REPORTS OF THE FINNISH ENVIRONMENT INSTITUTE 5 | 2010

# SYKE Proficiency Test 7/2009

**Phytoplankton**

**Kristiina Vuorio, Maija Huttunen, Seija Hällfors, Reija Jokipii, Marko Järvinen, Mirja Leivuori, Maija Niemelä and Markku Ilmakunnas**

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Helsinki 2010

FINNISH ENVIRONMENT INSTITUTE


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The organizer of the intercomparison test:
Finnish Environment Institute SYKE, Laboratories
Hakuninmaantie 6, 00430 Helsinki
phone +358 20 490 123, telefax +358 9 495 913

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# 1. INTRODUCTION

The Finnish Environment Institute (SYKE) is a national environmental reference laboratory established under the Environmental Protection Act (2000). The duties of SYKE include co-ordinating proficiency tests for analytical laboratories and other producers of environmental information. The proficiency testing service is part of the SYKE Laboratory Management System based on the EN ISO/IEC 17025 standard (2005). The SYKE proficiency testing service also conforms to the requirements of ISO/IEC Guide 43-1 (1997) and the ILAC G13:08 (2007) Guidelines for the Requirements for the Competence of Providers of Proficiency testing Schemes, (ISO 13528 (2005) and IUPAC Recommendations (Thompson et al. 2005). SYKE is the Proficiency Testing Provider No. PT01 accredited by the Finnish Accreditation Service (www.finas.fi). However, the organizing of phytoplankton proficiency test does not belong to the accredited scope.

SYKE organises phytoplankton proficiency tests every other year. The phytoplankton proficiency test SYKE 7/2009 is the second virtual proficiency test of SYKE based on filmed material. The first virtual phytoplankton intercomparison test was carried out in March 2007 in co-operation with Finnish Institute of Marine Research (present SYKE, Marine Research Centre) and University of Turku (Vuorio et al. 2007a). SYKE has also earlier, in co-operation with University of Turku, organised three informal phytoplankton intercomparison tests, two of which were national and one international test. These tests were based on natural water samples and laboratory strains of cyanobacteria (Vuorio et al. 2007b).

Phytoplankton analyses are routinely done by one analyst. Therefore, SYKE decided to organize the phytoplankton proficiency test at individual level. Thus the participants received personal test diploma including of the evaluation of their results.

# 2. ORGANISATION OF THE PROFICIENCY TEST

## 2.1. Responsibilities

| | |
|---|---|
| Contact person | Marko Järvinen, PhD, person in charge<br>Mirja Leivuori, coordinator |
| Expert panel | Marko Järvinen, PhD, Finnish Environment Institute, Freshwater Centre<br>Kristiina Vuorio, PhD, Finnish Environment Institute (SYKE), Freshwater Centre<br>Maija Niemelä, Finnish Environment Institute (SYKE), Freshwater Centre<br>Reija Jokipii, Finnish Environment Institute (SYKE), Freshwater Centre<br>Maija Huttunen, Finnish Environment Institute (SYKE), Marine Research Centre<br>Seija Hällfors, MSc, Finnish Environment Institute (SYKE), Marine Research Centre |
| Invited experts | Liisa Lepistö, Professor, lake phytoplankton identification<br>Guy Hällfors, Adjunct Professor, Baltic Sea phytoplankton identification |
| Address | Finnish Environment Institute (SYKE), Laboratories, Hakuninmaantie 6, FI-00430 Helsinki, Finland<br>Finnish Environment Institute (SYKE), Freshwater Centre, P.O. Box 140, FI-00251 Helsinki, Finland<br>Finnish Environment Institute (SYKE), Jyväskylä office, Survontie 9, FI-40500 Jyväskylä, Finland |

Finnish Environment Institute (SYKE), Marine Research Centre, Erik Palmenin aukio 1, FI-00560 Helsinki, Finland

Markku Ilmakunnas, layout of the report

E-mail: marko.jarvinen@ymparisto.fi
mirja.leivuori@ymparisto.fi
kristiina.vuorio@ymparisto.fi

## 2.2. Invitation and participants

The target groups of the proficiency test were consultants and environmental authorities who analyse phytoplankton samples from inland waters and/or the Baltic Sea, and phytoplankton analysts working in research institutes and universities.

Invitation to take part in the test was presented in the proficiency web page of SYKE (www.environment.fi/syke/proftest). In addition, personal invitations were sent to national and international phytoplankton expert laboratories and to European phytoplankton researchers and analysts using the e-mail lists of the Finnish phytoplankton society, EU Wiser project, HELCOM PEG-group, and EU Geographical Intercalibration Groups.

A total of 35 analysts (Appendix 1) from 23 organisations and 8 countries (Table 1) registered in the phytoplankton proficiency test. Participant no 28 cancelled participation after the material delivery.

Table 1. Number of participants and organisations of the SYKE 7/2009 test.

| Country | No of participants | No of organisations |
|---|---|---|
| Denmark | 3 | 2 |
| Estonia | 2 | 2 |
| Finland | 13 | 9 |
| Latvia | 1 | 1 |
| Norway | 2 | 1 |
| Romania | 2 | 2 |
| Sweden | 8 | 3 |
| United Kingdom | 3 | 3 |
| **Total** | **34** | **23** |

## 3. TIMETABLE

Invitation to participate in the test was announced on October 8, 2009. The registration deadline was October 30, 2009. The test material was posted on November 3, 2009. Participants were requested to return by e-mail the test results by November 27, 2009. Preliminary results were posted to participants on December 11, 2009. The participants were asked to give their comments concerning the preliminary test results by January 8, 2010.

# 4. TEST MATERIAL

The test integrated three components of the phytoplankton analysis: 1) the species identification, 2) phytoplankton counting and 3) the measurement of cell dimensions.

The test material included three DVD discs with digital images for the identification and counting tests and an Excel spreadsheet template for reporting the test results, and two 6 ml plastic tubes with preserved phytoplankton for the measurement test. The Excel spreadsheet also included detailed guidance for the test, both in Finnish and in English. The test material represented phytoplankton that typically occurs in freshwaters in the Northern Europe and in the Baltic Sea.

## 4.1. Phytoplankton identification test

The participants could take part both in the lake phytoplankton and the Baltic Sea phytoplankton identification tests or alternatively only one of the tests. Material for the phytoplankton identification was filmed using inverted microscopes with total magnifications of 250x, 750x and 1000x. The lake phytoplankton identification test consisted of 20 video-clips filmed from Lugol preserved samples using both light and phase contrast fields. A total of 21 taxa common in the Northern European freshwaters were to be identified (Fig. 1). The Baltic Sea phytoplankton identification test consisted of 20 video-clips filmed from Lugol preserved and live material using phase contrast fields and it represented a total of 22 identifiable taxa (Fig. 2). The requested minimum level of identification (species, genus, order) was indicated in the Excel spreadsheet template.

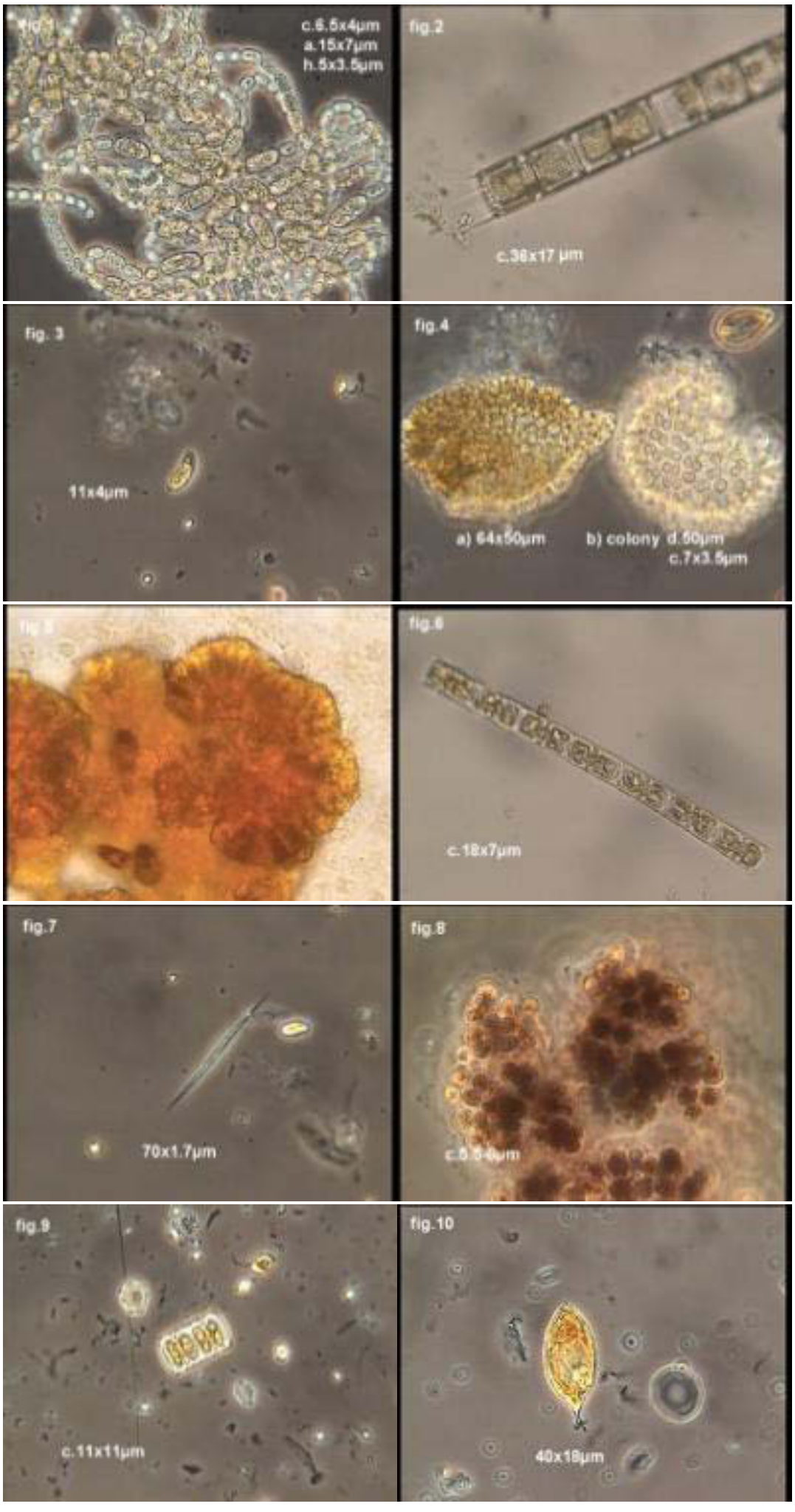
fig.1
c.6.5x4µm
a.15x7µm
h.5x3.5µm
fig.2
c.36x17 µm
fig. 3
11x4µm
fig.4
a) 64x50µm
b) colony d.50µm
c.7x3.5µm
fig.6
c.18x7µm
fig.7
70x1.7µm
fig.8
fig.9
c.11x11µm
fig.10
40x18µm

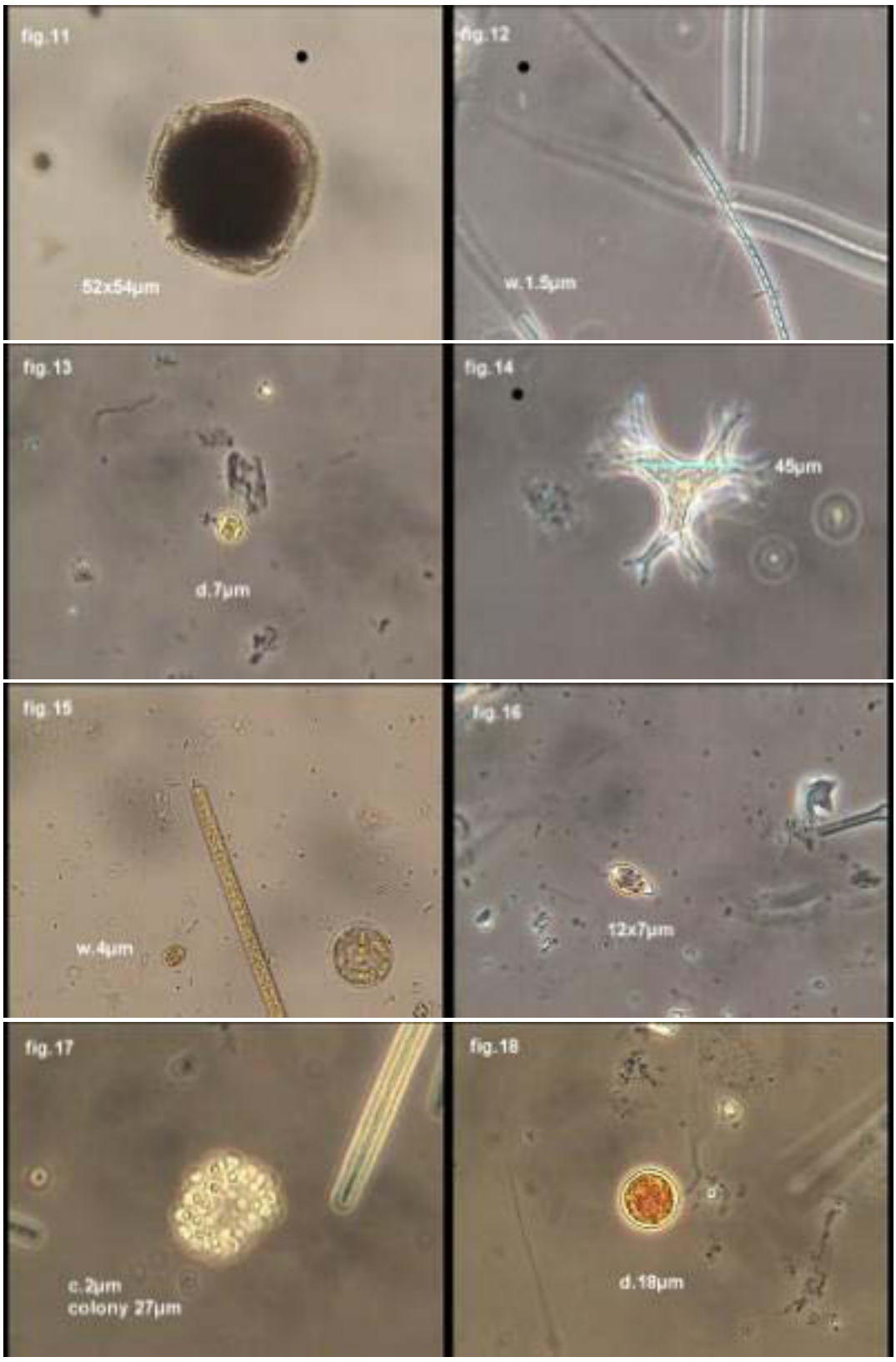
fig.11
52x54µm
fig.12
w.1.5µm
fig.13
d.7µm
fig.14
45µm
fig.15
w.4µm
fig.16
12x7µm
fig.17
c.2µm
colony 27µm
fig.18
d.18µm


Figure 1. Test material of the lake phytoplankton identification test comprised 20 video clips. Video clip number 4 comprised two taxa to be identified. Accepted identifications are given in Table 2. The resolution of the filmed material was higher than presented here in the example photographs.

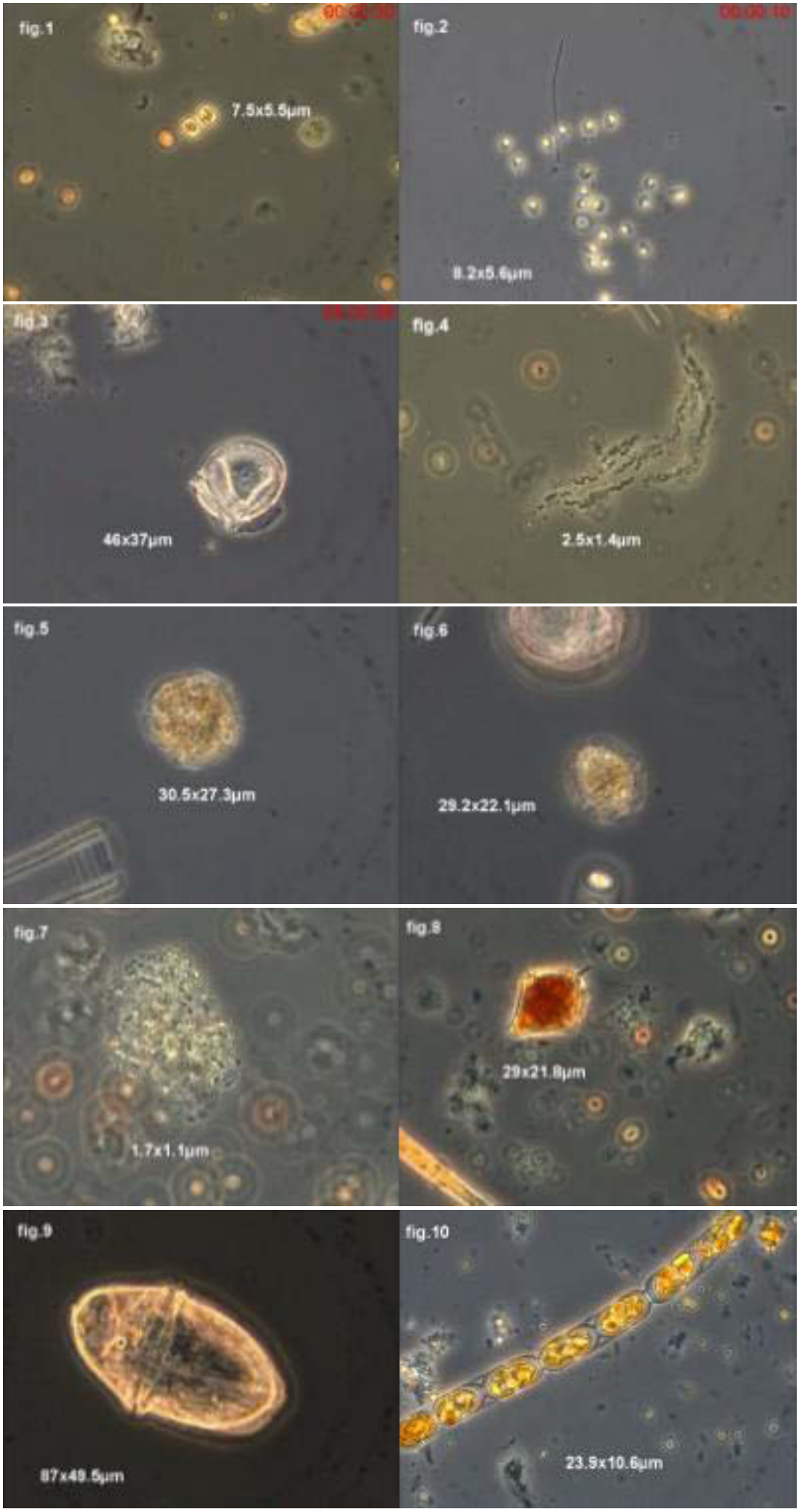
fig.1
7.5x5.5µm
fig.2
8.2x5.6µm
fig.3
46x37µm
fig.4
2.5x1.4µm
fig.5
30.5x27.3µm
fig.6
29.2x22.1µm
fig.7
1.7x1.1µm
fig.8
29x21.8µm
fig.9
87x49.5µm
fig.10
23.9x10.6µm

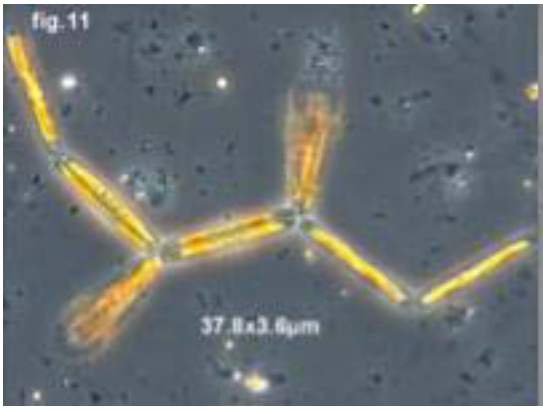
fig.11
37.8x3.6µm

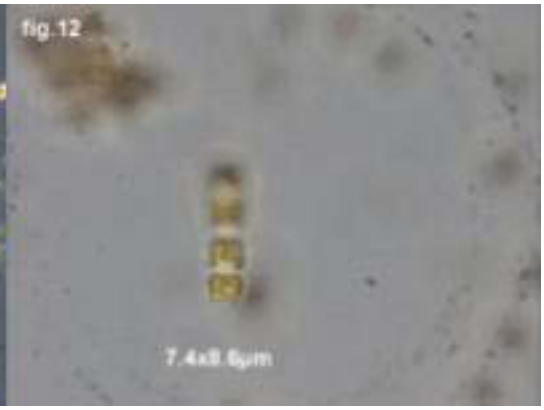
fig.12
7.4x8.6µm

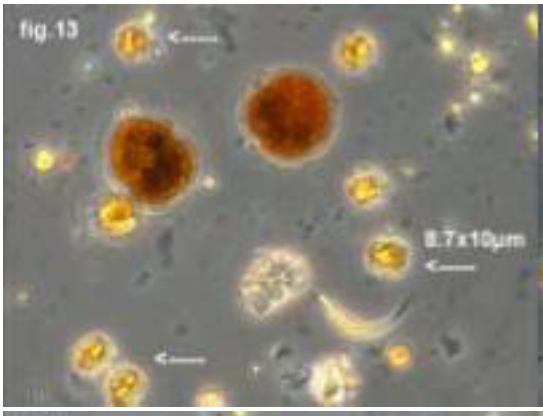
fig.13
8.7x10µm

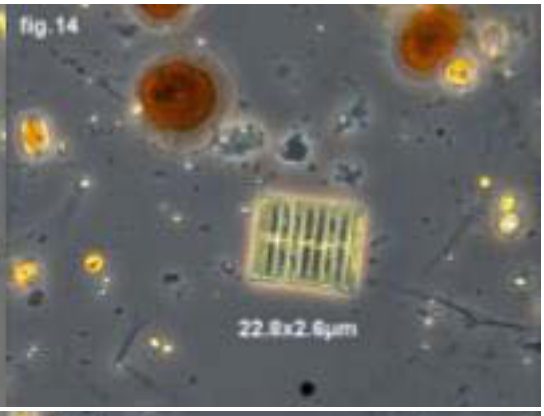
fig.14
22.8x2.6µm

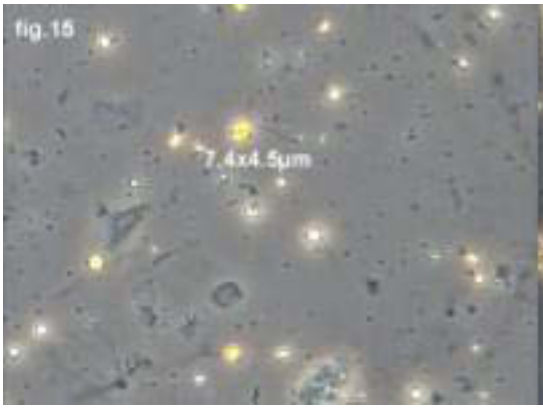
fig.15
7.4x4.5µm

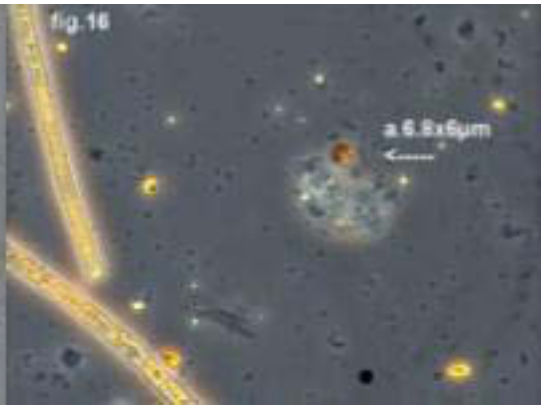
fig.16
a.6.8x6µm

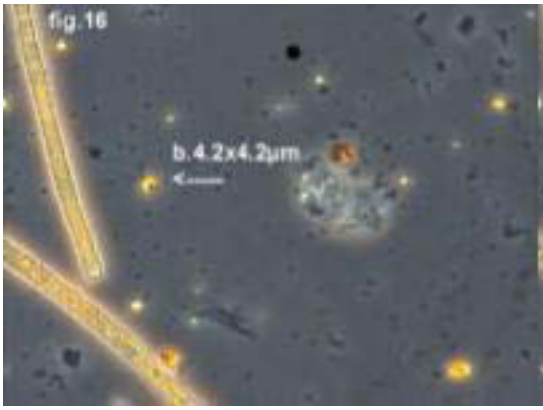
fig.16
b.4.2x4.2µm

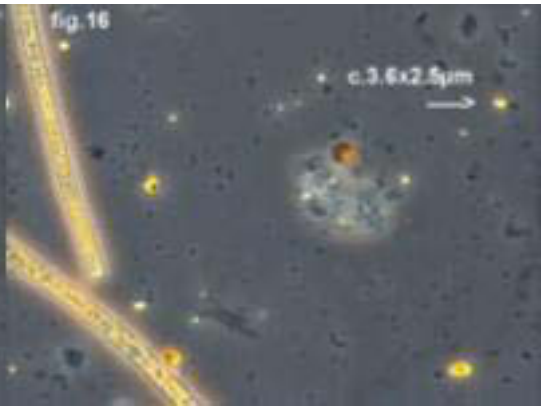
fig.16
c.3.6x2.5µm

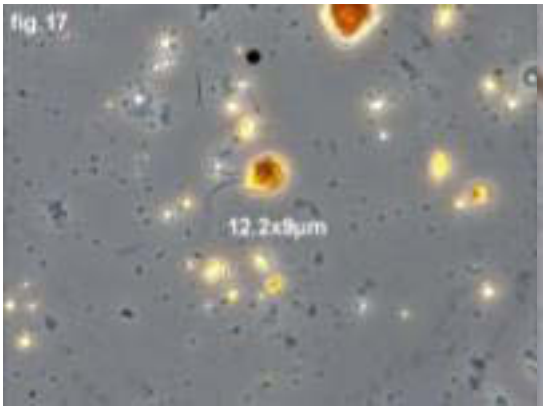
fig.17
12.2x9µm

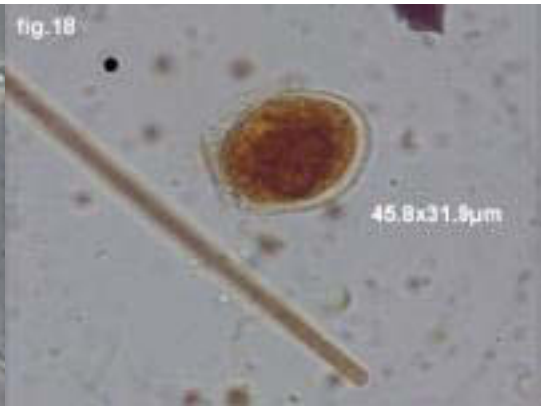
fig.18
45.8x31.9µm

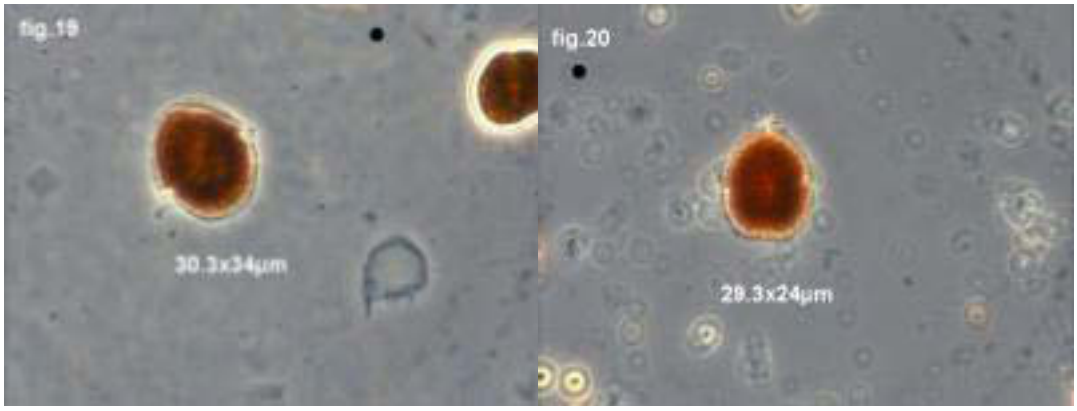


Figure 2. Test material of the Baltic Sea phytoplankton identification test comprised 20 video clips. The video clip number 16 comprised three taxa to be identified (shown here as separate photographs). Accepted identifications are given in Table 4. The resolution of the filmed material was higher than presented here in the example photographs.

### 4.2. Phytoplankton counting test

For the phytoplankton counting test 25 video-clips representing 25 fields of view in a microscope were filmed from a composite that was a mixture of natural lake phytoplankton and a laboratory culture. The natural lake phytoplankton consisted of the filamentous cyanobacterium *Aphanizomenon* sp. (Fig. 3) and the colonial cyanobacterium *Woronichinia naegeliana* (Unger) Elenkin 1933 (Fig. 4), and the laboratory culture of the cysts and mature cells of a marine dinoflagellate (Fig. 5). Prior to filming the composite sample was preserved with acid Lugol's solution and settled in Utermöhl settling chambers. Filming was performed using an inverted microscope with phase contrast illumination and a total magnification of 250x. The filmed material also contained other freshwater taxa originating from the lake material (fig. 6). These taxa were instructed to be ignored during the counting. Photographs of the requested taxa were presented in the Excel spreadsheet guidance.

Figure 3. The cyanobacterium *Aphanizomenon* sp. represented the filamentous taxa in the counting test.

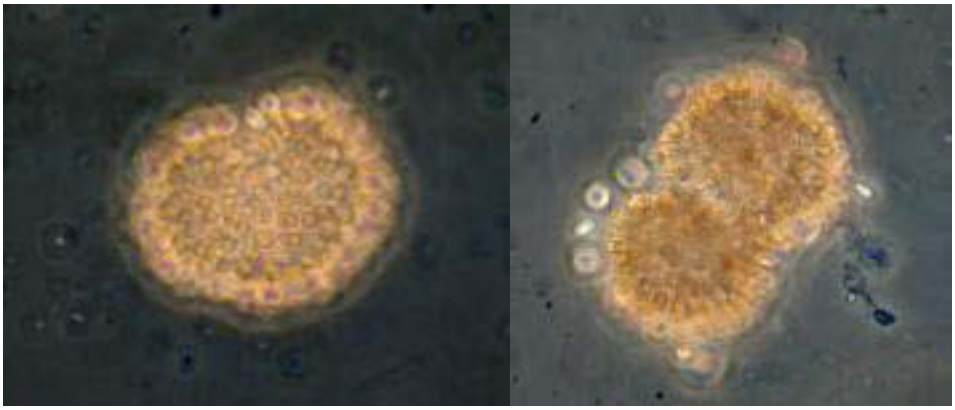

Figure 4. The cyanobacterium *Woronichinia naegeliana* represented the colony forming taxa in the counting test.

Figure 5. The dinoflagellate cysts represented the single-celled taxa to be counted together with the mature cells (not shown in the photographs) in the counting test.

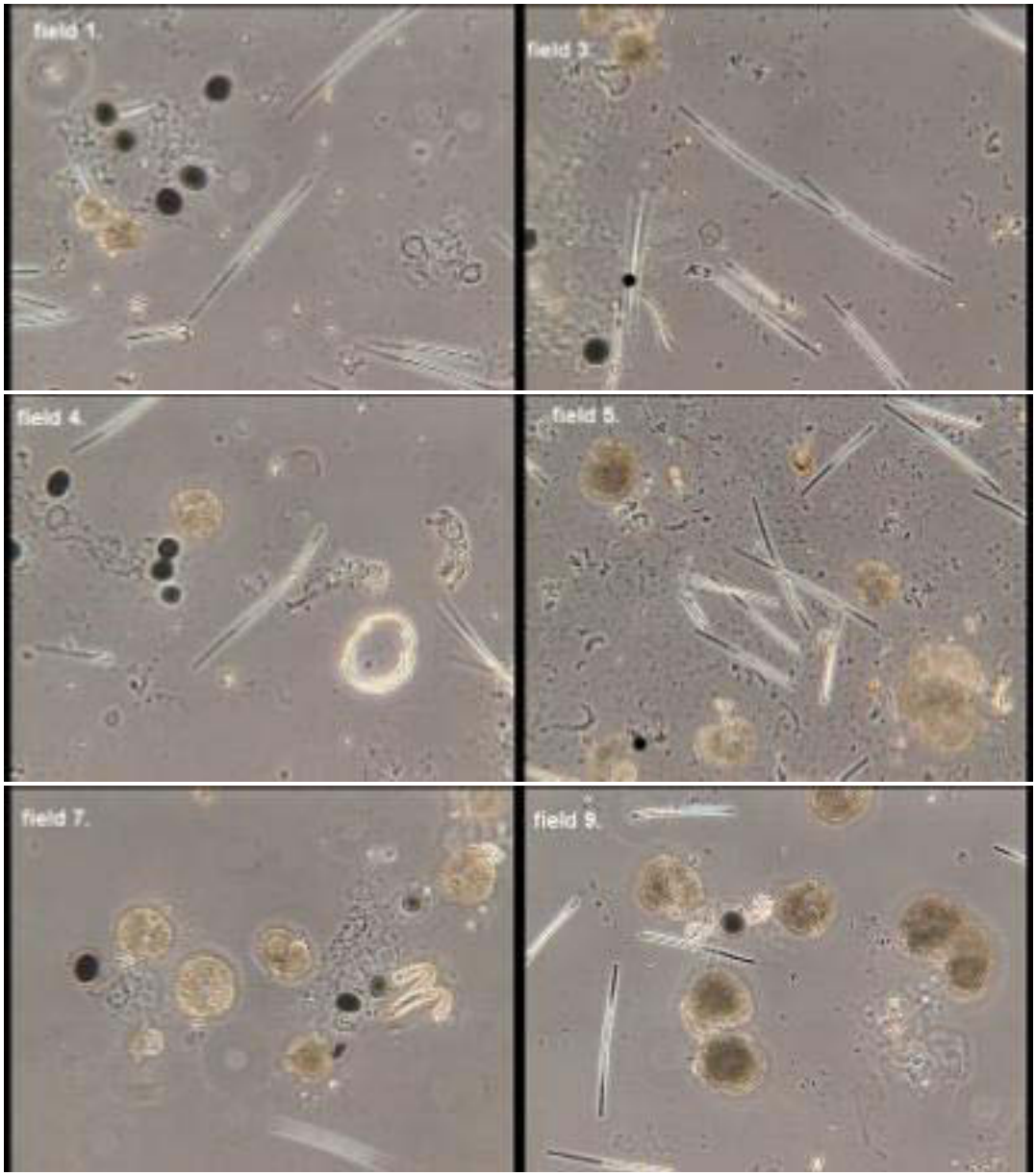


Figure 6. Example photographs taken from the video clips filmed for the phytoplankton counting test including the filamentous cyanobacterium *Aphanizomenon* sp., the colony forming cyanobacterium *Woronichinia naegeliana* and the single-celled dinoflagellate.

Participants were advised to perform the counting according to the guidelines presented in the EN 15204 standard (2006) (Fig. 7), and report their results on the Excel spreadsheet template included on the DVD. The counting unit for the filamentous *Aphanizomenon* was a filament irrespective of its length. For the colony forming *Woronichinia* the counting unit was a colony; irrespective of possible subcolonies, each colony was advised to be counted as one unit. The third counting unit was a single-celled dinoflagellate represented mostly by cysts. Both the cysts and the mature cells were advised to be counted as one counting unit. Participants were also asked to describe the details of the counting method used. For the reference material of the counting test, the members of the expert panel counted the requested taxa according to the EN 15204 standard (2006) and using all possible acceptable edge combinations.

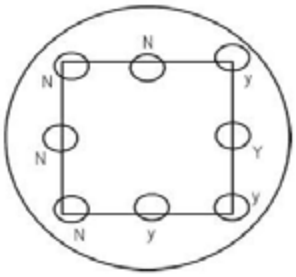


Figure 7. Recommendation of the rule for counting the cells on the edge of the counting grid as presented in the EN 15204 standard (2006) which was referred to in the SYKE 7/2009 test guidance (see also Olenina et al. 2006). For example the objects crossing the bottom and right hand grid are counted whilst those crossing both the top and left hand side of the grid are not counted. A key for the figure: Y = counted, N = not counted.

### 4.3. Biovolume estimation test

In the biovolume estimation test the dimensions of selected taxa were asked to be measured. For the biovolume estimation test the filamentous cyanobacterium *Aphanizomenon* sp. (Fig. 8), the colony forming cyanobacterium (*Microcystis wesenbergii*) (Fig. 9), both sampled from lakes, and a single-celled marine dinoflagellate (*Heterocapsa triquetra*) (Fig. 10) from a laboratory culture were pooled to a composite sample preserved with acid Lugol's solution. Two replicate samples containing ca. 6 ml of the sample were delivered to each participant. In addition to the taxa to be measured, the sample also included other algal species.

For the filamentous cyanobacterium the cell diameter of a growing cell located in the middle of the filament was advised to be measured. A total of 20 cells should be measured from different filaments, i.e. only one measurement per filament should have been performed. For the colonial cyanobacterium cell diameters of individual cells was advised to be measured. A total of 20 cells from different colonies should be measured, i.e. only one cell should be measured per colony. For the single-celled dinoflagellate both the cell height and cell width were advised to be measured from 30 mature individuals ignoring cysts. Results of the test were reported on the Excel spreadsheet according to guidance.

Fig 8. The cell diameter of the growing cell located in the middle of the filament of the filamentous cyanobacterium *Aphanizomenon* sp. was measured in the biovolume estimation test.

Fig 9. The cell diameter of the individual cells of the colony forming cyanobacterium (*Microcystis wesenbergii*) was measured in the biovolume estimation test.

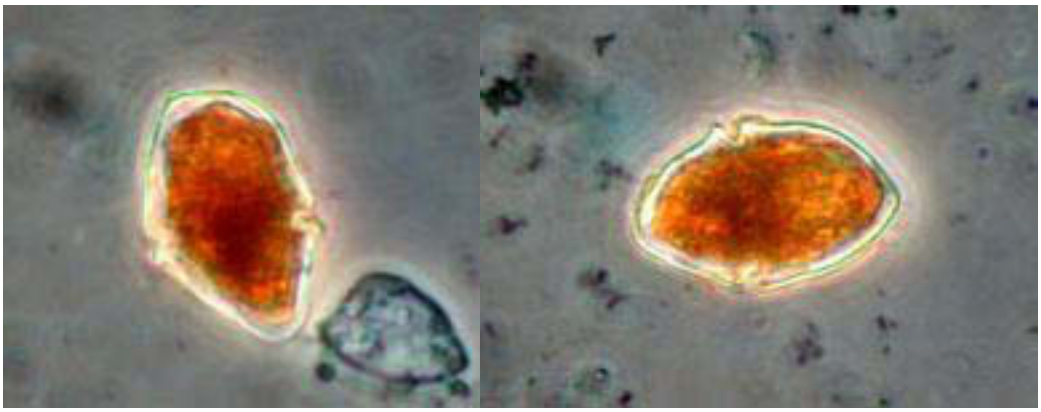

Fig 10. The cell height and the cell width of the single-celled dinoflagellate (*Heterocapsa triquetra*) were measured in the biovolume estimation.

## 5. STATISTICAL ANALYSES

Statistical analyses of the counting and biovolume components of the proficiency test material were carried out according to ISO 13528 (2005). Observations inconsistent with other observations, i.e. observations that were outside the 90% confidence limit, were interpreted as outliers. Thereafter, outliers were discarded on a case-by-case basis applying Hampel test. The robust mean values were used as assigned reference values and were evaluated applying robust statistics based on the assumption that the data are a sample from an essentially normal distribution contaminated with heavy tails and a small proportion of outliers. Therefore, normality of the results was not tested.

Uncertainty (u) of the assigned reference values was evaluated as follows: $u = 1.25*s_{rob}/\sqrt{n}$, in which $s_{rob}$ = robust standard deviation and n = number of results. The standard deviation ($s_p$) for the proficiency assessment was set at 10%. Criterion for the reliability of the assigned reference values was $u/s_p \leq 0.3$. This criterion was fulfilled in all statistical analysis of the test material. The criterion, $s_{rob} < 1.2*s_p$, was also fulfilled indicating that the z scores were reliable. Evaluation of performance for a single result was based on calculation of z-scores which are deviations of the individual test results from the assigned reference values (robust mean values) compared to the target dispersion 10%. For the proficiency assessment the z-scores were considered as follows: the result was considered satisfactory if $|z| \leq 2$, questionable if $2 < |z| < 3$ ) unsatisfactory if $|z| \geq 3$ .

For comparison of the individual test results of the counting test, verified values were also calculated by the expert panel. All possible combinations of the diagonal edges of a counting grid were considered when counting the objects on the edges of a counting grid according to EN 15204 (2006). See also Fig. 7.

# 6. RESULTS

## 6.1. Phytoplankton identification tests

The identification results of the participants were scored 3, 2, 1 or 0 according to the correctness of the answer (Tables 3 and 5). The quality target in both the lake and the Baltic Sea phytoplankton identification test was set at 75% of the maximum scores. Synonyms were accepted. Identification at lower level (e.g. genus level if the species level identification was requested) was awarded with 2 points. Correct species level identification gave 3 points also when the genus level identification was requested. If the taxon to be identified was closely related and resembled closely the suggested taxon, 1 or 2 points were awarded depending on the degree of difficulty of identification or how close relatives the taxa in question were.

### 6.1.1. Lake phytoplankton identification test

Altogether 25 analysts took part this part of the test. The requested taxa represented typical species in Northern-European freshwaters ranging from common to relatively uncommon in occurrence. The correctness of the identification of each taxon, originally carried out by the expert panel, was verified by the invited expert Professor Liisa Lepistö. The awarded scores are presented in Table 3. Two of the taxa were identified correctly by all participants (Fig. 11). The good quality target was set to 75% of the maximum scores, i.e. 47 of the maximum of 63 points. Twenty analysts reached the good quality target with personal scores at least 75% of the maximum score (Fig. 12). None of the participants received the maximum score.

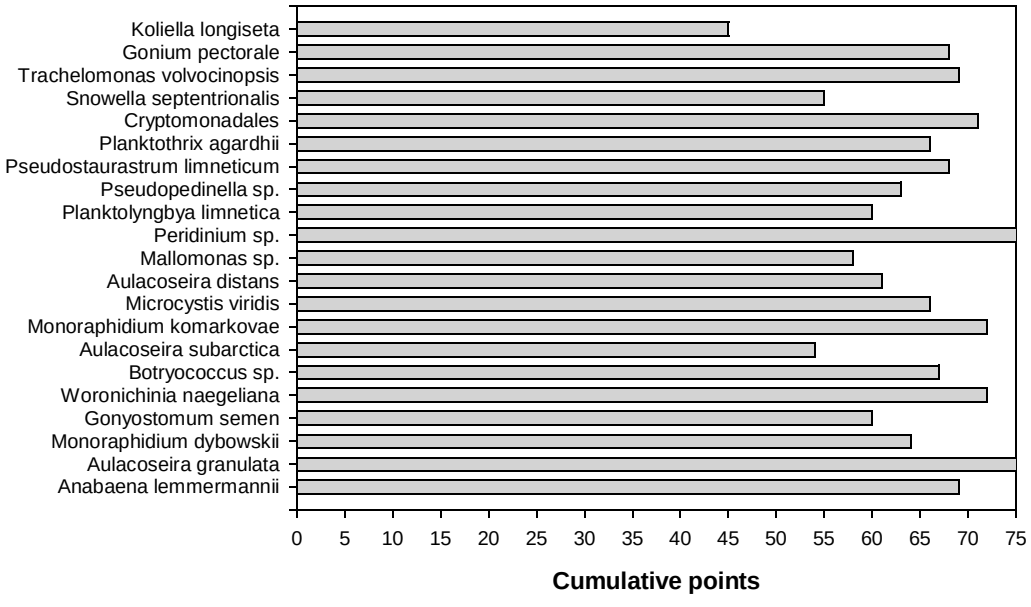


Figure 11. Cumulative points for each taxon in the lake phytoplankton identification test. Maximum score of 75 represents correct identification by all participants.

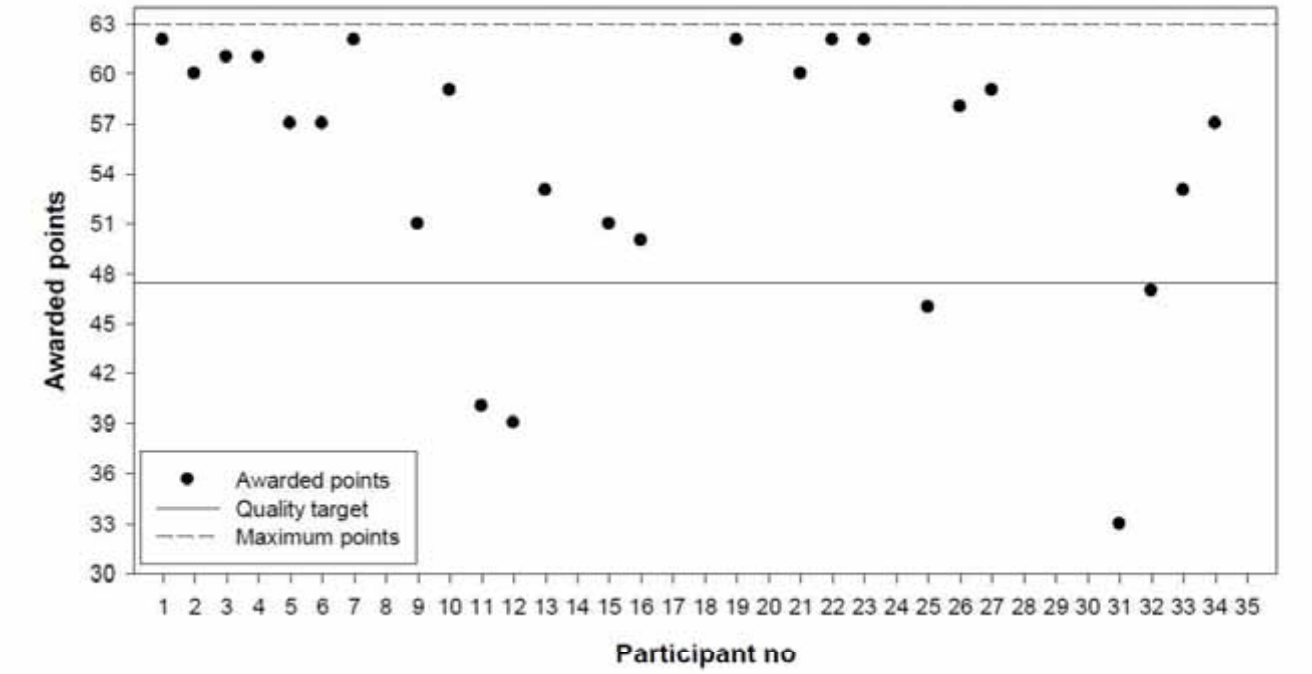


Figure 12. Results of the lake phytoplankton identification test. The quality target was set to 47 points (≥75%) of the maximum of 63 points.

Table 2: Suggested correct identifications including the accepted synonyms for the lake phytoplankton identification test.

| Video no | Correct identification | Identification level |
|---|---|---|
| 1 | *Anabaena lemmermannii* P. Richter 1903<br>[*Anabaena flos-aquae* f. *lemmermannii* (P. Richter) Canabaeus 1929]<br>[*Anabaena utermoehlii* Geitler 1925] | Species |
| 2 | *Aulacoseira granulata* (Ehrenberg) Simonsen 1979<br>[*Gallionella granulata* Ehrenberg 1943]<br>[*Melosira granulata* (Ehrenberg) Ralfs 1861] | Species |
| 3 | *Monoraphidium dybowskii* (Woloszynska) Hindák & Komárková-Legnerová 1969<br>[*Keratococcus dybowskii* Woloszynska 1917] | Species |
| 4a | *Gonyostomum semen* (Ehrenberg) Diesing | Species |
| 4b | *Woronichinia naegeliana* (Unger) Elenkin 1933<br>[*Coelosphaerium naegelianum* Unger 1854]<br>[*Gomphosphaeria naegeliana* (Unger) Lemmermann 1907] | Species |
| 5 | *Botryococcus* sp. Kützing | Genus |
| 6 | *Aulacoseira subarctica* (O. Müller) Haworth 1988<br>*Melosira italica* subsp. *subarctica* O. Müller<br>*Aulacoseira italica* ssp. *subarctica* (O Müller) Simonsen 1979 | Species |
| 7 | *Monoraphidium komarkovae* Nygaard 1979<br>[*Monoraphidium setiforme* (Nygaard) Komárková-Legnerová 1969] | Species |
| 8 | *Microcystis viridis* (A. Braun) Lemmermann 1902<br>[*Polycystis viridis* A. Braun in Rabenhorst 1862]<br>[*Microcystis aeruginosa f. viridis* (A. Braun in Rabenhorst) Elenkin 1938]<br>[*Diplocystis viridis* (A. Braun) Komárek 1958] | Species |
| 9 | *Aulacoseira distans* (Ehrenberg) Simonsen 1979<br>[*Gallionella distans* Ehrenberg 1936]<br>[*Melosira distans* (Ehrenberg) Kützing 1844] | Species |
| 10 | *Mallomonas* sp. Perty<br>*Mallomonas caudata* Ivanov emend. Krieger<br>[*Mallomonas fastigata* Zacharias] | Genus |
| 11 | *Peridinium* sp. Ehrenberg<br>*Peridinium willei* Huitfeld-Kaas | Genus |
| 12 | *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg 1992<br>[*Lyngbya limnetica* Lemmermann 1898]<br>[*Planktolyngbya subtilis* (W. West) Anagnostidis & Komárek 1988] | Species |
| 13 | *Pseudopedinella* sp. | Genus |
| 14 | *Pseudostaurastrum limneticum* (Borge) R. Chodat<br>[*Tetraedron limneticum* Borge 1900] | Species |
| 15 | *Planktothrix agardhii* Anagnostidis & Komárek 1988<br>[*Oscillatoria agardhii* Gomont 1892] | Species |
| 16 | Cryptomonadales<br>*Rhodomonas lens* Pascher & Ruttner | Order |
| 17 | *Snowella septentrionalis* Komárek & Hindák 1988 | Species |
| 18 | *Trachelomonas volvocinopsis* Swirenko | Species |
| 19 | *Gonium pectorale* O.F. Müller | Species |
| 20 | *Koliella longiseta* Hindák | Species |

Table 3: Identification results suggested by the participants for each taxon and the corresponding awarded scores in the lake phytoplankton identification test.

| Video no | Taxon | Points |
|---|---|---|
| 1 | *Anabaena lemmermannii* | 3 |
| | *Anabaena lemmermannii* var. *lemmermannii* | 3 |
| | *Anabaena lemmermannii* var. *minor* | 3 |
| | *Anabaena flos-aquae* | 2 |
| 2 | *Aulacoseira granulata* | 3 |
| | *Aulacoseira granulata* var. *granulata* | 3 |
| | *Melosira granulata* var. *granulata* | 3 |
| 3 | *Monoraphidium dybowskii* | 3 |
| | *Monoraphidium minutum* | 2 |
| | *Chromulina vagans* | 0 |
| | *Closterium* sp. | 0 |
| 4a | *Gonyostomum semen* | 3 |
| | *Botryococcus braunii* | 0 |
| | *Gomphosphaeria lacustris* | 0 |
| | *Microcrocis geminate* | 0 |
| | *Woronichinia naegeliana* | 0 |
| 4b | *Woronichinia naegeliana* | 3 |
| | *Woronichinia karelica* | 2 |
| | *Gomphosphaeria aponina* | 1 |
| 5 | *Botryococcus* sp. | 3 |
| | *Botryococcus braunii* | 2 |
| | *Botryococcus protuberans* | 1 |
| | *Botryococcus terribilis* | 1 |
| 6 | *Aulacoseira subarctica* | 3 |
| | *Aulacoseira italica* | 2 |
| | *Aulacoseira valida* | 2 |
| | *Aulacoseira ambigua* | 1 |
| | *Aulacoseira islandica* | 1 |
| 7 | *Monoraphidium komarkovae* | 3 |
| | *Monoraphidium griffithii* | 3 |
| | *Koliella spiculiformis* | 0 |
| 8 | *Microcystis viridis* | 3 |
| | *Microcystis aeruginosa* | 1 |
| | *Microcystis botrys* | 1 |
| | *Microcystis wesenbergii* | 1 |
| | *Porphyrium purpureum* | 0 |
| 9 | *Aulacoseira distans* | 3 |
| | *Melosira distans* | 3 |
| | *Aulacoseira alpigena* | 2 |
| | *Aulacoseira* sp. | 2 |
| | *Aulacoseira* cf. *alpigena* | 2 |
| | *Aulacoseira lacustris* | 1 |
| | *Stephanodiscus hantzschii* | 0 |
| 10 | *Mallomonas* sp. | 3 |
| | *Mallomonas caudata* | 3 |
| | ??(*Mallomonas* sp.) | 1 |
| | *Ankyra* sp. | 0 |
| | *Characium* sp. | 0 |
| | *Cryptomonas* sp. | 0 |
| | *Korshikoviella* sp. | 0 |
| | *Spumella* sp. | 0 |
| 11 | *Peridinium* sp. | 3 |
| | *Peridinium willei* | 3 |

| | | |
|---|---|---|
| 12 | *Planktolyngbya limnetica* | 3 |
| | *Lyngbya limnetica* | 3 |
| | cf. *Leptolyngbya* sp. | 1 |
| | *Leptolyngbya tenuis* | 1 |
| | *Lyngbya lagerheimii* | 1 |
| | *Phormidium tenue* | 0 |
| | *Pseudanabaena* sp. | 0 |
| 13 | *Pseudopedinella* sp. | 3 |
| | *Chrysochromulina* sp. | 0 |
| | *Ochromonas* sp. | 0 |
| 14 | *Pseudostaurastrum limneticum* | 3 |
| | *Pseudostaurastrum limnetica* | 3 |
| | *Pseudostaurastrum* sp. | 2 |
| | *Pseudostaurastrum* cf. *enorme* | 2 |
| | *Pseudostaurastrum hastatum* | 2 |
| | *Staurastrum paracosum* | 0 |
| 15 | *Planktothrix agardhii* | 3 |
| | *Oscillatoria agardhii* | 3 |
| | *Planktothrix* sp. | 2 |
| | *Planktothrix suspensa* | 2 |
| | *Oscillatoria ornata* | 0 |
| 16 | Cryptomonadales | 3 |
| | Pyrenomonadales | 3 |
| | *Rhodomonas lens* | 3 |
| | Cryptophyta | 2 |
| | Chromulinales | 0 |
| 17 | *Snowella septentrionalis* | 3 |
| | *Snowella* cf. *septentrionalis* | 3 |
| | *Snowella* sp. | 3 |
| | *Snowella litoralis* | 2 |
| | *Aphanocapsa* sp. | 0 |
| | *Coelomoron* sp. | 0 |
| | *Gomphosphaeria* sp. | 0 |
| | *Woronichinia elorantae* | 0 |
| | *Woronichinia* sp. | 0 |
| 18 | *Trachelomonas volvocinopsis* | 3 |
| | *Trachelomonas volvocina* | 2 |
| 19 | *Gonium pectorale* | 3 |
| | *Gonium* sp. | 2 |
| | *Pandorina morum* | 0 |
| 20 | *Koliella longiseta* | 3 |
| | *Koliella spirotaenia* | 2 |
| | *Koliella elongate* | 2 |
| | ??(*Koliella* sp.) | 1 |
| | *Monoraphidium komarkovae* | 0 |
| | Nostocales | 0 |
| | *Planktolyngbya* sp. | 0 |

### 6.1.2. Baltic Sea phytoplankton identification test

Altogether 18 analysts took part this part of the test. The requested taxa represented typical species in the northern Baltic Sea ranging from common to relatively uncommon in occurrence. The correctness of the identification of each taxon, originally carried out by the expert panel, was verified by the invited expert Adjunct Professor Guy Hällfors (Table 4). The awarded scores are presented in Table 5. Only one of the given taxa was identified correctly by all participants. None of the participants received the maximum score of 66, however, 12 analysts reached good quality target with at least 75% of the maximum score (Fig. 14).

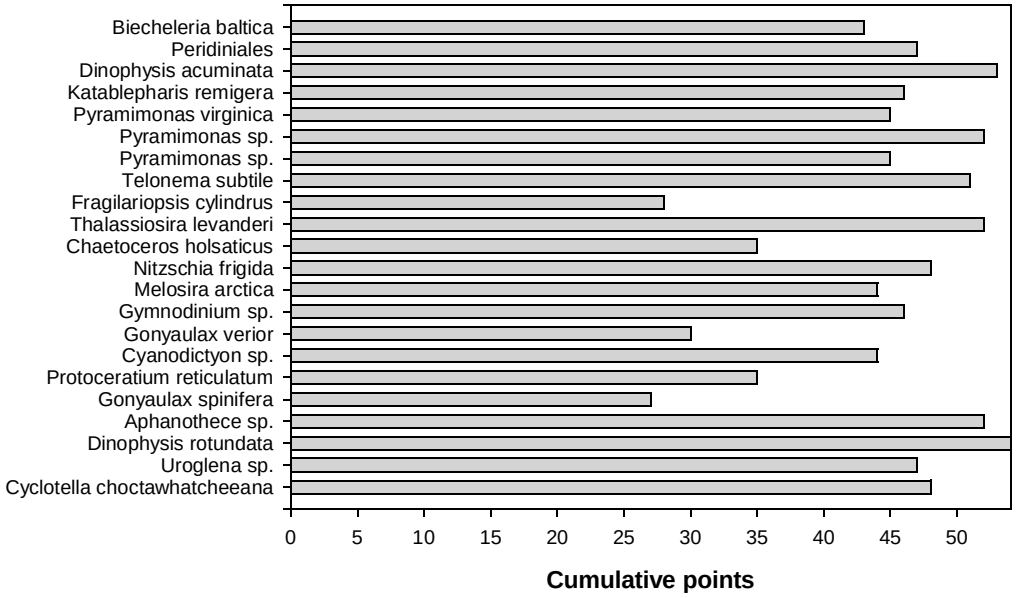


Figure 13. The cumulative points for each taxon in the Baltic Sea phytoplankton identification test. Maximum score of 54 represents a correct identification by all participants.

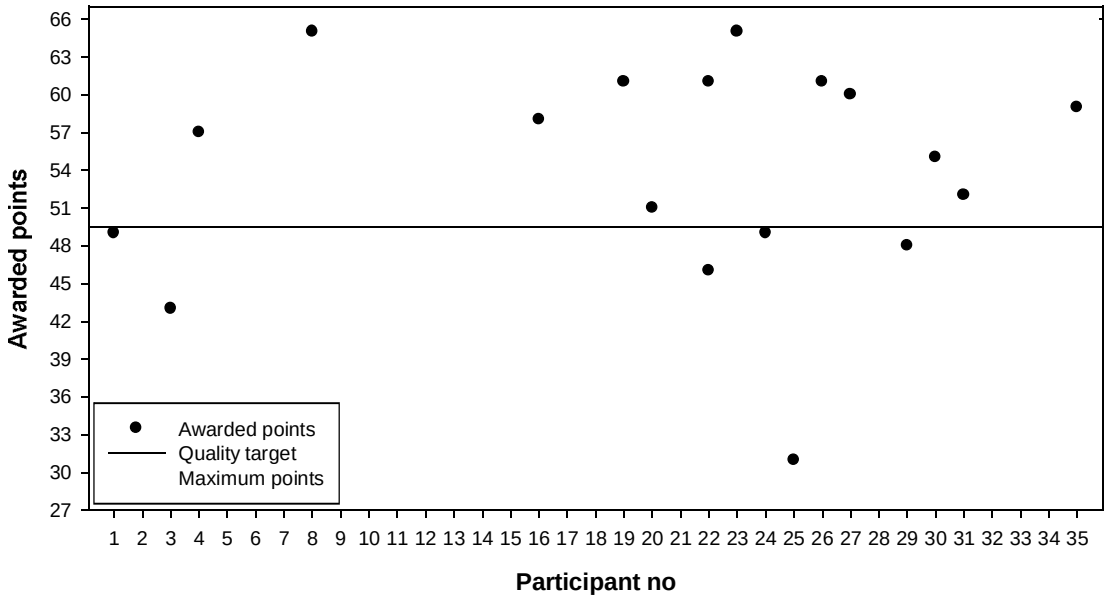


Figure 14. The results of the Baltic Sea phytoplankton identification test. The quality target was set to 50 points (≥75%) of the maximum of 66 points.

Table 4: Suggested correct identifications for the Baltic Sea phytoplankton identification test and the synonyms as presented in the Checklist of Baltic Sea Phytoplankton Species (Hällfors 2004).

| Video no | Correct identification | Identification level |
|---|---|---|
| 1 | *Cyclotella choctawhatcheeana* Prasad *in* Prasad, Nienow & Livingston 1990<br>[*Cyclotella caspia* auct.]<br>[non *Cyclotella caspia* sensu Grunow 1878]<br>[?*Thalassiosira nana* Lohmann 1908 *p.p.*]<br>[*Cyclotella hakanssoniae* Wendker 1990] | Species |
| 2 | *Uroglena* Ehrenberg 1835<br>*Uroglena americana* Calkins 1892<br>[*Uroglenopsis americana* (Calkins) Lemmermann 1899] | Genus |
| 3 | *Dinophysis rotundata* Claperède & Lachmann 1859<br>[*Dinophysis laevis* Claparède & Lachmann 1859]<br>[*Phalachroma rotundatum* (Claparède & Lachmann) Kofoid & Michener 1911]<br>[*Prodinophysis rotundata* (Claparède & Lachmann) Balech 1944]<br>[*Dinophysis whittingae* Balech 1971]<br>[?*Phalachroma rudgei* Murray & Whitting 1899] | Species |
| 4 | *Aphanothece* sp.<br>*Aphanothece parallelliformis* Cronberg 2003 | Genus |
| 5 | *Gonyaulax spinifera* (Claparède & Lachmann) Diesing 1866<br>[*Peridinium spiniferum* Claparède & Lachmann 1859]<br>[*Peridinium* sp. Levander 1894]<br>[*Peridinium levanderi* Lemmermann 1900]<br>[*Gonyaulax levanderi* (Lemmermann) Paulsen 1907] | Species |
| 6 | *Protoceratium reticulatum* (Claparède & Lachmann) Bütschli 1885<br>[*Peridinium reticulatum* Claparède & Lachmann 1859]<br>[*Protoceratium aceros* Bergh 1881]<br>[*Gonyaulax grindleyi* Reinecke 1967] | Species |
| 7 | *Cyanodictyon* sp.<br>*Cyanodictyon balticum* Cronberg 2003 | Genus |
| 8 | *Gonyaulax verior* Sournia 1973<br>[*Amylax diacantha* Meunier 1919]<br>[*Gonyaulax longispina* Lebour 1925]<br>[*Gonyaulax diacantha* (Meunier) Schiller 1937]<br>[non *Gonyaulax diacantha* Athanassopoulos 1931] | Species |
| 9 | *Gymnodinium* sp.<br>*Gymnodinium gracile* Bergh 1881<br>[*Gymnodinium roseum* Lohmann 1908]<br>[non *Gymnodinium roseum* Dogiel 1907]<br>[*Gymnodinium lohmannii* Paulsen 1908]<br>[*Gymnodinium abbreviatum* Kofoid & Swezy 1921] | Genus |
| 10 | *Melosira arctica* (Ehrenberg) Dickie *ex* Ralfs *in* Pritschard 1861<br>[*Gaillionella arctica* Ehrenberg 1853]<br>[*Melosira hyperborea* Grunow *in* Van Heurck 1882]<br>[*Melosira arctica* v. *bornholmiensis* Cleve-Euler 1935] | Species |
| 11 | *Nitzschia frigida* Grunow *in* Cleve & Grunow 1880<br>[*Nitzschia polaris* auct.] | Species |
| 12 | *Chaetoceros holsaticus* Schütt 1895<br>[*Chaetoceros leve* Schütt 1895]<br>[*Chaetoceros balticum* P.T. Cleve 1896]<br>[*Chaetoceros granii* P.T. Cleve 1900] | Species |
| 13 | Thalassiosira *levanderi* van Goor 1924<br>[*Coscinodiscus levanderi* (Van Goor) Cleve-Euler 1951] | Species |
| 14 | *Fragilariopsis cylindrus* (Grunow) W. Krieger *in* Helmcke & Krieger 1954<br>[*Fragilaria cylindrus* Grunow *in* Cleve & Möller 1882 (*F. cylindrica*. auct.)]<br>[*Nitschia cylindrus* (Grunow *in* Cleve & Möller) Hasle 1972] | Species |
| 15 | *Telonema subtile* Griessmann 1913 | Species |

| | | |
|---|---|---|
| 16a | *Pyramimonas* sp. | Genus |
| 16b | *Pyramimonas* sp. | Genus |
| 16c | *Pyramimonas virginica* Pennick 1977 | Species |
| 17 | *Katablepharis remigera* (Vors) Clay & Kugrens 1999<br>[*Leucocryptos remigera* Vors 1992 | Species |
| 18 | *Dinophysis acuminata* Claparède & Lachmann 1859<br>[*Dinophysis rotundata* Levander 1894, 1901]<br>[*Dinophysis ovum* v. *baltica* Paulsen 1908]<br>[*Dinophysis arctica* sensu Wołoszyńska 1928]<br>[*Dinophysis baltica* (Paulsen) Wołoszyńska 1928]<br>[*Dinophysis cassubica* Wołoszyńska 1928]<br>[*Dinophysis levanderi* Wołoszyńska 1928]<br>[*Dinophysis paulseni* Wołoszyńska 1928]<br>[*Dinophysis boehmii* Paulsen 1949]<br>[*Dinophysis borealis* Paulsen 1949]<br>[*Dinophysis lachmannii* Paulsen 1949]<br>[?*Dinophysis skagii* Paulsen 1949]<br>[*Dinophysis ovum* auct.? (*cf.* Pankow 1990) | Species |
| 19 | Peridiniales<br>*Kryptoperidinum foliaceum* (Stein) Lindemann 1924<br>[*Glenodinium foliaceum* Stein 1883]<br>[*Peridinium umbo* Sjöstedt 1924]<br>[*Phyllodinium scutellaris* Conrad 1926] | Order |
| 20 | *Biecheleria baltica* Moestrup, Lindberg et Daugberg 2009<br>[*Woloszynskia halophila* sensu Elbrächter et Kremp 2005]<br>[non *Gymnodinium halophilum* Biecheler (1952, 33, figs X-XIV)] | Species |

Table 5: Identification results suggested by the participants for each taxon and the corresponding scores in the Baltic Sea phytoplankton identification test.

| Video no | Taxon | Points |
|---|---|---|
| 1 | *Cyclotella choctawhatcheeana* | 3 |
| | *Thalassiosira levanderi* | 0 |
| 2 | *Uroglena* sp. | 3 |
| | *Uroglena americana* | 3 |
| | *Ochromonas* sp. | 2 |
| | *Hemiselmis* sp. | 0 |
| 3 | *Dinophysis rotundata* | 3 |
| 4 | *Aphanothece* sp. | 3 |
| | *Aphanothece parallelliformis* | 3 |
| | *Cyanodictyon* sp. | 1 |
| 5 | *Gonyaulax spinifera* | 3 |
| | *Peridiniella catenata* | 0 |
| | *Peridiniopsis umbonatum* | 0 |
| | *Peridinium sp.* | 0 |
| | *Protoperidinium brevipes* | 0 |
| | *Protoperidinium pellucidum* | 0 |
| 6 | *Protoceratium reticulatum* | 3 |
| | *Gonyaulax grindley* | 3 |
| | *Gonyaulax* sp. | 2 |
| | *Alexandrium* sp. | 0 |
| | *Heterocapsa triquetra* | 0 |
| | *Peridinium inconspicuum* | 0 |
| | *Woloszynskia halophila* | 0 |
| | *Woloszynskia pascheri* | 0 |
| 7 | *Cyanodictyon* sp. | 3 |
| | *Cyanodictyon balticum* | 3 |
| | *Cyanodictyon planctonicum* | 1 |
| | *Aphanothece* sp. | 1 |
| 8 | *Gonyaulax verior* | 3 |
| | *Protoperidinium bipes* | 0 |
| | *Protoperidinium brevipes* | 0 |
| 9 | *Gymnodinium* sp. | 3 |
| | *Gymnodinium gracile* | 3 |
| | *Gymnodinium abbreviatum* | 3 |
| | *Gymnodinium fuscum* | 2 |
| | *Gyrodinium fissum* | 1 |
| | *Gyrodinium* sp. | 1 |
| 10 | *Melosira arctica* | 3 |
| | *Melosira* cf. *lineata* | 1 |
| | *Melosira lineata* | 1 |
| | *Melosira nummuloides* | 1 |
| 11 | *Nitzschia frigida* | 3 |
| | *Thalassionema nitzschioides* | 0 |
| 12 | *Chaetoceros holsaticus* | 3 |
| | *Chaetoceros* sp. | 2 |
| | *Chaetoceros wighamii* | 1 |
| 13 | *Thalassiosira levanderi* | 3 |
| | *Thalassiosira baltica* | 1 |
| 14 | *Fragilariopsis cylindrus* | 3 |
| | *Nitzschia cylindra* | 3 |
| | *Achnanthes taeniata* | 0 |
| | *Navicula vanhoeffenii* | 0 |
| 15 | *Telonema subtile* | 3 |
| | *Sphaerellopsis fluviatilis* | 0 |
| 16a | *Pyramimonas* sp. | 3 |
| | *Tetraselmis* sp. | 0 |
| 16b | *Pyramimonas* sp. | 3 |
| | *Pyramimonas orientalis* | 1 |
| 16c | *Pyramimonas virginica* | 3 |
| | *Hemiselmis virescens* | 0 |

| | | |
|---|---|---|
| 17 | *Katablepharis remigera* | 3 |
| | *Katablepharis* sp. | 2 |
| | Autotrophic flagellate | 0 |
| 18 | *Dinophysis acuminata* | 3 |
| | *Dinophysis norvegica* | 2 |
| 19 | Peridiniales | 3 |
| | *Kryptoperidinium foliaceum* | 3 |
| | Gonyaulacales | 2 |
| | Gymnodiniales | 2 |
| | *Alexandrium ostenfeldii* | 0 |
| 20 | *Biecheleria baltica* | 3 |
| | *Woloszynskia halophila* | 3 |
| | *Scripsiella hangoei* | 2 |
| | *Durinskia baltica* | 1 |
| | *Glenodinium paululum* | 1 |
| | *Gymnodinium* sp. | 1 |

## 6.2. Phytoplankton counting test

All 34 participants took part the counting test. Most participants carried out the counting test according to the EN 15204 (2006) as requested in the test guidance. Altogether 21 of the participants counted objects on the lower and right hand side edges, as presented in the standard example on page 14 (see Fig. 7 and Tables 7-9). Other acceptable combinations were used by 7 participants. However, a total of 6 participants were not aware of a proper counting procedure. Individual results were compared to robust mean value from which the outliers were removed according to Hampel test (Table 6). In all, 30 participants performed all components of the counting test satisfactorily ( $|z\ score| < 2$), and only one participant failed to perform all the components ( $|z\ score| > 3$; Tables 7-9, Figs 15-17). Three of the participants failed to perform the filament and colony counts ( $|z\ score| > 3$). If the participant reported more than one set of counts (based on different edge combinations), only one set of counts per participant was included in the test.

Table 6. Parameters calculated from the counting test material. Robust mean value from which the outliers were removed was decided to be used as an assigned reference value (in bold). For comparison the count results (mean ± SD) of the expert panel are also presented.

| **Assigned reference value** | **Filament** | **Colony** | **Cell** |
|---|---|---|---|
| Median (all results) | 163 | 67 | 64 |
| Mean value (all results) | 160 | 70 | 63 |
| **Robust mean (no of outliers)** | **163 (3)** | **67 (3)** | **64 (1)** |
| Robust mean (all results) | 163 | 69 | 63 |
| Expert value ± SD | | | |
| Lower + right edges | 164 ± 0.8 | 69 ± 0.5 | 63 ± 0.5 |
| Upper + right edges | 149 ± 1.2 | 72 ± 0.8 | 60 ± 0.5 |
| Lower + left edges | 155 ± 1.2 | 72 ± 0.0 | 66 ± 0.5 |
| Upper + left edges | 142 ± 0.9 | 76 ± 0.8 | 63 ± 0.8 |

Table 7: Methods used and results (including N = number of participants, mean ± SD, median, minimum and maximum value) by the participans in the counting test of the filamentous cyanobacterium *Aphanizomenon* sp. from 25 video clips.

| Method | N | Mean ± SD | Median | Min | Max |
|---|---|---|---|---|---|
| Lower + right | 21 | 162 ± 7.5 | 15 | 138 | 170 |
| Upper + right | 3 | 150 ± 1.7 | 151 | 148 | 152 |
| Lower + left | 1 | 158 | | | |
| Upper + left | 3 | 151 ± 12.6 | 144 | 141 | 169 |
| Upper + lower | 1 | 147 | | | |
| More than half inside | 1 | 156 | | | |
| Partial filaments added | 1 | 163 | | | |
| Entirely inside | 1 | 113 | | | |
| All counted | 2 | 197 | | 196 | 197 |

Table 8: Methods used and results (including N = number of participants, mean ± SD, median, minimum and maximum value) by the participans in the counting test of the colony forming cyanobacterium *Woronichinia naegeliana* from 25 video clips.

| Method | N | Mean ± SD | Median | Min | Max |
|---|---|---|---|---|---|
| Lower + right | 21 | 65 ± 1.9 | 66 | 62 | 69 |
| Upper + right | 3 | 83 ± 20.1 | 70 | 67 | 111 |
| Lower + left | 1 | 69 | | | |
| Upper + left | 3 | 71 ± 2.4 | 73 | 68 | 73 |
| Upper + lower | 1 | 126 | | | |
| More than half inside | 2 | 74 | | 66 | 81 |
| Entirely inside | 1 | 38 | | | |
| All counted | 2 | 79 | | 77 | 81 |

Table 9: Methods used and results (including N = number of participants, mean ± SD, median, minimum and maximum value) by the participans in the counting test of the single-celled dinoflagellate cysts and mature cells from 25 video clips.

| Method | N | Mean ±SD | Median | Min | Max |
|---|---|---|---|---|---|
| Lower + right | 21 | 63 ± 1.9 | 63 | 59 | 66 |
| Upper + right | 3 | 64 ± 1.7 | 63 | 62 | 64 |
| Lower + left | 1 | 68 | | | |
| Upper + left | 3 | 66 ± 1.2 | 66 | 64 | 67 |
| Upper+lower | 1 | 65 | | | |
| More than half inside | 2 | 64 | | 61 | 66 |
| Entirely inside | 1 | 38 | | | |
| All counted | 2 | 69 | | 69 | 69 |

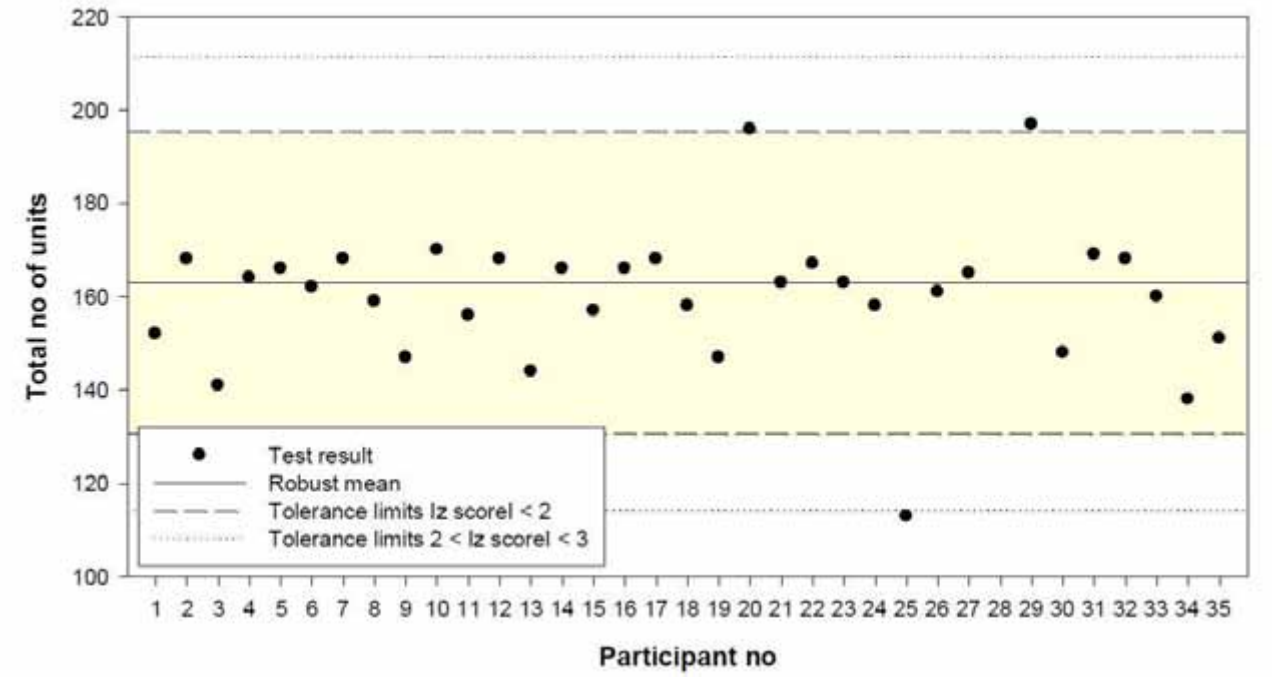


Figure 15. Evaluation of results of each participant of the counting test for the filamentous cyanobacterium *Aphanizomenon* sp. from 25 video clips. $|z\ score| < 2$ = satisfactory (area in yellow), $2 < |z\ score| < 3$ = questionable and $|z\ score| > 3$ = unsatisfactory.

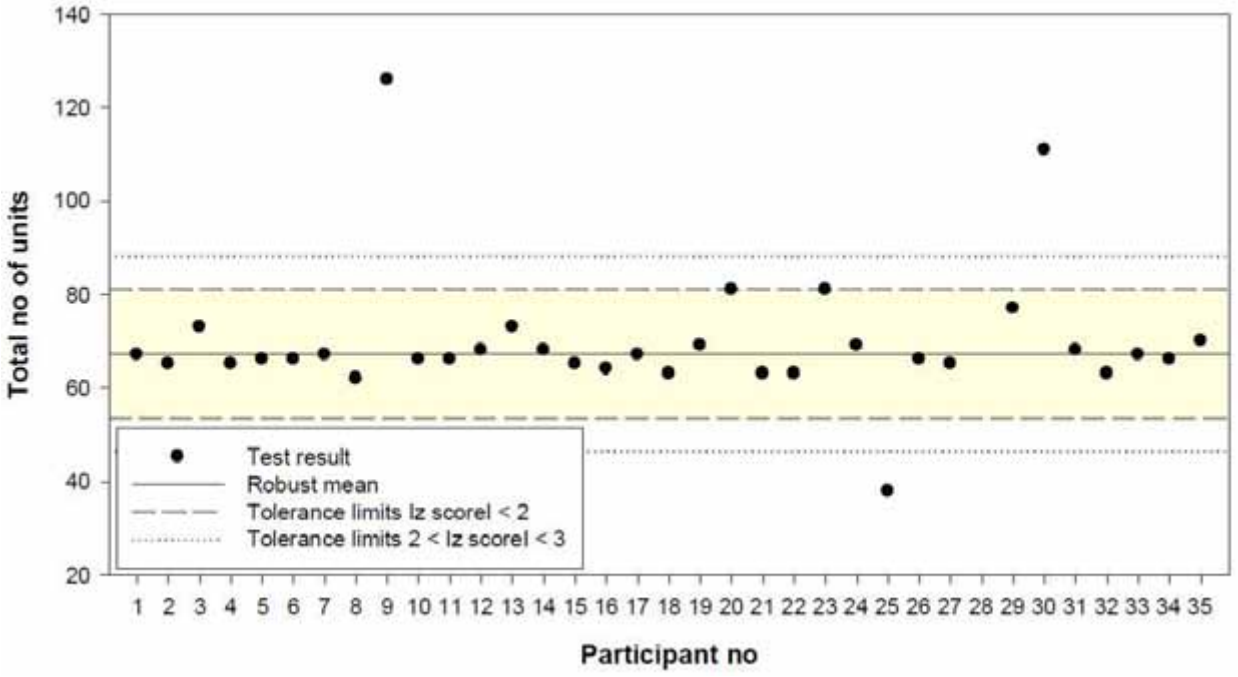


Figure 16. Evaluation of results of the participants of the counting test for the colony forming cyanobacterium *Woronichinia naegeliana* from 25 video clips. $|z\ score| < 2$ = satisfactory (area in yellow), $2 < |z\ score| < 3$ = questionable and $|z\ score| > 3$ = unsatisfactory.

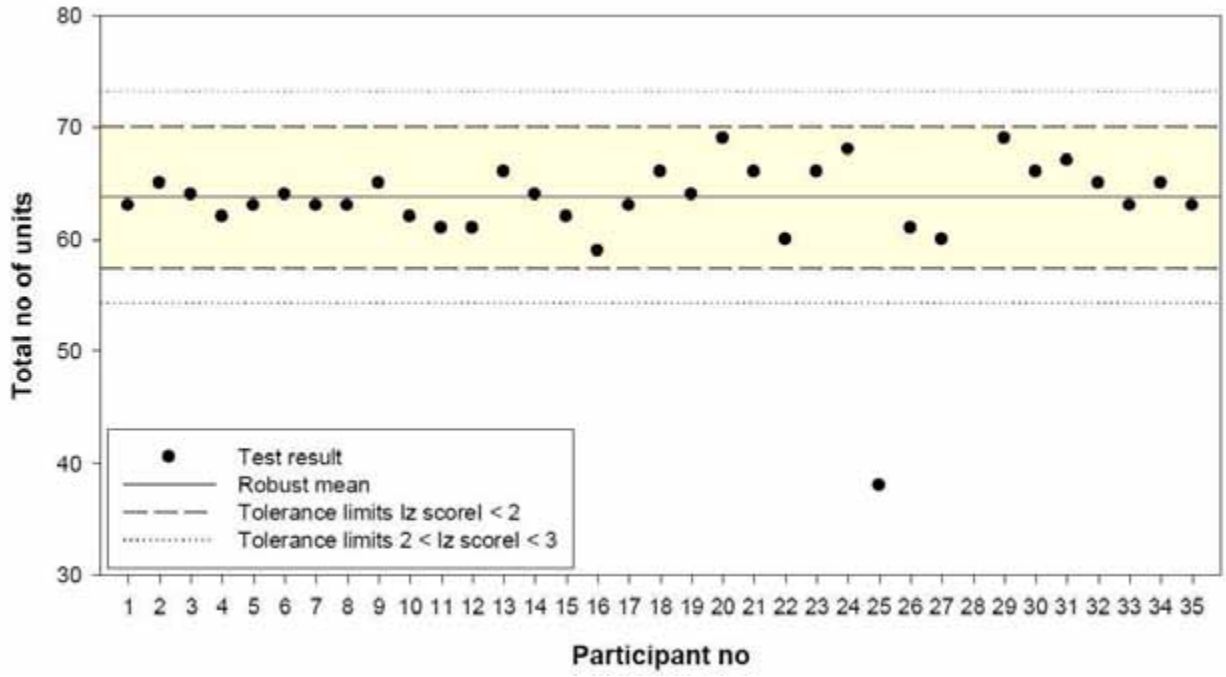


Figure 17. Evaluation of results of the participants of the counting test for the single-celled dinoflagellate cysts from 25 video clips. $|z\ score| < 2$ = satisfactory (area in yellow), $2 < |z\ score| < 3$ = questionable and $|z\ score| > 3$ = unsatisfactory.

### 6.3. Biovolume estimation test

All 34 participants measured the requested cell dimensions. Most participants used a calibrated ocular micrometer in measurements. However, 10 participants made the measurements using an image analyser programme. Individual results were compared to the robust mean value (= assigned reference value) from which the outliers were removed according to Hampel test. The results were evaluated using z-scores. In all, 31 of the participants performed all measurements satisfactorily. Only one participant failed to perform measurements of all three taxa (Figs 18-20). If more measurements were made than requested, these measurements were not included in the test. Phase contrast illumination was used by 22 participants, 4 used bright field illumination and 4 both phase contrast and bright field illumination, 2 participants used differential interference contrast illumination and 2 participants did not give the information. Magnifications used for the measurements of the filament and cell diameters varied from 400x to 1260 x and dimensions of the dinoflagellate cyst were measured using magnifications from 200x to 1260x. The ocular micrometer scales for the filament and cell diameter measurements ranged from 0.82 to 3.4 µm and for the dinoflagellate cyst height and cyst width measurements the ocular micrometer scales ranged from 0.82 to 5 µm.

Table 10. Calculated parameters from the measurement test material. Robust mean value from which the outliers were removed was decided to be used as an assigned reference value (in bold). For comparison the count results of the expert panel are also presented. Aphanizz = *Aphanizomenon* sp., Micr wes = *Microcystis wesenbergii* and Hete tri = *Heterocapsa triquetra*, d = diameter, h = height and w = width.

| **Assigned reference value** | **Aphanizz filament d** | **Micr wes cell d** | **Hete tri cell h** | **Hete tri cell w** |
|---|---|---|---|---|
| Median (all results) | 3.0 | 5.0 | 28.0 | 18.9 |
| Mean value (all results) | 3.1 | 5.0 | 28.4 | 19.1 |
| **Robust mean (no of outliers)** | **3.0 (2)** | **5.0 (1)** | **28.1 (1)** | **18.9 (1)** |
| Robust mean (all results) | 3.2 | 5.1 | 28.5 | 19.3 |
| Expert value ± SD, n = 3 (lower + right edges) | 3.2 ± 0.3 | 4.9 ± 0.5 | 27.7 ± 3.4 | 18.8 ± 2.0 |

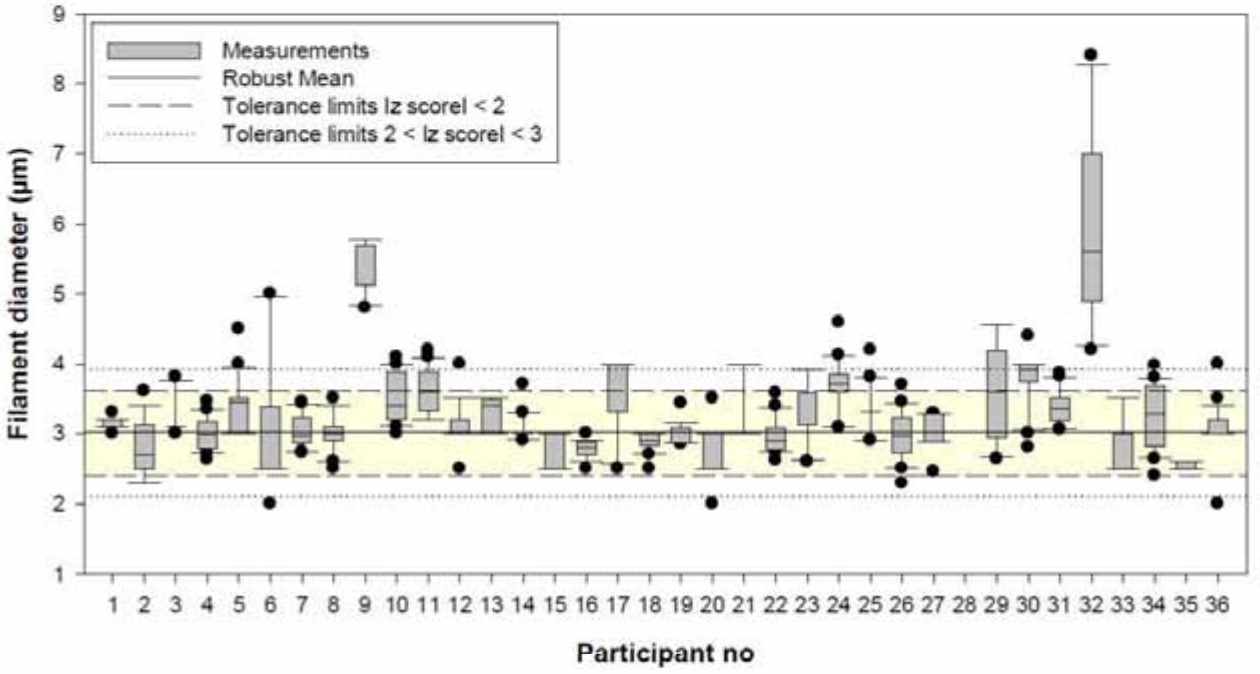


Figure 18. Box plot presentation (including median value, box boundaries = 25th and 75th percentile, error bars = 10th and 90th percentiles and ●= outlying points) of the measurement results for the diameter of the cyanobacterium *Aphanizomenon* sp. Participant no 36 = Expert reference measurements (n=40, two experts, measurements performed using ocular micrometer, magnifications 788x and 1000x with ocular micrometer scales 1.6 and 1.8). $|z\ score| < 2$ = satisfactory (area in yellow), $2 < |z\ score| < 3$ = questionable and $|z\ score| > 3$ = unsatisfactory.

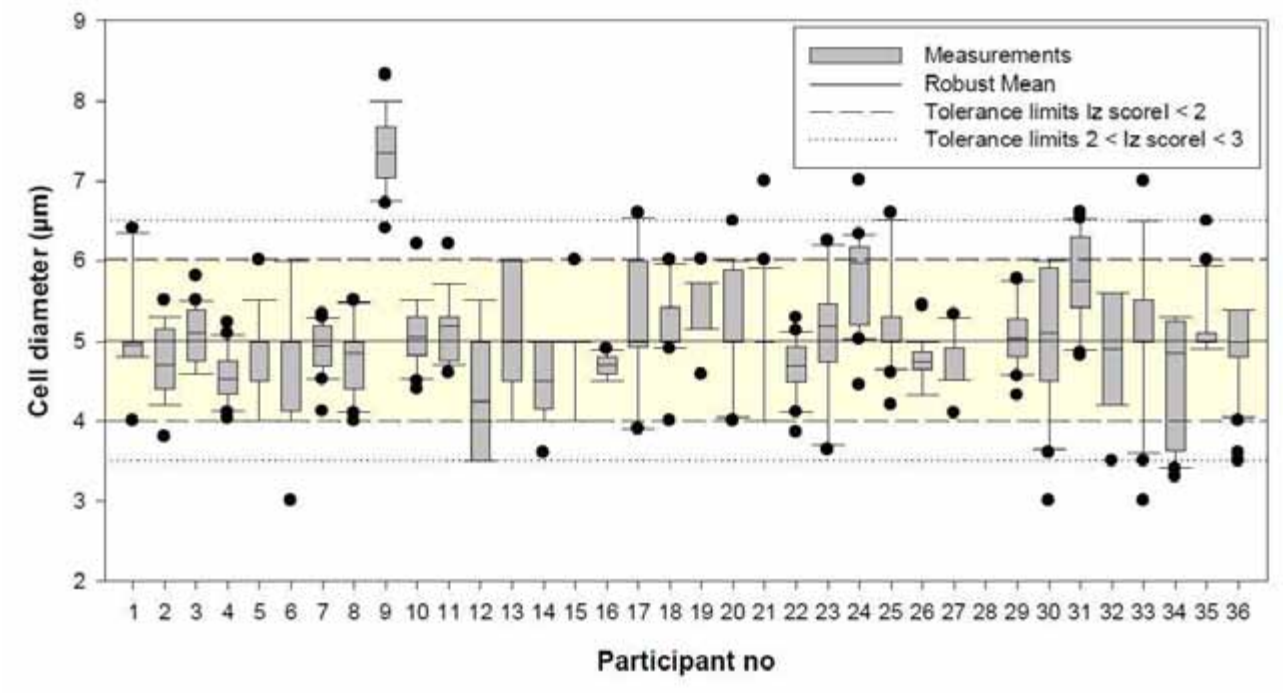


Figure 19. Box plot presentation (including median value, box boundaries = 25th and 75th percentile, error bars = 10th and 90th percentiles and ●= outlying points) of the measurement results for the diameter of the cyanobacterium *Microcystis wesenbergii*. Participant no 36 = Expert reference measurements (n=40, two experts, measurements performed using ocular micrometer, magnifications 788x and 1000x with ocular micrometer scales 1.6 and 1.8). $|z\ score| < 2$ = satisfactory (area in yellow), $2 < |z\ score| < 3$ = questionable and $|z\ score| > 3$ = unsatisfactory.

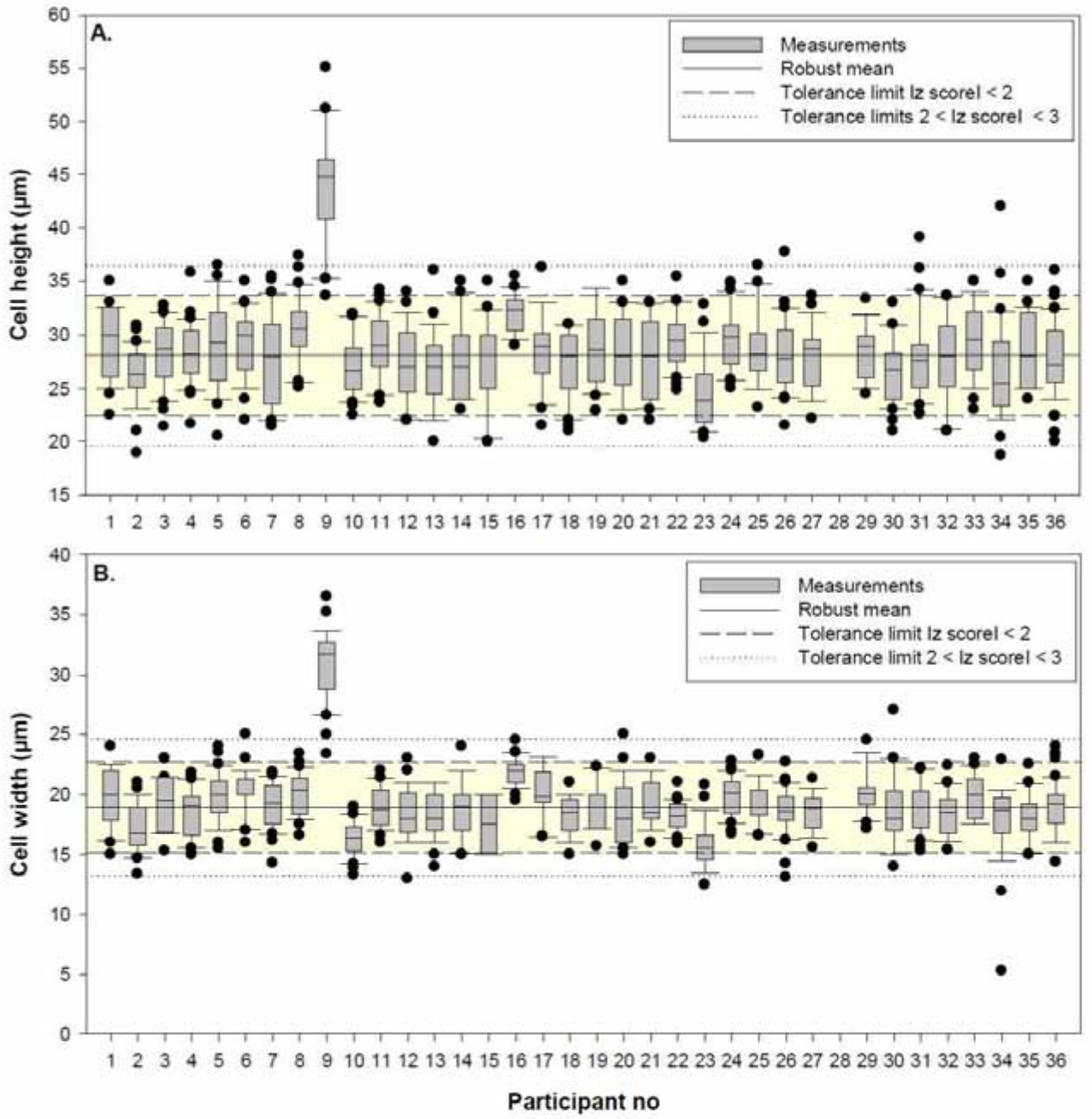


Figure 20. Box plot presentation (including median value, box boundaries = 25th and 75th percentile, error bars = 10th and 90th percentiles and ●= outlying points) of the measurement results of the diameter of the dinoflagellate *Heterocapsa triquetra*: A) height and B) width. Participant no 36 = Expert reference measurements (n=40, two experts, measurements performed using ocular micrometer, magnifications 788x and 1000x with ocular micrometer scales 1.6 and 1.8). $|z\ score| < 2$ = satisfactory (area in yellow), $2 < |z\ score| < 3$ = questionable and $|z\ score| > 3$ = unsatisfactory.

In addition to the biovolume measurement test, participants were asked to give the preferred shapes and equations for the biovolume determinations (Table 10). This part of the test was not evaluated, nor included in the test diploma. We asked this information to get an overview of the equations used for the biovolume calculations of each taxon in the absence of accepted standard for the phytoplankton biovolume determinations. For both *Aphanizomenon* and *Microcystis*, two geometric shapes and equations were suggested. For *Heterocapsa* four different geometric shapes and five different equations were suggested. The draft proposal CEN TC230 WG2 TG3: Phytoplankton biovolume determination (in preparation) and the Olenina et al. (2006) suggest the following geometric shapes and equations: cylinder (= circle based cylinder) $V = \pi * d^2 * h/4$ for *Aphanizomenon*, sphere $V = \pi * d^3/6$ for *Microcystis*. The CEN draft proposal suggests three different possibilities for *Heterocapsa*: two cones (= double

cone) $V = \pi*d^2*h/12$, cone + half sphere $V = (\pi*d^2/12)*(h+d/2)$ and rotational ellipsoid (= oval cylinder, cylinder on elliptic base, oval based cylinder) $V = \pi*d^3*h/6$, whereas in the Olenina et al. (2006) only the shape of the double cone is used.

Table 10. Given suggestions for preferred geometric shapes and equations for each taxon measured in the biovolume measurement component.

| Taxon | Geometric shape | Equation | n |
|---|---|---|---|
| *Aphanizomenon* (cell dimensions) | Cylinder | $V= \pi*d^2*h/4$ | 32 |
| | Rotational ellipsoid | $V= \pi*d^2*h/6$ | 2 |
| *Microcystis* (cell dimensions) | Sphere | $V= \pi*d^3/6$ | 33 |
| | Rotational ellipsoid | $V= \pi*d^2*h/6$ | 1 |
| *Heterocapsa triquetra* | Double cone | $V= \pi*d^2*h/12$ | 10 |
| | Rotational ellipsoid | $V= \pi*d^2*h/6$ | 9 |
| | Flattened ellipsoid | $V= \pi*d_1*d_2*h/6$ | 9 |
| | Cone + half sphere | $V= \pi*d^2*h/12$ | 5 |
| | Cone + half sphere | $V= \pi*(d^2/12)*(d/2+1)$ | 1 |

## 7. EVALUATION OF PERFORMANCE AND DISCUSSION

Phytoplankton analysis results are used for example for the assessment of the ecological status of water bodies. Therefore, phytoplankton analyses require effective quality control procedures for assuring the validity of analysis results. A widely accepted way to monitor validity is to take part in proficiency testing schemes. The primary aim of the SYKE 7/2009 phytoplankton proficiency test was to help individual laboratories and institutes to monitor the reliability of their analyses and take remedial measures where necessary to improve the quality of results. In the phytoplankton analysis the expertise of the analyst has a major importance. Therefore the test was carried out at an individual level, and the diploma also includes the name of the analyst who participated in the test.

Traditional phytoplankton proficiency tests with natural samples typically include several sources of error. The first source of error may arise from the unhomogenous material delivered to participants. Secondly, additional errors may arise from the sample preparation, e.g. from an inadequate homogenizing of samples and uneven settling. Virtual testing is an excellent method to minimise these errors and to produce as identical and homogenous material as possible, especially for the identification and counting tests.

The phytoplankton identification tests proved more difficult than expected. Altogether 80% of the participants in the lake phytoplankton identification test reached the quality target of 75%. The corresponding percentage in the Baltic Sea phytoplankton identification test was 67%. One reason for the high number of unsatisfactory results may be that some participants normally work with phytoplankton dissimilar to the taxa presented in the test. It is also possible that some participants were not familiar with or could not use the most recent identification literature. The taxa that proved most difficult to identify were *Koliella longiseta*, *Aulacoseira subarctica* and *Snowella septentrionalis* in the lake phytoplankton test, and the two *Gonyaulax* species and *Fragilariopsis cylindrus* in the Baltic Sea identification test. These species are all common representatives of the northern waters.

The success in the counting test was good and 91% of the participants performed all parts of the counting test satisfactorily. Detailed guidance on how to perform the counting test was not given, but participants were asked to follow the EN 15204 (2006) standard. The reason for this was that we wanted to screen how many of the participants follow the standard counting rules. Only those participants who did not follow the instructions given in the standard failed in the statistical test to perform the counting test satisfactorily. Filament counts of the cyanobacterium *Aphanizomenon* involved high variation. The main

reason for the high variation was the different combinations of the perpendicular edges taken into account, especially because many of the *Aphanizomenon* filaments were crossing the edges. The variation in the results was lower in the dinoflagellate counts because most cells did not cross the edges. The presence of subcolonies in the colony forming cyanobacterium *Woronichinia naegeliana* increased variation in the colony counts. It seems that some participants did not follow the guidance not to take possible subcolonies into account.

Altogether 88% of the participants performed all parts of the measurement test successfully. Only one participant failed to perform all three parts. Participants who used ocular micrometer and image analyser programme performed equally well in this part of the test. Errors in the measurements may arise e.g. from an incorrect calibration of ocular micrometers. The choice of formula was screened, because, in addition to the measurements of dimensions, the differences in the biovolume estimations may arise from the choice of the geometric shape. This emphasises the current need for a commonly accepted standard for biovolume determinations.

The overall success in the phytoplankton proficiency test demonstrated excellent phytoplankton identification skills by a large number of participants. A majority of the participants was also able to perform phytoplankton counts and measurements satisfactorily. The results of the proficiency test highlighted the importance to follow the CEN guidance in the quantitative phytoplankton analysis. Individual analysts benefit from participating external quality assurance to maintain the quality and further improve and harmonise the reliability of the phytoplankton analysis results.

The percentage (78%) of participants who reached the good quality target in the current lake phytoplankton identification test was similar to that (80%) of the first test SYKE 11/2006 (Vuorio et al. 2007a). However, in the current Baltic Sea phytoplankton identification test the percentage (67%) of participants with a satisfactory performance was lower than in the first virtual phytoplankton proficiency test (90%). This is most likely due to the smaller number of identifiable taxa (10) in the first Baltic Sea phytoplankton identification test.

## 8. COMMENTS SENT BY THE PARTICIPANTS

No comments concerning the preliminary test results were received by the deadline of January 2010. However, after the test material delivery on March 2009, a few questions and comments concerning the execution of the test were received. The comments did not deal the phytoplankton identification, albeit one participant considered the number of 20 taxa in the identification test to be too low.

A few participants were not familiar with the EN 15204 (2006) standard and asked for more detailed guidance on how to execute the counting part of the test. In the replies, the test organiser referred to the guidance presented on the Excel spreadsheet, but the standard or the figure (Fig. 7) showing the recommended rule were not delivered to participants. Another question concerned the counting of subcolonies of the colony forming cyanobacterium *Woronichinia naegeliana*, when the two colonies were clearly separate but had a common mucilage. The counting procedure was left for the participant to decide, because in this test material it had no effect on the test result. The question on how to count the intensely vacuolized terminal cells of the filamentous cyanobacterium *Aphanizomenon* visible at the edges of the counting field, was also left for the participant to decide. We did not expect the participant to count single cells on the edges of the view as filaments, because in such cases it was not possible to distinguish whether it was a single cell or the end cell of a filament. These cells were not many and the decision did not affect the individual evaluation of this component either.

One of the participants suggested that more detailed descriptions of the quality of the microscopes used should have been included as well as information about the literature used in the identifications.

We agree that a listing of the used identification literature would help to study and explain the outcome of the identification test results. This reporting activity is considered to be added in the next SYKE phytoplankton proficiency test. On the whole, the comments concerning the test were positive and no reclamations of damaged material were received.

## 9. SUMMARY

The Finnish Environment Institute (SYKE) organised the second virtual proficiency test of SYKE based on filmed material. A total of 34 analysts from 23 organisations and 8 countries took part the test. The test material represented phytoplankton that typically occurs in freshwaters in the Northern Europe and in the Baltic Sea.

The test integrated three components: 1) phytoplankton species identification, 2) phytoplankton counting and 3) the measurement of cell dimensions. The lake phytoplankton identification test consisted of 20 video-clips of 21 taxa and the Baltic Sea phytoplankton identification test consisted of 20 video-clips of 22 taxa. For the phytoplankton counting test 25 video-clips representing 25 fields of view in a microscope were filmed. In the measurement test dimensions of three selected taxa were asked to be measured from a Lugol preserved composite sample.

In the lake phytoplankton identification test altogether 80% of the participants reached the good quality target of 75%. The corresponding percentage in the Baltic Sea phytoplankton identification test was 67%. The success in the counting test was good and 91% of the participants performed all three parts of the counting test satisfactorily. Altogether 88% of the participants performed all three parts of the measurement test successfully.

The majority of the participants demonstrated excellent phytoplankton identification skills and were also able to perform phytoplankton counts and measurements satisfactorily. The results of the proficiency test highlighted the importance to follow the CEN guidance in the quantitative phytoplankton analysis and emphasises the current need for commonly accepted standard for biovolume determinations.

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Vuorio, K., Lepistö, L. and Holopainen, A.-L. (2007b) Intercalibrations of freshwater phytoplankton analyses. Boreal Environment Research 12: 561-569.

# PARTICIPANTS IN THE PROFICIENCY TEST SYKE 7/2009

| Organisation | Country |
|---|---|
| GRONTMIJ/Carl Bro<br>Trine Warming Perlt | Denmark |
| Orbicon/Leif Hansen<br>Jacob Peter Jacobsen, Bettina Jensen | Denmark |
| Estonian Marine Institute<br>Kaire Toming | Estonia |
| Estonian University of Life Sciences, Institute of Acrigultural and Environmental Sciences, Centre for Limnology<br>Aimar Rakko | Estonia |
| Company Zwerver<br>Satu Zwerver | Finland |
| City of Helsinki Environment Centre<br>Marjut Räsänen | Finland |
| Private consultant<br>Sanna Kankainen | Finland |
| Pöyry Environment Ltd<br>Eeva-Leena Anttila | Finland |
| Finnish Environment Institute (SYKE)<br>Heidi Hällfors, Sirpa Lehtinen, Päivi Sillman, Minna Ylä-Jarkko | Finland |
| University of Helsinki, Lammi biological station<br>Elina Peltomaa | Finland |
| University of Jyväskylä, Institute for Environmental Research<br>Arja Palomäki | Finland |
| Water and Environment Research of south-West finland<br>Sanna Autio, Hanna Turkki | Finland |
| Ecomonitor Ltd<br>Raino-Lars Albert | Finland |
| Latvian Istitute of Aquatic Ecology, Marine Monitoring Center<br>Iveta Jürgensone | Latvia |
| Norwegian Institute for Water Research<br>Birger Skjelbred, Camilla H.C. Hagman | Norway |
| Laboratorul Regional de Calitatea Apei<br>Levente Nagy | Romania |
| National Administration "Romania Waters", Prut River Brancs-Water quality Lab Iasi<br>Stefan Miron | Romania |
| SMHI, Oceanographic Unit<br>Ann-Turi Skjevik | Sweden |
| SLU, Insitution för vatten och miljö<br>Eva Herlitz | Sweden |
| WEAB Ab<br>Lars Edler | Sweden |
| Medins Biology AB<br>Ingrid Hårding, Annika Liungman, Carin Nilson, Irene Sundberg, Jan-Erik Svensson | Sweden |
| Beacon Biological<br>Sarah Pritchard | United Kingdom |
| Ecology lab, East Kilbride, Scottish Environment Protection Agency<br>Pauline Lang | United Kingdom |
| ENSIS Ltd<br>Gina Henderson | United Kingdom |

# Documentation page

| | | |
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| Author(s) | Kristiina Vuorio, Maija Huttunen, Seija Hällfors, Reija Jokipii, Marko Järvinen, Mirja Leivuori, Maija Niemelä and Markku Ilmakunnas | |
| Title of publication | SYKE Proficiency Test 7/2009<br>Phytoplankton | |
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| Abstract | The Finnish Environment Institute (SYKE) organised in 2009 the second virtual proficiency test based on filmed material. A total of 34 analysts from 23 organisations and eight countries took part the test. The test material represented phytoplankton that typically occurs in freshwaters in the Northern Europe and in the Baltic Sea.<br><br>The test included three components: 1) phytoplankton species identification, 2) phytoplankton counting and 3) the measurement of cell dimensions. The lake phytoplankton identification test consisted of 20 video-clips with 21 taxa and the Baltic Sea phytoplankton identification test consisted of 20 video-clips with 22 taxa. For the phytoplankton counting test 25 video-clips representing 25 fields of view in a microscope were filmed. In the measurement test the dimensions (diameter, width and/or height) of three selected taxa were asked to be measured from a Lugol preserved composite sample.<br><br>In the lake phytoplankton identification test altogether 80% of the participants reached the good quality target of 75% of the maximum score. The corresponding percentage in the Baltic Sea phytoplankton identification test was 67 %. The success in the counting test was good and 91% of the participants performed all three parts of the counting test successfully. Altogether 88% of the participants performed all three parts of the measurement test successfully.<br><br>Majority of the participants demonstrated excellent phytoplankton identification skills and were also able to perform phytoplankton counts and measurements successfully. The results of the proficiency test highlighted the importance to follow the CEN guidance in the quantitative phytoplankton analysis and also emphasises the current need for a new standard for the biovolume determinations. | |
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| Tekijä(t) | Kristiina Vuorio, Maija Huttunen, Seija Hällfors, Reija Jokipii, Marko Järvinen, Mirja Leivuori, Maija Niemelä ja Markku Ilmakunnas | |
| Julkaisun nimi | SYKE Vertailukoe 7/2009<br>Kasviplankton | |
| Julkaisun osat/ muut saman projektin tuottamat julkaisut | Julkaisu on saatavana vain internetistä.<br>www.ymparisto.fi/julkaisut | |
| Tiivistelmä | Suomen ympäristökeskus (SYKE) järjesti vuonna 2009 järjestyksessään toisen kasviplanktonin vertailukokeen, joka perustui virtuaaliseen materiaaliin. Yhteensä 34 kasviplanktonlaskijaa 23 eri organisaatiosta ja kahdeksasta eri maasta osallistui testiin. Testimateriaali edusti tyypillistä pohjoiseurooppalaista makeitten vesien ja Itämeren kasviplanktonia.<br><br>Testi koostui kolmesta komponentista: 1) kasviplanktonin lajintunnistus, 2) kasviplanktonin laskenta ja 3) kasviplanktonsolujen dimensioiden mittaus. Järvikasviplanktonin lajintunnistusosiota varten filmattiin 20 videota, joissa esiintyi 21 tunnistettavaa taksonia. Itämeren kasviplanktonin tunnistusta varten kuvattiin niin ikään 20 videota, joissa esiintyi yhteensä 22 eri taksonia. Laskentatestiä varten kuvattiin 25 otosta, jotka esittivät 25 näkymää mikroskoopissa. Soludimensioiden mittausta varten osallistujille toimitettiin Lugolin liuoksella säilötty näyte, josta tuli mitata kolmen eri taksonin solujen halkaisija, leveys ja/tai pituus.<br><br>Järvikasviplanktonin lajintunnistustestissä 80 % osallistujista saavutti tavoitetason 75 % maksimipistemäärästä. Vastaava prosenttiluku Itämeren lajintunnistusosiossa oli 67 %. Osallistujista 91 % suoritti kaikki kolme laskentatestin osiota hyväksyttävästi. Soludimensioiden mittaustestissä 88 % osallistujista menestyi hyväksyttävästi kaikkien kolmen taksonin mittauksissa.<br><br>Suurin osa testiin osallistuneista suoriutui kaikista testin komponenteista hyvin. Menestyminen laskentatestissä edellytti hyväksytyn EN-15204 standardin noudattamista. Eurooppalaisen biomassastandardin puuttumisen takia mittausosiossa ehdotettujen solutilavuuksien määrittämiseen ehdotettiin useita eri geometrisia kaavoja. | |
| Asiasanat | vertailukoe, kasviplankton, järvet, Itämeri, lajintunnistus, laskenta, biovolyymin mittaus | |
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# Presentationsblad

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| Publikationens titel | SYKE Provningsjämförelse 7/2009<br>Växtplankton | |
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| Sammandrag | Finlands Miljöcentral (SYKE) genomförde den andra växtplankton provningsjämförelsen baserade sig på virtuell filmad material. Sammanlagt 34 experter från 23 organisationer och åtta europeiska länder deltog i provningsjämförelsen. Testmaterialet bestod av typiskt växtplankton i nordeuropeiska sötvatten och Östersjön.<br><br>Jämförelsen bestod av tre olika komponenter: 1) växtplankton identifieringstest, 2) växtplankton räkningstest och 3) mätningstest av cell dimensioner. För identifiering av sötvattentaxa filmades 20 videotagningar med 21 olika taxa och för identifiering av brackvattentaxa filmades 22 olika taxa. För det tekniska räkningstestet filmades 25 videotagningar av ett prov som innehöll tre utvalda taxa. För mätningstestet (celldiameter, -bredd och/eller -höjd) ett Lugol inlagd prov som innehöll tre utvalda taxa skickades för deltagarna.<br><br>I identifieringstestet av sötvattentaxa 80 % av deltagarna nådde bra kvalitet nivå 75 % av den maximala poängsumman. Motsvarande siffran i identifieringstestet av brackvattentaxa var 67 %. Framgången i räkningstestet var bra och 91 % av deltagarna genomförde räkningstestet godtagbart. Sammanlagt 88 % av deltagarna genomförde mätningstestet godtagbart.<br><br>Majoriteten av deltagarna visade utmärkta identifieringskunskaper och klarade sig utmärkt i alla tre komponenter av testet. Resultatet av provningsjämförelsen betonade betydelsen att överensstämma enligt accepterade CEN standarder. Bristfälligheten av accepterade CEN standarden för bestämningen av biovolymer syntes i mängden av föreslag av geometriska formulär. | |
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