuals by email. Due to severe influenza, some participants requested and were given additional time to provide their results. Detailed results on the participants' test success as well as the diplomas

were provided to participants on March, 31st via mail, after all results were received by the organizers. To ensure participant anonymity, final results were handled only by the contact persons in charge of the test. Participants were also given random numbers to ensure anonymity in this report. Participants were asked to contact Kristian Meissner or Henrik Nygård if they disagreed with the detailed result. All complaints regarding the Baltic test were dealt with by April 13th 2016. Complaints regarding the freshwater material were noted in spring but the final decision on their validity was taken in November after it became evident that the intended validation through genetic identification was not reliable. There were two reasons why the genetic identification was not used: i) a large portion of the sent material had too little DNA to be extracted due to preservation in too low alcohol content and ii) incidents of cross-contamination of DNA samples became evident. This information altered the verification of complaints for the freshwater material. Instead of responding only to requests for verification made by participants, all identification errors were checked for possible faults on the organizers part. After complaint validity and error structure were assessed changes to final scores and updated certificates were sent out to affected participants in November. A total of ten corrective changes were made affecting 8 of the 22 participants.

3.3 Test material and evaluation criteria

Information about the required taxonomic resolution of the tests was given on the Profest website and in the information letter.

The taxonomic resolution required for freshwater macroinvertebrate identification is based on the requirements set for Finnish national freshwater macroinvertebrate monitoring [5; List 1 and 2]. Northern Baltic Sea macroinvertebrate identification is based on the requirements of the BQI and BBI indexes, which are used in Sweden and Finland, respectively [6, 7; Attachment 3.3.1].

The test material was composed of sample material gathered during different national monitoring and research projects. Samples were initially conserved in ethanol, but part of the Baltic material was originally conserved in formalin. Some of the samples in the lake profundal subtest had been dyed with Rose Bengal during previous research projects. All freshwater material was assembled by taxonomic experts and spot checked by the contact person prior to sample assembly and then transferred to one sample vial filled with 80 % ethanol. The lotic and lake littoral subtests contained 50 individuals belonging to a fixed set of 41 and 28 taxa, respectively. The lake profundal subtest contained 33 individuals belonging to a fixed set of 20 taxa. Participants received at least one specimen of all the fixed taxa whereas the rest of the individuals were randomly assigned to any of the taxa. Each freshwater specimen was packed into a separate vial.

The Baltic material was photographed under a microscope. The magnification differed depending on the size of the organism and a scale bar was added to all pictures as reference of the size. Several pictures were taken of the same individual from different angles and with different magnification to ensure that all important species characteristics were captured. Pictures of the same individual were collected in same folder and the set of pictures therefore corresponded to one identification task. In the final check of the pictures, pictures from the

same taxon were added in case the characteristics important for identification of the species were doubtful. This was the case for a few amphipod species. The North-Eastern Baltic test contained 50 sets of pictures, belonging to 38 taxa.

All samples were accompanied with detailed instructions to participants. Instructions included how and when to report results, where to send the samples for the genetic identification as well as a request to the participants to list the taxonomic keys used to reach their results. The Excel files which acted as answer sheets were e-mailed to the participants after the samples were sent out. The answer sheets contained a full list of all the taxa required by the standard taxonomic resolution of the Finnish Environment Institute for macroinvertebrates of the boreal freshwater [5] and North-Eastern Baltic [6, 7] regions. Participants used the spreadsheet to mark the corresponding sample vial numbers or picture set number for the taxa they had keyed in the test.

4 Results

The results of each participant as expressed in the diplomas are given in Table 2. While overall average success of all participants was high (i.e. above 95 % in all lake freshwater subtests), there was variation between participants (Figure 1). The average results were 97 % for lake littoral, 95 % for lake profundal, 93 % for lotic and 94 % for North-Eastern Baltic Sea, coastal.

The most often misidentified taxa in the lake littoral subtest was *Capnia sp.* with 27 % of all sent specimens misidentified (Table 3). In the lake profundal subtest, *Stictochironomus rosenchoeldi* was the most commonly misidentified species with 29 % of the individuals keyed incorrectly (Table 4). *Hydropsyche saxonica* was the most frequently misidentified taxa of the lotic subtest with 50 % of the specimens misidentified (Table 5) followed by *Brachyptera risi* (42 %). The most frequently misidentified species in the Baltic test (Table 6) were the alien amphipod *Gammarus tigrinus* (33%) (Figure 2a), followed by the gastropod *Peringia ulvae* (28 %) (Figure 2b). Polychaetes were generally well identified and only one incorrect identification was reported (Tables 6 and 7).

In total, 19 Baltic taxa, 18 lake littoral taxa, 11 lake profundal taxa and 20 lotic taxa were always correctly identified. These amounted to 50 %, 64 %, 55 % and 49 % of all taxa in the subtests, respectively (Table 7).

Table 2. Correctly identified taxons (%) of each participant. Participant numbers have been randomly assigned.

Participant nr	Lake littoral	Lake Profundal	Lotic	Baltic coastal
1	88	100	88	96
2	96	100	98	100
3				94
4		91	90	86
5			96	
6				96
7	100		100	94
8	96	88	88	88
9	96	94	86	98
10	100		98	
11				100
12		91		
13				96
14				98
15				96
16		100		
17				100
18				90
19		97	94	
20	100	100	100	94
21				94
22		94		94
Number of participants (n)	7	10	10	17
Mean	97	95	93	95
SE	1,1	1,3	1,7	1,0

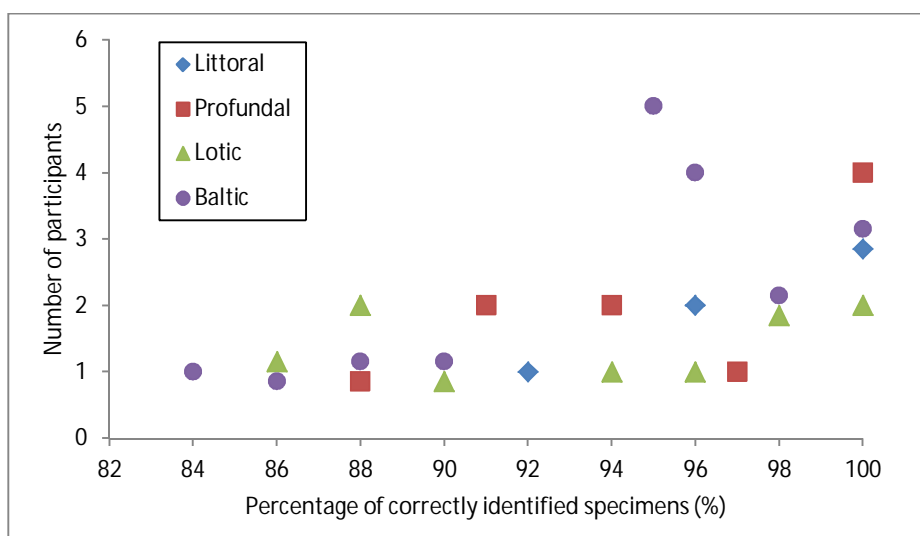


Figure 1. The distribution of test success in the different subtests. For clarity, symbols are slightly offset.

Table 3. The number of misidentified lake littoral taxa in the test material. The proportion of misidentified specimens to the number of specimens sent to participants is given in brackets. The false taxa identities assigned to the misidentified species are also provided.

Taxa	Misidentified	False taxa identities
HIRUDINEA		
<i>Helobdella stagnalis</i>	1 (0.09)	<i>Erpobdella sp.</i>
GASTROPODA		
Lymnaeidae		
<i>Radix balthica (L. peregra)</i>	2 (0.13)	<i>Myxas glutinosa</i>
EPHEMEROPTERA		
Leptophlebiidae		
<i>Leptophlebia sp.</i>	1 (0.08)	<i>Paraleptophlebia sp.</i>
PLECOPTERA		
Capniidae		
<i>Capnia sp.</i>	3 (0.27)	<i>Nemoura sp. (1), Leuctra sp. (2)</i>
TRICHOPTERA		
Polycentropodidae		
<i>Polycentropus flavomaculatus</i>	2 (0.14)	<i>Neureclipsis bimaculata</i>
Psychomyiidae		
<i>Tinodes waeneri</i>	1 (0.09)	<i>Psychomyia pusilla</i>
Hydroptilidae		
ODONATA		
Aeshnidae		
<i>Aeshna grandis</i>	1 (0.11)	<i>A. juncea</i>

Table 4. List of the number of misidentified lake profundal taxa in the test material. The proportion of misidentified specimens to the number of specimens sent to participants is given in brackets. The false taxa identities assigned to the misidentified species are also provided.

Taxa	Misidentified	False taxa identities
OLIGOCHAETA		
Tubificidae		
<i>Limnodrilus spp.</i>	2 (0.11)	<i>Potamothrix/Tubifex spp.</i>
BIVALVIA		
Sphaeriidae		
<i>Pisidium spp.</i>	1 (0.05)	<i>Sphaerium spp.</i>
DIPTERA		
Tanypodinae		
<i>Procladius spp.</i>	1 (0.05)	<i>Macropelopia spp.</i>
Orthoclaadiinae		
<i>Cricotopus spp.</i>	2 (0.2)	<i>Potthastia longimana (1), Orthocladus sp. (1)</i>
<i>Psectrocladius spp.</i>	1 (0.06)	<i>Heterotrissocladius marcidus</i>
<i>Zalutschia zalutschicola</i>	1 (0.08)	<i>Heterotrissocladius subpilosus</i>
Chironominae		
<i>Stictochironomus rosenschoeldi</i>	4 (0.29)	<i>Sergentina coracina (3), Polypedilum (1)</i>
Tanytarsini		
<i>Cladotanytarsus mancus</i>	3 (0.18)	<i>Tanytarsus spp. (2), Paratanytarsus sp. (1)</i>

Table 5. List of the number of misidentified lotic taxa in the test material. The proportion of misidentified specimens of the number of specimens sent to participants is given in brackets. The false taxa identities assigned to the misidentified species are also provided.

Taxa	Misidentified	False taxa identities
EPHEMEROPTERA		
Baetidae		
<i>B. vernus group</i>	1 (0.11)	<i>Baetis rhodani</i>
Siphonuridae		
<i>Ameletus inopinatus</i>	2 (0.2)	<i>B. muticus</i> (1), <i>Parameletus sp.</i> (1)
Leptophlebiidae		
<i>Leptophlebia sp.</i>	2 (0.17)	<i>Paraleptophlebia sp.</i>
Ephemerillidae		
<i>Ephemerella aroni (aurivillii)</i>	1 (0.1)	<i>Seratella (Ephemerella) ignita</i>
PLECOPTERA		
Perlodidae		
<i>Diura sp.</i>	1 (0.1)	<i>Arcynopteryx compacta</i>
Chloroperlidae		
<i>Brachyptera risi</i>	5 (0.42)	<i>Nemoura sp.</i> (4), <i>Rhabdiopteryx acuminata</i> (1)
Nemouridae		
<i>Amphinemura borealis</i>	1 (0.09)	<i>A. sulcicollis</i>
<i>Protonemura sp.</i>	1 (0.08)	<i>Nemoura sp.</i>
Capniidae		
<i>Capnopsis schilleri</i>	1 (0.09)	<i>Siphonoperla burmeisteri</i>
Leuctridae		
<i>Leuctra sp.</i>	1 (0.08)	<i>Capnia sp.</i>
TRICHOPTERA		
Hydropsychidae		
<i>H. saxonica</i>	6 (0.5)	<i>Hydropsyche augustipennis</i>
Polycentropodidae		
<i>Neureclipsis bimaculata</i>	1 (0.08)	<i>Holocentropus dubius</i>
Glossosomatidae		
<i>Agapetus sp.</i>	1 (0.1)	<i>Lype phaeopa</i>
Rhyacophilidae		
<i>R. nubila</i>	3 (0.14)	<i>R. fasciata</i>
Brachycentridae		
<i>Micrasema gelidum</i>	2 (0.18)	<i>M. setiferum</i> (1), <i>Sericostoma personatum</i> (1)
COLEOPTERA		
Elmidae		
<i>Elmis aenea</i>	1 (0.04)	<i>Normandia nitens</i>
<i>Oulimnius tuberculatus</i>	1 (0.04)	<i>Limnius volckmari</i>

Table 6. The number of misidentified Baltic taxa in the test material. The proportion of misidentified specimens of the number of specimens sent to participants is given in brackets. The false taxa identities assigned to the misidentified species are also provided.

Taxa	Misidentified	False taxa identities
PLATYHELMINTHES		
<i>Turbellaria</i> spp.	4 (0.22)	<i>Limapontia capitata</i> (4)
NEMATODA	1 (0.06)	<i>Prostoma graecense</i>
ANNELIDA		
Polychaeta		
<i>Pygospio elegans</i>	1 (0.06)	<i>Boccardiella ligerica</i>
Oligochaeta	1 (0.03)	Tipulidae
MOLLUSCA		
Gastropoda		
<i>Bithynia tentaculata</i>	1 (0.06)	Not identified
<i>Peringia ulvae</i>	5 (0.28)	<i>Ecrobia ventrosa</i> (2), <i>Potamopyrgus antipodarum</i> (3)
<i>Physa fontinalis</i>	1 (0.06)	Lymnaeidae
Lymnaeidae	4 (0.11)	<i>Bithynia tentaculata</i> , <i>Ecrobia ventrosa</i> (3)
Bivalvia		
<i>Mya arenaria</i>	1 (0.06)	<i>Macoma balthica</i>
<i>Macoma balthica</i>	6 (0.17)	<i>Mya arenaria</i> (2), <i>Pisidium</i> spp. (4)
ARTHROPODA		
Mysida		
<i>Neomysis integer</i>	3 (0.17)	<i>Mysis mixta</i> (2), <i>Hemimysis anomala</i>
Isopoda		
<i>Saduria entomon</i>	2 (0.06)	<i>Idotea granulosa</i> (2)
Amphipoda		
<i>Monoporeia affinis</i>	1 (0.02)	<i>Leptocheirus pilosus</i>
<i>Pontoporeia femorata</i>	1 (0.02)	<i>Bathyporeia pilosa</i>
<i>Gammarus oceanicus</i>	4 (0.22)	<i>Gammarus locusta</i> (3), <i>Calliopius laevisculus</i>
<i>Gammarus zaddachi</i>	3 (0.17)	<i>Gammarus salinus</i> (2), <i>G. tigrinus</i>
<i>Gammarus salinus</i>	4 (0.22)	<i>Gammarus zaddachi</i> (3), <i>G. oceanicus</i>
<i>Gammarus tigrinus</i>	6 (0.33)	<i>Gammarus oceanicus</i> (2), <i>G. locusta</i> , <i>G. salinus</i> , <i>G. zaddachi</i>
<i>Gammarus locusta</i>	2 (0.11)	<i>Gammarus salinus</i> , <i>G. oceanicus</i>



Figure 2. The most frequently misidentified species in the Baltic test was the alien amphipod *Gammarus tigrinus* (A), followed by the gastropod *Peringia ulvae* (B).

Table 7. Summary of correctly identified Baltic, lake littoral, lake profundal and lotic taxa. The number of total individuals is given in brackets.

Baltic taxa	Lake littoral taxa	Lake profundal taxa	Lotic taxa
HYDROOIA <i>Cordylophora caspia</i> (18)	HIRUDINEA <i>Erpobdella</i> sp. (12)	DIPTERA <i>Chaoborus flavicans</i> (18)	BIVALVIA <i>Sphaerium</i> sp. (11)
PRIAPULIDA <i>Halicryptus spinulosus</i> (36)	GASTROPODA <i>Gyraulus</i> sp. (9)	Ceratopogonidae (15) <i>Propiloscerus jacuticus</i> (22)	EPHEMEROPTERA <i>Kageronia fuscogrisea</i> (10)
POLYCHAETA <i>Hediste diversicolor</i> (18) <i>Bylgides sarsi</i> (36) <i>Fabricia stellaris</i> (18) <i>Marenzelleria</i> spp. (36)	EPHEMEROPTERA <i>Heptagenia dalearica</i> (19) <i>Kageronia fuscogrisea</i> (16) <i>Ephemera vulgata</i> (14) <i>Caenis horaria</i> (14)	<i>Chironomus anthracinus</i> (18) <i>Chironomus plumosus</i> (25) <i>Chironomus salinarius</i> (<i>neocorax</i>) (19) <i>Cryptochironomus defectus</i> (14)	<i>Ephemerella mucronata</i> (10) PLECOPTERA <i>Isoperla</i> sp. (10) <i>Taeniopteryx nebulosa</i> (12) <i>Nemoura</i> sp. (9)
GASTROPODA <i>Theodoxus fluviatilis</i> (18) <i>Valvata piscinalis</i> (18) <i>Potamopyrgus antipodarum</i> (18)	HETEROPTERA <i>Micronectinae</i> sp. (12) <i>Corixinae</i> sp. (18)	<i>Demicryptochironomus</i> <i>vulneratus</i> (12) <i>Dicrotendipes</i> spp. (14) <i>Microtendipes pedellus</i> (14) <i>Pseudochironomus prasinatus</i> (16)	MEGALOPTERA <i>Sialis</i> sp. (12) TRICHOPTERA <i>Hydropsyche pellucidula</i> <i>Hydropsyche siltalai</i> (10) <i>Polycentropus</i> <i>Polycentropus irroratus</i> (14) <i>Plectrocnemia</i> sp. (11) <i>Tinodes waeneri</i> (10) <i>Oxyethira</i> sp. (11) <i>Silo pallipes</i> (10) <i>Micrasema setiferum</i> (11) <i>Lepidostoma hirtum</i> (12)
BIVALVIA <i>Mytilus trossulus</i> (18) <i>Dreissena polymorpha</i> (18) <i>Cerastoderma glaucum</i> (18)	TRICHOPTERA <i>Agraylea</i> sp. (11) <i>Limnephilidae</i> sp. (13) <i>Lepidostoma hirtum</i> (10) <i>Athripsodes</i> sp. (11) <i>Mystacides</i> sp. (13) <i>Oecetis</i> sp. (13)		
ACARI <i>Hydrachnidia</i> (18)	ODONATA <i>Erythromma najas</i> (10)		
OSTRACODA (18)	COLEOPTERA <i>Oulimnius tuberculatus</i> (14)		COLEOPTERA <i>Hydraena</i> sp. (22) <i>Limnius volckmari</i> (11)
CIRRIPIEDIA <i>Amphibalanus improvisus</i> (18)	DIPTERA Ceratopogonidae (14) Tabanidae (13)		DIPTERA Simuliidae (10)
MYSIDA <i>Mysis relicta</i> (18)			
ISOPODA <i>Idotea balthica</i> (18)			
AMPHIPODA <i>Corophium volutator</i> (18)			
DIPTERA Chironomidae (36)			

5 Evaluation of performance and discussion

In the lake littoral subtest, six participants out of seven scored higher than 95% correct and three scored perfect results (100 %). Half of the ten participants in the lake profundal subtest achieved a score higher than 95 %, with four participants scoring a perfect result (100 %). In the lotic subtest, two participants out of ten scored perfect results (100 %) and again half of the participants scored more than 95 % correct. In the Baltic subtest, three participants identified all tasks correctly, whereas half of participants scored more than 95 %. Taxa misidentification affected roughly half of all used taxa. The highest fraction of correctly identified taxa was found in the lake littoral subtest where two thirds of the used taxa were always correctly

identified. Mostly, taxa were misidentified once or twice, but some taxa clearly seemed to cause more difficulties than others. In the Baltic coastal test, *Gammarus* spp. was the most frequent wrongly identified taxa (11-33 %, depending on the species). However, only once a misidentification was assigned to another genus, indicating that while the genus *Gammarus* is well identified, the correct species is difficult to assign. Currently, the benthic index used in Finland (BBI), requires separation of species but not necessarily their correct identification and thus the observed issue has probably not affected the use of BBI. Other frequently misidentified taxa were *Peringia ulvae*, *Turbellaria* sp. and surprisingly also *Macoma balthica*. In lotic samples, *Hydropsyche saxonica* was incorrectly identified in half of the cases and *Brachyptera risi* almost equally often (42 %). Thus, while overall lotic error rates indicate reliable taxonomic results, the clustered misidentification of some species indicate the need for focused taxonomic training of experts.

To address the problem of expert training, the Finnish Limnological Society founded a subgroup for benthic macroinvertebrates, which has regularly arranged taxonomic workshops. These workshops have attracted high popularity and attendance. The aforementioned results for both Baltic as well as lotic misidentifications clearly demonstrate the demand for such workshops to maintain the high quality of professional and semi-professional taxonomic identifications. The organizers of this test highly encourage participation in such workshops by experts involved with routine keying.

5.1 Feedback from the proficiency test

Less than half of the participants gave feedback regarding the test. Feedback on the arrangements and timetables of the test was mostly positive. In some feedback the test fee was considered too high. Feedback on the test material concentrated on the quality of provided specimens. While most found the test material to be adequate, some participants felt that the provided specimens were too small or in too poor condition for detailed identification. Some participants noted that identification of such specimens was possible only at the genus level. As a reaction to feedback from previous the proficiency test, all freshwater specimens in the proficiency were delivered in separate vials in order to increase their detectability. Participants generally commended Proftest SYKE on organizing this kind of tests as they felt it alerted them to potential personal knowledge gaps in taxonomic skills.

In an attempt to avoid problems related to poor specimen quality in test samples and to ensure equality among all participants, the provider featured digitized samples in the Baltic test for the first time. Feedback from the participants acknowledged the benefits of the use of same set of pictures for all participants, namely because this enables equal evaluation. However, most of the participants also found the identification from pictures unusual. The main mentioned problems were the inability to turn and twist the specimens and the difficulty to perceive the size of the specimen, restricting the use of 'hands-on' experience. As keying of digitized samples was mostly applauded by participants it will be the future direction of macroinvertebrate proficiency testing.

Despite efforts to provide error free material to participants, some sample preparation errors became apparent with the freshwater material. Some specimens were reported to have been in

poor condition immediately after they had arrived and some replacement individuals were provided to the participants free of charge, given there was time to do so without further delaying the test. When initial results were sent to participants, a few participants suspected that some test taxa had been incorrectly keyed by the test organizer. Complaints regarding freshwater taxa identity were intended to be objectively solved by genetic identification. Unfortunately the DNA based tests were not reliable enough to be used in the assessment of the validity of complaints regarding the identity of specimens. Despite this setback great care was taken to reanalyze all possible faults that could have occurred with the sample material. In cases of doubt the case was settled to the advantage of the participant. While this might have caused a false correction to the advantage of the participant in freshwater tests, the impact of these changes on the overall result is minute, since these changes affected only 10 of the 1180 freshwater specimens sent out to participants. Thus, even in the worst case the organizer error rate in the freshwater test was less than 1 ‰. All mistakes on the organizers part resulted in the issuing of new, corrected diplomas to affected participants.

Despite some issues with the physical sample material, the organizers were overall content with the test. In future tests sample preparation will have to be more stringently supervised or replaced entirely with digital samples to avoid organizer mistakes. Set timetables for sample distribution and reports on initial results were largely met and internal communication between the different branches and offices of Proftest SYKE functioned well. A delay in the organizers response to test complaints and in the provision of final diplomas to the participants was noted.

A preliminary evaluation of the reported taxonomic keys used in keying the test material revealed large variation amongst the keys used for Baltic coastal identification. Unfortunately, no conclusions on the use of either Baltic or freshwater taxonomic literature can be made due to the scarcity of literature lists provided by participants.

In future tests, more effort to market the test to both an international audience and students will be undertaken.

6 Summary and conclusion

The Finnish Environment Institute (SYKE) conducted a benthic macroinvertebrate taxa identification proficiency test in which 22 participants took part. Participants could choose between four macroinvertebrate identification subtests; Baltic North-Eastern, lake littoral, lake profundal and lotic. In the lake littoral subtest, six participants out of seven scored higher than 95 % correct and three participants scored perfect results (100 %). Half of the ten participants in the lake profundal subtest achieved a score higher than 95 %, with four participants scoring a perfect result. In the lotic subtest, two participants out of ten scored 100 % and half of the participants scored more than 95 % correct. In the Baltic test the average result was 94 % correctly identified taxa.

In total, 64% of the lake littoral taxa, 55% of the lake profundal taxa and 49% of the lotic taxa were always correctly identified. In the Baltic test, the average proportion of misidentified taxa was 8%, whereas 50% of the test taxa were always correctly identified. The results clearly

demonstrate the need for recurring taxonomic identification tests for lotic, lentic profundal and littoral and Baltic coastal macroinvertebrates. There are also some needs for the development of quality assurance in proficiency tests and macroinvertebrate identification. Future aims include that tests will be conducted on a regular basis and the inclusion of digitized samples in all future tests. Further, increased international and semi-professional (e.g. student) participation are clear major future goals.

7 Summary and conclusion in Finnish

Profest SYKE järjesti pohjaeläintunnistuksen vertailukokeen, johon osallistui 22 määrittäjää. Osallistajat voivat osallistua seuraaviin pohjaeläintunnistusosioihin; koillisen Itämeren-, järvilitoraali-, järvisyvänteiden tai virtavesien taksonien tunnistustestiin. Järvien littoraalitesissä kuusi osallistujaa seitsemästä ylittivät 95 % oikein ja kolme osallistujaa määrittivät kaiken oikein (100 %). Puolet syväntetaksonien tunnistustesteihin osallistuneista tunnistivat 95 % oikein, joista neljällä oli kaikki oikein. Virtavesien tunnistustestissä kaksi osallistujaa kymmenestä tunnisti kaikki oikein ja puolet yli 95 % oikein. Itämeren osiossa tunnistettiin keskimäärin 94 % oikein.

Kaikkiaan 64 % järvien litoraalitaksoneista, 55 % järvisyväntetaksoneista ja 49 % virtavesien taksoneista tunnistettiin aina oikein. Itämeriosiossa keskimääräiset väärintunnistetut taksonit edustivat 8 % ja 50 % taksoneista tunnistettiin aina oikein. Tulokset osoittavat selvästi tarpeen säännöllisesti toistuville virtavesien, syvänteiden ja littorali sekä Itämeritaksoneiden pohjaeläin-vertailukokeille. Lisäksi tunnistettiin tarve pätevyyskokeiden laadunvarmistuksen kehittämiseen ja pohjaeläimien tunnistamiseen. Digitaalisten aineistojen lisääminen, kansainvälisten osallistujien ja opiskelijoiden lisääminen ovat tulevien vertailukokeiden tavoitteet.

REFERENCES

1. SFS-EN ISO 17043, 2010. Conformity assessment – General requirements for Proficiency Testing.
2. ISO 13528, 2005. Statistical methods for use in proficiency testing by interlaboratory comparisons.
3. Thompson, M., Ellison, S. L. R., Wood, R., 2006. The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry laboratories (IUPAC Technical report). Pure Appl. Chem. 78: 145-196, www.iupac.org.
4. Meissner, K., Björklöf, K., Jaale, M., Könönen, K., Rissanen, J. and Leivuori, M. (2012). Proficiency Test SYKE 7/2011, Boreal lake littoral and NE Baltic benthic macroinvertebrate taxonomic identification. Reports of Finnish environment institute 16. <https://helda.helsinki.fi/handle/10138/39786>
5. Meissner, K., Aroviita, J., Hellsten, S., Järvinen, M., Karjalainen, S. M. Kuoppala, M., Mykrä, H. ja Vuori, K-M. 2010. Jokien ja järvien biologinen seuranta – näytteenotosta tiedon tallentamiseen. Online guidance. <http://www.ymparisto.fi/download/noname/%7BB948034F-7F9D-4EAB-A153-92FA2DDEDBBE%7D/29725>
6. Perus J., Bonsdorff E., Bäck S., Lax H.-G., Villnäs A. and Westberg V. (2007). Zoobenthos as indicators of ecological status in coastal brackish waters: a comparative study from the Baltic Sea. *Ambio* 36: 250-256
7. Vuori K.-M., Mitikka S, Vuoristo H (2009) Pintavesien ekologisen tilan luokittelu. Ympäristöhallinnon ohjeita 3:2009. <http://hdl.handle.net/10138/41785>.



ISBN 978-952-11-4658-9 (PDF)

ISSN 1796-1726 (online)