



# GOF2014 INTERCALIBRATION EXERCISE

Report of the physical-chemical-biological field intercalibration on sampling and analytical procedures in the frames of the Gulf of Finland year 2014

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## INTRODUCTION

Estonia, Finland and Russia carried out an international field oceanographic intercalibration targeting to clarify the possible differences in the analytical results of the laboratories of the representative monitoring authorities in the countries. The participating institutes and contacting persons were

- Estonian Marine Institute (EMI) / Andres Jaanus, EST
- Finnish Environment Institute (SYKE) / Mika Raateoja, FIN
- Marine Systems Institute (MSI) / Inga Lips, EST
- North-West Interregional Territorial Administration for Hydrometeorology and Environmental Monitoring (Hydromet) / Tatjana Zagrebina, RUS
- Russian State Hydrometeorological University (RSHU) / Tatjana Eremina, RUS
- St. Petersburg State University (SPbSU) / Alexander Antsulevich, RUS

### 1<sup>ST</sup> FIELD INTERCALIBRATION ON THE 28<sup>TH</sup> OF AUGUST, 2013

Location: station LL3A, 60°04.03' N 26°20.80' E, nominal depth 68 m

Participating institutes: SYKE (FIN), Hydromet (RUS), RSHU (RUS) and SPbSU (RUS) on board R/V Aranda

Sampling depths: 1, 32, 60 m

The design of this intercalibration – samples taken and divided by SYKE and analysed by Hydromet, RSHU, SPbSU and SYKE – targets to clarify the differences in the analytical results caused by various analytical procedures of the participating institutes. The tested parameters were nutrients ( $\text{PO}_4^{3-}$ , TOTP,  $\text{NO}_2^-$ ,  $\text{NO}_2^- + \text{NO}_3^-$ , TOTN,  $\text{SiO}_4^-$ ), Chlorophyll *a* and phytoplankton biomass and composition.

It is assumed that the sub-samples were homogeneous and that their handling and storing did not have an effect on the results. However, freezing and melting the water samples, even though carefully instructed, causes some unpredicted changes to the dissolved and total nutrient concentrations (for example, to the fine particulate organic fraction).

### 2<sup>ND</sup> INTERCALIBRATION ON THE 19<sup>TH</sup> OF SEPTEMBER, 2013

Location: station LL7, 59°50.79'N 24°50.27'E, nominal depth 100 m

Participating institutes: MSI and EMI (EST) on board R/V Salme and SYKE (FIN) on board R/V Aranda. MSI, EMI (EST) and SYKE (FIN) received samples from both of the vessels. Hydromet (RUS) and SPbSU (RUS) received zooplankton samples from R/V Aranda.

Sample depths: 1, 28 and 80 m

The design of this intercalibration – samples taken either by MSI or SYKE and analysed by EMI, MSI or SYKE – target to clarify the differences in the analytical results caused by i) the 100-m distance between the sampling sites. This was done by comparing the two determinations made by SYKE from its own sample taken on board R/V Aranda and from MSI's sample taken on board R/V Salme and vice versa, ii) various analytical procedures of the participating institutes.

The melting procedure of the frozen samples was carefully instructed. However, the difference between the results also includes the changes caused by differences in sample storing and treatment prior to analysis. The parameters to test were nutrients ( $\text{PO}_4^{3-}$ , TOTP,  $\text{NO}_2^-$ ,  $\text{NO}_2^- + \text{NO}_3^-$ , TOTN,  $\text{SiO}_4^-$ ), Chlorophyll *a*, phytoplankton and zooplankton biomass and composition. Some parameters ( $\text{NH}_4^+$ ,  $\text{O}_2$ , pH) are sensitive to the gas exchange process and thus those parameters were analysed onboard.

Again, it is assumed that the sub-samples were homogeneous (not probably so with zooplankton samples; each were taken with a separate net cast) and that their handling and storing did not have an effect on the results. However, freezing and melting the water samples, even though carefully-instructed, causes

some unpredicted changes to the dissolved and total nutrient concentrations (for example, to the fine particulate organic fraction).

For phytoplankton, we also tested the difference in the two ways of taking the water samples: pooling equal amounts of water from the depths of 1, 2.5, 5, 7.5 and 10 m or 1, 5, and 10 m.

Additionally, CTD systems of the vessels were compared using the parameters temperature, salinity, in vivo Chlorophyll *a* fluorescence and O<sub>2</sub>.

## **PART 1: HYDROGRAPHICAL AND CHEMICAL PARAMETERS**

### **GENERAL COMMENTS**

The shortcoming in the intercalibration of nutrient determinations was the lack of commercial reference materials. By analyzing the same reference materials in all laboratories we could have estimated how close to the true concentration each laboratory will get with their analytical methods. This would have helped in evaluating what causes the differences observed in the results. This, however, will be done in the Feb, 2014 by SYKE ProfTest proficiency testing services.

In the lack of reference materials as standpoints for comparison, the best option available for the comparison of the results was to use an average value of each parameter as the “true concentration”, and compare the average results of each of the laboratories to that value. This average concentration of all laboratories, the “true concentration”, was an average of all institutes’ average values.

Deviation of each laboratory’s nutrient results from the “true concentration” can be put into perspective by having information of laboratory’s measurement uncertainty. At this point, however, this information was not received from all the laboratories and hence this comparison was not made. Furthermore, it was also unclear whether each laboratory had used commercial reference materials in their own analysis, which methods were used, and what were the detection limits.

In addition, to evaluate the possible reasons and explanations for the observed deviations in the results and the differences among the laboratories, the impact of freezing and especially the melting is elemental. The melting process was carefully instructed, but still there are many aspects (duration, room temperature, water path if used in the melting process) introducing error to the estimations, especially to those parameters dependent on gas exchange and those separating dissolved and particulate phases of nutrients.

In general, the results from total nutrient determinations varied among laboratories more than did the determinations of dissolved nutrients. This may result from differences in sample treatment and storing practices and storing time before analysis. However, this information was not available in this stage of reporting, so their potential impacts on the results cannot be estimated. For example, it was unclear, whether the samples were pretreated (filtrated, centrifuged or settled) before determination of dissolved nutrients. Presence of fine particulate material in the samples (or subsamples) separated for determination of total concentration of nutrients may have also affected the total nitrogen and total phosphorus. In that case, it is important that the original bulk sample divided into subsamples was homogeneous and that subsamples in each laboratory were shaken or settled equally long times before separating the subsamples for analysis. Regarding the low nutrient concentrations, comparison of concentrations is challenged by the fact that different laboratories have different detection limits and those were not always informed.

Considering total phosphorus determinations in the September test, it is noteworthy that laboratories from EMI and MSI reported total concentrations of phosphorus that were lower than the  $\text{PO}_4^{3-}$  results from the same samples (in samples taken from 28 m and 80 m water depths). This same phenomenon has been identified with low TOTP and  $\text{PO}_4^{3-}$  values also elsewhere. The reason, why TOTP concentrations sometimes are smaller than concentration of  $\text{PO}_4^{3-}$ , is not clear.

## 1<sup>ST</sup> FIELD INTERCALIBRATION ON THE 28<sup>TH</sup> OF AUGUST, 2013

### Nitrogen

Comparison of various dissolved nitrogen species was challenging because of the variable practices in determining and reporting  $\text{NO}_3^-$  and  $\text{NO}_2^-$  concentrations. SYKE determined  $\text{NO}_2^-$  and the sum of  $\text{NO}_2^-$  and  $\text{NO}_3^-$ , from which  $\text{NO}_3^-$  concentration could be calculated. Other laboratories reported directly  $\text{NO}_3^-$ .

$\text{NO}_2^-$  results were difficult to compare because of the low ambient  $\text{NO}_2^-$  levels often leading to estimations at or below the detection limit. Hence, this part is not summarized here.

SPbSU's nitrogen ( $\text{NO}_3^-$  and TOTN) determinations were markedly lower than those provided by the other laboratories, except at 1 m for  $\text{NO}_3^-$ .

For  $\text{NO}_3^-$ , the differences between the other institutes were moderate at 32 and 60 m (2 to 22 % from the study average) with values increasing in the line RSHU < Hydromet < SYKE (Table 1). There was a marked deviation between the institutes at 1 m without any distinct pattern. This cannot be explained with a low concentration level as some of the laboratories reported pronounced values.

RSHU did not report TOTN as they do not measure it routinely. For TOTN, the difference between Hydromet and SYKE was moderate (5 to 10 % from the study average, i.e., 10 to 14 % from each other) with Hydromet having higher values than SYKE (Table 2).

### Phosphorus

RSHU's phosphorus ( $\text{PO}_4^{3-}$  and TOTP) determinations at 1 and 32 m were markedly higher than those provided by the other laboratories.

For  $\text{PO}_4^{3-}$ , the differences between the other institutes were moderate at 32 and 60 m (1 to 32 %) with values increasing in the line SPbSU < Hydromet < SYKE (< RSHU at 60 m, Table 3). At 1 m, there was much difference between the institutes. The concentration level, however, was quite low.

For TOTP, the differences between the other institutes were moderate (0 to 26 %) with values increasing in the line SPbSU < Hydromet < SYKE (< RSHU at 60 m, Table 4). At 1 m, there was much difference between the institutes, but again, the concentration level was low.

### Silica

For  $\text{SiO}_4^-$ , SPbSU's determinations at 1 m were markedly lower than those provided by the other laboratories. Otherwise, the difference between the institutes were moderate (3 to 22 %, Table 5) with no clear mutual order of the institutes.

### Chlorophyll *a*

For Chlorophyll *a*, the differences between the institutes were moderate (1 to 28 %, Table 6) with values increasing in the line RSHU < Hydromet < SYKE < SPbSU.

## 2<sup>ND</sup> INTERCALIBRATION ON THE 19<sup>TH</sup> OF SEPTEMBER, 2013

### Nitrogen

$\text{NO}_2^-$  results were difficult to compare because of the low ambient nitrite levels often leading to estimations at or below the detection limit. Hence, only the 80-m part was reported (Table 7), and in this water depth,  $\text{NO}_2^-$  was reported only by SYKE. At 80 m, the difference caused by the two sampling sites 100 m away from each other was small (8 %, Table X).

For  $\text{NO}_2^- + \text{NO}_3^-$ , the differences between the institutes were moderate (typically < 20 %) with EMI having clearly higher level at 1 m and lower level at 28 m (Table 8). The difference due to the two sampling sites

was small (1 to 2 %) in the highest concentration (at 28 m) and moderate otherwise (11 to 30 %, Table 17).

For TOTN, the difference between the institutes was small (typically < 10 %, Table 9). The difference due to the two sampling sites was small (0 to 7 %, Table 17).

### **Phosphorus**

EMI's  $\text{PO}_4^{3-}$  determinations, especially at 1 m, were markedly higher than those provided by the other laboratories (Table 10). Generally, MSI and SYKE had similar results while EMI had somewhat higher concentrations also at 28 and 80 m even though not as much higher than at 1 m. The difference due to the two sampling sites was small (1 to 4 % with one exception close to the detection limit, Table 17).

EMI's TOTP determinations at 1 and 28 m were markedly higher than those provided by the other laboratories (Table 11). At 1 m, also MSI and SYKE differed considerably from each other. The difference due to the two sampling sites was small (1 to 14 %, Table 17).

### **Silica**

For  $\text{SiO}_4^-$ , the differences between the institutes were moderate (typically < 20 %) with EMI having slightly lower values throughout the test (Table 12). The difference due to the two sampling sites was small (1 to 10 %) and moderate (17 to 38 %) at 80 m (Table 17).

### **Oxygen**

MSI's  $\text{O}_2$  determinations at 80 m were markedly higher than those provided by the other laboratories.

The difference between the institutes was small except at 80 m where the difference was high (Table 13). At 80 m however, the concentration level was very low.

### **pH**

For pH, the difference between the institutes was small (Table 14).

### **Ammonium**

For  $\text{NH}_4^+$ , the difference between the institutes was moderate (typically < 20 %, Table 15) except at 28 m where the difference was high).

### **Chlorophyll *a***

For Chlorophyll *a*, the difference between the institutes was small to moderate (typically < 20 %, Table 16) with SYKE having the lowest estimates. The difference due to the two sampling sites was moderate (1 to 24 %).

### **CTD-data**

Temperature and salinity profiles were much alike for EMI, MSI, and SYKE with EMI's device responding a little slower to the temperature anomalies (Figure 1). However, larger differences were observed in the deep density values. As these differences are apparently not based on temperature or salinity estimations they are probably linked to the ways of calculating water density.

Pronounced variation took place in the  $\text{O}_2$  profiles in terms of both the absolute level in the surface mixed layer and the response to the oxyclines. SYKE reported lower  $\text{O}_2$  level in the surface mixed layer than the other institutes but seemed to respond more properly to the variations in the  $\text{O}_2$  field.

*In vivo* Chl *a* fluorescence were much alike in the surface mixed layer but below that SYKE exhibited clearly higher baseline values.

## THE IMPACT OF THE SAMPLING PLACE

In general, the measurement error due to the 100 m distance between the two sampling places was small in this comparison and clearly lower than the uncertainty in analytical determination when all determinations are taken into account (Table 17). Larger percentual differences at 1 m were usually explained by low ambient concentration levels. The difference in  $\text{SiO}_4^-$  at 1m, however, can be regarded large. At 80 m,  $\text{SiO}_4^-$  and  $\text{NO}_2^- + \text{NO}_3^-$  levels had a large difference despite the substantial concentration. Chlorophyll *a* estimates varied markedly at 20 m, but that difference was most likely of natural origin; the main density gradient located at around that depth.

## RECOMMENDATIONS

In the case that the intercalibration will be repeated later on, some recommendations can be pointed out. Depending on the method and equipment used, nutrient analytics, in general, allow determination of very small concentrations and microbiological, biological and chemical processes occurring after sampling affect the concentrations. Thus, differences in sampling, sample storing and handling prior to analysis can be expected to affect greatly the results of nutrient determinations between different laboratories. To ensure comparability of the samples, it is important that the participating laboratories get very detailed instructions for sampling and storing (e.g., storing times and temperatures) and well as practices in preparing samples for analyses (for example, melting time of frozen samples and treatment of samples containing particulate material). Identical forms to help in documenting the detailed information about the sampling, sample storing and sample treatment prior to analyses could be helpful.

Furthermore, it would be very beneficial to include commercial reference samples with known nutrient concentrations into each analysis. This would offer a possibility to estimate which of the determinations are closest to the true concentrations and help to avoid the problem arising from using average concentrations as reference values. The problem, for example, can be that one big deviation from the true concentration shifts the average concentration into wrong direction and causes incorrect deviation to the other determinations.



Table 1.  $\text{NO}_3^-$  results ( $\mu\text{mol l}^{-1}$ ).

Institute	Sample depth	Average within institute	Standard deviation within institute	Average (n=4) of all institutes	Deviation of institutional average from the study average*	
					$\mu\text{mol l}^{-1}$	%
	m	$\mu\text{mol l}^{-1}$		$\mu\text{mol l}^{-1}$	$\mu\text{mol l}^{-1}$	%
SYKE	1	0,10 <sup>1</sup>	0,00	0,43	-0,33	77
Hydromet		0,36 <sup>1</sup>	0,00		-0,07	16
RSHU		0,55	0,15		+0,12	28
SPbSU <sup>2</sup>		0,71	0,20		+0,28	65
SYKE	32	6,69	0,06	4,59 (5,76)	+2,10 (+0,93)	46 (16)
Hydromet		6,11	0,36		+1,52 (+0,35)	33 (6)
RSHU		4,47	1,26		-0,08 (-1,29)	2 (22)
SPbSU <sup>2</sup>		1,07	0,00		-3,52	78
SYKE	60	9,65	0,32	6,83 (8,75)	+2,82 (+0,90)	41 (10)
Hydromet		8,57	0,71		+1,74 (-0,18)	25 (2)
RSHU		8,02	0,68		+1,19 (-0,73)	17 (8)
SPbSU <sup>2</sup>		1,07	0,00		-5,76	84

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)

<sup>1</sup> = reported values below the detection limit, hence the detection limit was used

<sup>2</sup> = two replicates instead of three

( ) = values calculated without SBpSU's results. The ratio of SPbSU to the average of the others is 0.19 at 32 m and 0.12 at 60 m. At 1 m, the broad range of results and the use of values of the limit of detection do not require the exclusion of any institute.

Table 2. TOTN results ( $\mu\text{mol l}^{-1}$ ).

Institute	Sample depth	Average within institute	Standard deviation within institute	Average (n=3) of all institutes	Deviation of institutional average from the study average*	
					$\mu\text{mol l}^{-1}$	%
	m	$\mu\text{mol l}^{-1}$		$\mu\text{mol l}^{-1}$	$\mu\text{mol l}^{-1}$	%
SYKE	1	26,69	0,18	22,77 (28,10)	+3,92 (-1,41)	17 (5)
Hydromet		29,50	0,82		+6,73 (+1,40)	30 (5)
RSHU <sup>2</sup>						
SPbSU <sup>1</sup>		12,13	0,20		-10,64	47
SYKE	32	27,32	1,77	24,47 (29,37)	+2,85 (-2,05)	12 (7)
Hydromet		31,41	2,57		+6,94 (+2,04)	28 (7)
RSHU <sup>2</sup>						
SPbSU <sup>1</sup>		14,67	0,76		-10,10	41
SYKE	60	29,01	0,24	26,05 (32,23)	+2,96 (-3,22)	11 (10)
Hydromet		35,45	2,89		+9,40 (+3,22)	36 (10)
RSHU <sup>2</sup>						
SPbSU <sup>1</sup>		13,70	0,40		-12,35	47

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)

<sup>1</sup> = two replicates instead of three

<sup>2</sup> = TOTN is not determined routinely in RSHU, so they did not participate in this test

( ) = values calculated without SPbSU's results. The ratio of SPbSU to the average of the others is 0.22 at 1 m, 0.25 at 32 m, and 0.21 at 60 m.

Table 3.  $PO_4^{3-}$  results ( $\mu\text{mol l}^{-1}$ ).

Institute	Sample depth	Average within institute	Standard deviation within institute	Average (n=4) of all institutes	Deviation of institutional average from the study average*	
					$\mu\text{mol l}^{-1}$	%
	m	$\mu\text{mol l}^{-1}$		$\mu\text{mol l}^{-1}$		
SYKE	1	0,08	0,01	0,25 (0,11)	-0,17 (-0,03)	68 (27)
Hydromet		0,16 <sup>1</sup>	0,00		-0,09 (+0,05)	36 (45)
RSHU		0,67	0,04		+0,42	168
SPbSU <sup>2</sup>		0,10	0,00		-0,15 (-0,01)	60 (9)
SYKE	32	0,79	0,05	0,87 (0,67)	-0,08 (+0,12)	9 (18)
Hydromet		0,68	0,00		-0,19 (+0,01)	22 (1)
RSHU		1,47	0,02		+0,80	92
SPbSU <sup>2</sup>		0,53	0,02		-0,34 (-0,14)	39 (21)
SYKE	60	3,43	0,22	3,10	+0,33	11
Hydromet		2,95	0,19		-0,15	5
RSHU		3,91	0,31		+0,81	26
SPbSU <sup>2</sup>		2,10	0,00		-1,00	32

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the other institutes, the results are also managed without this institute's input (in parentheses)

<sup>1</sup> = reported values below the detection limit, hence the detection limit was used

<sup>2</sup> = two replicates instead of three

( ) = values calculated without RSHU's results. The ratio of RSHU to the average of the others is 5.91 at 1 m and 2.20 at 32 m.

Table 4. TOTP results ( $\mu\text{mol l}^{-1}$ ).

Institute	Sample depth	Average within institute	Standard deviation within institute	Average (n=4) of all institutes	Deviation of institutional average from the study average*	
					$\mu\text{mol l}^{-1}$	%
	m	$\mu\text{mol l}^{-1}$		$\mu\text{mol l}^{-1}$	$\mu\text{mol l}^{-1}$	%
SYKE	1	0,41	0,02	0,83 (0,28)	-0,42 (+0,13)	51 (46)
Hydromet		0,16 <sup>1</sup>	0,00		-0,67 (-0,12)	81 (43)
RSHU		2,49	0,15		+1,66	200
SPbSU <sup>2</sup>		0,26	0,00		-0,57 (-0,02)	69 (7)
SYKE	32	0,98	0,09	1,30 (0,86)	-0,32 (+0,12)	25 (14)
Hydromet		0,82	0,02		-0,48 (-0,04)	37 (5)
RSHU		2,63	0,04		+1,33	102
SPbSU <sup>2</sup>		0,77	0,00		+0,53 (-0,09)	41 (10)
SYKE	60	3,61	0,27	3,61	0,00	0
Hydromet		3,25	0,10		-0,36	10
RSHU		4,54	0,24		+0,93	26
SPbSU <sup>2</sup>		3,04	0,00		-0,57	16

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)

<sup>1</sup> = reported values below the detection limit, hence the detection limit was used

<sup>2</sup> = two replicates instead of three

( ) = values calculated without RSHU's results. The ratio of RSHU to the average of the others is 9.00 at 1 m and 3.07 at 32 m.

Table 5.  $\text{SiO}_4$  results ( $\mu\text{mol l}^{-1}$ ).

Institute	Sample depth	Average within institute	Standard deviation within institute	Average (n=4) of all institutes	Deviation of institutional average from the study average*	
					$\mu\text{mol l}^{-1}$	%
	m	$\mu\text{mol l}^{-1}$		$\mu\text{mol l}^{-1}$	$\mu\text{mol l}^{-1}$	%
SYKE	1	5,28	0,12	5,48 (6,59)	-0,20 (-1,31)	4 (20)
Hydromet		8,07	0,21		+2,61 (+1,48)	48 (22)
RSHU		6,41	0,42		+0,93 (-0,18)	17 (3)
SPbSU <sup>1</sup>		2,14	0,15		-3,30	60
SYKE	32	16,69	0,60	17,30	-0,61	4
Hydromet		19,34	0,21		+2,04	12
RSHU		18,56	0,51		+1,26	7
SPbSU <sup>1</sup>		14,63	0,20		-2,67	15
SYKE	60	42,51	1,84	40,34	+2,17	5
Hydromet		47,70	1,23		+7,36	18
RSHU		31,84	0,72		-8,50	21
SPbSU <sup>1</sup>		39,30	1,31		-1,04	3

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)

<sup>1</sup> = two replicates instead of three

( ) = values calculated without SPbSU's results. The ratio of SPbSU to the average of the others is 0.32 at 1 m.

Table 6. Chlorophyll a results ( $\mu\text{g l}^{-1}$ ).

Institute	Sample depth	Average within institute	Standard deviation within institute	Average (n=4) of all institutes	Deviation of institutional average from the study average*	
					$\mu\text{g l}^{-1}$	%
	m	$\mu\text{g l}^{-1}$		$\mu\text{g l}^{-1}$	$\mu\text{g l}^{-1}$	%
SYKE	1	6,68	0,15	6,27	+0,41	7
Hydromet		6,33	0,31		+0,06	1
RSHU		5,02	0,17		-1,25	20
SPbSU <sup>1</sup>		7,05	0,21		+0,78	12
SYKE	5	6,08	0,17	6,22	+0,14	2
Hydromet		5,82	1,06		-0,40	6
RSHU		5,02	0,35		-1,20	19
SPbSU <sup>1</sup>		7,95	0,21		+1,73	28
SYKE	10	3,89	0,08	3,79	+0,10	3
Hydromet <sup>1</sup>		3,46	0,21		-0,33	9
RSHU		3,41	0,35		-0,38	10
SPbSU <sup>1</sup>		4,40	0,14		+0,61	16
SYKE	20	1,52	0,05	1,67	-0,15	9
Hydromet <sup>1</sup>		1,81	0,42		+0,14	8
RSHU		1,61	0,17		-0,06	4
SPbSU <sup>1</sup>		1,75	0,07		+0,08	5

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)

<sup>1</sup> = two replicates instead of three

Table 7.  $\text{NO}_2^-$  results ( $\mu\text{mol l}^{-1}$ ).

Institute		Sample depth	Average within institute	Standard deviation within institute	Average (n=2) of all institutes	Deviation of institutional average from the study average*	
Sampled	Determined					$\mu\text{mol l}^{-1}$	%
		m	$\mu\text{mol l}^{-1}$		$\mu\text{mol l}^{-1}$		
SYKE	SYKE	80	0,12	0,01	0,13	-0,01	8
MSI	SYKE		0,13	0,01		+0,01	8

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)

Table 8.  $\text{NO}_2^- + \text{NO}_3^-$  results ( $\mu\text{mol l}^{-1}$ ).

Institute		Sample depth	Average within institute	Standard deviation within institute	Average (n=6) of all institutes	Deviation of institutional average from the study average*	
Sampled	Determined					m	$\mu\text{mol l}^{-1}$
SYKE	SYKE	1	0,24	0,02	0,28	-0,04	14
MSI	SYKE		0,27	0,03		-0,01	4
EMI	EMI		0,38	0,03		+0,10	36
SYKE	EMI		0,32	0,04		+0,04	14
MSI	MSI		0,29	0,23		+0,01	4
SYKE	MSI		0,20	0,00		-0,08	28
SYKE	SYKE	28	3,51	0,04	3,14	+0,37	12
MSI	SYKE		3,53	0,04		+0,39	12
EMI	EMI		2,55	0,21		-0,59	19
SYKE	EMI		2,59	0,07		-0,55	18
MSI	MSI		3,22	0,19		+0,08	3
SYKE	MSI		3,48	0,02		+0,34	11
SYKE	SYKE	80	0,76	0,02	0,89	-0,13	15
MSI	SYKE		0,97	0,01		+0,08	9
EMI	EMI		1,10	0,14		+0,21	24
SYKE	EMI		0,77	0,10		-0,12	13
MSI	MSI		0,97	0,03		+0,08	9
SYKE	MSI		0,76	0,00		-0,13	15

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)

Table 9. TOTN results ( $\mu\text{mol l}^{-1}$ ).

Institute		Sample depth	Average within institute	Standard deviation within institute	Average (n=6) of all institutes	Deviation of institutional average from the study average*	
Sampled	Determined	m	$\mu\text{mol l}^{-1}$		$\mu\text{mol l}^{-1}$	$\mu\text{mol l}^{-1}$	%
SYKE	SYKE	1	22,57	0,39	22,79	-0,22	1
MSI	SYKE		23,03	0,30		+0,25	1
EMI	EMI		23,12	0,47		+0,33	1
SYKE	EMI		21,94	0,21		-0,85	4
MSI	MSI		25,62	9,88		+2,83	12
SYKE	MSI		20,50	0,09		-2,29	10
SYKE	SYKE	28	22,81	0,10	22,68	+0,13	1
MSI	SYKE		23,46	0,15		+0,78	3
EMI	EMI		22,14	1,23		-0,54	2
SYKE	EMI		22,45	0,30		-0,23	1
MSI	MSI		23,23	2,22		+0,55	2
SYKE	MSI		21,96	0,32		-0,72	3
SYKE	SYKE	80	28,05	0,06	26,11	+1,94	7
MSI	SYKE		28,13	0,58		+2,02	8
EMI	EMI		26,87	0,45		+0,76	3
SYKE	EMI		24,94	2,14		-1,17	4
MSI	MSI		22,51	3,44		-3,60	14
SYKE	MSI		26,18	0,10		+0,07	0

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)



Table 10.  $PO_4^-$  results ( $\mu\text{mol l}^{-1}$ ).

Institute		Sample depth	Average within institute	Standard deviation within institute	Average (n=6) of all institutes	Deviation of institutional average from the study average*	
Sampled	Determined	m	$\mu\text{mol l}^{-1}$		$\mu\text{mol l}^{-1}$	$\mu\text{mol l}^{-1}$	%
SYKE	SYKE	1	0,09	0,01	0,20 (0,12)	-0,11 (-0,03)	55 (25)
MSI	SYKE		0,11	0,01		-0,09 (-0,01)	45 (8)
EMI	EMI		0,37	0,04		+0,17	85
SYKE	EMI		0,38	0,06		+0,18	90
MSI	MSI		0,14	0,02		-0,06 (+0,02)	30 (17)
SYKE	MSI		0,12	0,01		-0,08 (0,00)	40 (0)
SYKE	SYKE	28	0,62	0,01	0,74	-0,12	16
MSI	SYKE		0,64	0,01		-0,10	14
EMI	EMI		0,95	0,05		+0,21	28
SYKE	EMI		0,98	0,03		+0,24	32
MSI	MSI		0,62	0,01		-0,12	16
SYKE	MSI		0,65	0,00		-0,09	12
SYKE	SYKE	80	4,48	0,02	4,64	-0,16	3
MSI	SYKE		4,58	0,04		-0,06	1
EMI	EMI		4,92	0,05		+0,28	6
SYKE	EMI		4,95	0,23		+0,31	7
MSI	MSI		4,41	0,10		-0,23	5
SYKE	MSI		4,50	0,06		-0,14	3

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from of the average of the other institutes, the results are also managed without this institute's input (in parentheses)

( ) = values calculated without EMI's results. The ratio of EMI to the average of the others is 3.26 at 1 m.

Table 11. TOTP results ( $\mu\text{mol l}^{-1}$ ).

Institute		Sample depth	Average within institute	Standard deviation within institute	Average (n=6) of all institutes	Deviation of institutional average from the study average*	
Sampled	Determined	m	$\mu\text{mol l}^{-1}$		$\mu\text{mol l}^{-1}$	$\mu\text{mol l}^{-1}$	%
SYKE	SYKE	1	0,36	0,02	0,70 (0,30)	-0,34 (+0,06)	49 (20)
MSI	SYKE		0,41	0,03		-0,29 (+0,11)	41 (37)
EMI	EMI		1,60	0,08		+0,90	129
SYKE	EMI		1,39	0,11		+0,69	99
MSI	MSI		0,23	0,05		-0,47 (-0,07)	67 (23)
SYKE	MSI		0,18	0,01		-0,52 (-0,12)	74 (40)
SYKE	SYKE	28	0,79	0,03	1,05 (0,74)	-0,26 (+0,05)	25 (7)
MSI	SYKE		0,78	0,03		-0,27 (+0,04)	26 (5)
EMI	EMI		1,75	0,08		+0,70	67
SYKE	EMI		1,62	0,12		+0,57	54
MSI	MSI		0,69	0,02		-0,46 (-0,05)	44 (7)
SYKE	MSI		0,69	0,01		-0,46 (-0,05)	44 (7)
SYKE	SYKE	80	4,99	0,00	4,58	+0,41	9
MSI	SYKE		4,85	0,06		+0,27	6
EMI	EMI		4,19	0,28		-0,39	9
SYKE	EMI		4,77	0,11		+0,19	4
MSI	MSI		4,00	0,41		-0,58	13
SYKE	MSI		4,65	0,02		+0,07	2

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)

( ) = values calculated without EMI's results. The ratio of EMI to the average of the others is 5.07 at 1 m, and 2.28 at 28 m.

Table 12.  $\text{SiO}_4^-$  results ( $\mu\text{mol l}^{-1}$ ).

Institute		Sample depth	Average within institute	Standard deviation within institute	Average (n=6) of all institutes	Deviation of institutional average from the study average*	
Sampled	Determined	m	$\mu\text{mol l}^{-1}$		$\mu\text{mol l}^{-1}$	$\mu\text{mol l}^{-1}$	%
SYKE	SYKE	1	8,13	0,025	7,88	+0,25	3
MSI	SYKE		8,92	0,271		+1,04	13
EMI	EMI		6,70	0,126		-1,18	15
SYKE	EMI		6,11	0,193		-1,77	22
MSI	MSI		8,65	0,191		+0,77	10
SYKE	MSI		8,78	0,044		+0,90	11
SYKE	SYKE	28	15,61	0,322	14,45	+1,16	8
MSI	SYKE		15,81	0,197		+1,36	9
EMI	EMI		12,96	0,541		-1,49	10
SYKE	EMI		12,19	0,202		-2,26	16
MSI	MSI		14,40	0,151		-0,05	0
SYKE	MSI		15,74	0,426		+1,29	9
SYKE	SYKE	80	56,02	0,674	48,55	+6,47	13
MSI	SYKE		46,59	0,993		-1,96	4
EMI	EMI		33,52	1,149		-15,03	31
SYKE	EMI		46,09	0,847		-2,46	5
MSI	MSI		52,50	0,821		+3,95	8
SYKE	MSI		56,58	0,461		+8,03	17

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)

Table 13. O<sub>2</sub> results (mg l<sup>-1</sup>).

Institute		Sample depth	Average within institute	Standard deviation within institute	Average (n=3) of all institutes	Deviation of institutional average from the study average*	
Sampled	Determined					m	mg l <sup>-1</sup>
EMI	EMI	1	8,90	0,00	8,86	+0,04	0
SYKE	SYKE		8,63	0,01		-0,23	3
MSI	MSI		9,05	0,09		+0,19	2
EMI	EMI	28	7,53	0,06	7,82	-0,29	4
SYKE	SYKE		7,76	0,02		-0,06	1
MSI	MSI		8,17	0,05		+0,35	4
EMI	EMI	80	0,43	0,06	0,57 (0,37)	-0,14 (+0,06)	25 (16)
SYKE	SYKE		0,20	0,02		-0,37 (-0,17)	65 (46)
MSI	MSI		1,08	0,09		+0,51	89

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)

( ) = values calculated without MSI's results. The ratio of MSI to the average of the others is 2.91 at 80 m

Table 14. pH results.

Institute		Sample depth	Average within institute	Standard deviation within institute	Average (n=3) of all institutes	Deviation of institutional average from the study average*	
Sampled	Determined	m					%
EMI	EMI	1	7,94	0,02	7,97	-0,03	0
SYKE	SYKE		8,01	0,00		+0,04	1
MSI	MSI		7,96	0,01		-0,01	0
EMI	EMI	28	7,58	0,02	7,63	-0,05	1
SYKE	SYKE		7,58	0,01		-0,05	1
MSI	MSI		7,72	0,04		+0,09	1
EMI	EMI	80	7,32	0,01	7,32	0,00	0
SYKE	SYKE		7,22	0,02		-0,10	1
MSI	MSI		7,43	0,03		+0,11	1

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)

Table 15.  $\text{NH}_4^+$  results ( $\mu\text{mol l}^{-1}$ ).

Institute		Sample depth	Average within institute	Standard deviation within institute	Average (n=3) of all institutes	Deviation of institutional average from the study average*	
Sampled	Determined					m	$\mu\text{mol l}^{-1}$
EMI	EMI	1	0,50	0,03	0,42	+0,08	19
SYKE	SYKE		0,42	0,00		0,00	0
MSI	MSI		0,34	0,04		-0,08	19
EMI	EMI	28	0,50	0,03	0,35	+0,15	43
SYKE	SYKE <sup>1</sup>		0,25	0,00		-0,10	29
MSI	MSI <sup>1</sup>		0,30	0,00		-0,05	14
EMI	EMI	80	8,01	0,05	8,17	-0,16	2
SYKE	SYKE		9,37	0,19		+1,20	15
MSI	MSI		7,13	0,12		-1,04	13

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)

<sup>1</sup> = reported values below the detection limit, hence the detection limit was used

Table 16. Chlorophyll a results ( $\mu\text{g l}^{-1}$ ).

Institute		Sample depth	Average within institute	Standard deviation within institute	Average (n=6) of all institutes	Deviation of institutional average from the study average*	
Sampled	Determined					m	$\mu\text{g l}^{-1}$
SYKE	SYKE	1	3,27	0,08	3,41	-0,14	4
MSI	SYKE		2,87	0,22		-0,54	16
EMI	EMI		3,87	0,21		+0,46	13
SYKE	EMI		3,55	0,16		+0,14	4
MSI	MSI		3,49	0,12		+0,08	2
SYKE	MSI		3,41	0,17		0,00	0
SYKE	SYKE	5	3,25	0,06	3,35	-0,10	3
MSI	SYKE		2,46	0,21		-0,89	27
EMI	EMI		3,67	0,26		+0,32	10
SYKE	EMI		3,65	0,22		+0,30	9
MSI	MSI		3,45	0,14		+0,10	3
SYKE	MSI		3,61	0,00		+0,26	8
SYKE	SYKE	10	3,45	0,04	3,48	-0,03	1
MSI	SYKE		2,99	0,33		-0,49	14
EMI	EMI		3,68	0,39		+0,19	5
SYKE	EMI		3,72	0,06		+0,24	7
MSI	MSI		3,45	0,07		-0,03	1
SYKE	MSI		3,61	0,00		+0,13	4
SYKE	SYKE	20	0,51	0,05	0,60	-0,09	15
MSI	SYKE		0,56	0,14		-0,04	7
EMI	EMI		0,69	0,09		+0,09	15
SYKE	EMI		0,81	0,12		+0,21	35
MSI	MSI		0,60	0,00		0,00	0
SYKE	MSI		0,40	0,17		-0,20	33

\* For concentration, + / - denotes for deviation to higher / lower values. Whenever the institute's estimate deviates more than two-fold (higher or lower) from the average of the other institutes, the results are also managed without this institute's input (in parentheses)

Table 17. Difference in the results due to sampling place. The distance between EMI and MSI (R/V Salme) and SYKE (R/V Aranda) at LL7 was about 100 m. For each depth, the two values refer to the difference between the samples taken on board both of the vessels, as analyzed by SYKE (upper), EMI (middle) and MSI (lower). Also shown is the percentage of this difference from the lower of the two determined concentrations. The sampling depths for Chlorophyll a are shown in parentheses.

Depth		NO <sub>2</sub> <sup>-</sup>		NO <sub>2</sub> <sup>-</sup> +NO <sub>3</sub> <sup>-</sup>		TOTN		PO <sub>4</sub> <sup>-</sup>		TOTP		SiO <sub>4</sub> <sup>-</sup>		Chlorophyll a	
		μmol l <sup>-1</sup>	%	μmol l <sup>-1</sup>	%	μmol l <sup>-1</sup>	%	μmol l <sup>-1</sup>	%	μmol l <sup>-1</sup>	%	μmol l <sup>-1</sup>	%	μg l <sup>-1</sup>	%
1	SYKE			0,03	11	0,46	2	0,02	26	0,05	14	0,79	10	0,40	12
	EMI			0,06	16	1,18	5	0,01	3	0,21	13	0,59	9	0,32	8
	MSI			0,01	5	5,12	25	0,02	17	0,05	28	0,13	2	0,08	2
28 (5)	SYKE			0,02	1	0,65	3	0,02	4	0,01	1	0,20	1	0,79	24
	EMI			0,04	2	0,31	1	0,03	3	0,13	7	0,77	6	0,02	1
	MSI			0,26	8	1,27	6	0,03	5	0,00	0	1,34	9	0,16	5
80 (10)	SYKE	0,01	8	0,21	28	0,08	0	0,10	2	0,14	3	9,43	17	0,46	13
	EMI			0,33	30	1,93	7	0,03	1	0,58	14	12,57	38	0,04	1
	MSI			0,21	28	3,67	16	0,09	2	0,65	16	4,08	8	0,16	5
20	SYKE													0,05	10
	EMI													0,12	17
	MSI													0,20	50



Figure 1. Comparison of CTD-data between MSI (red), EMI (black) and SYKE (blue).

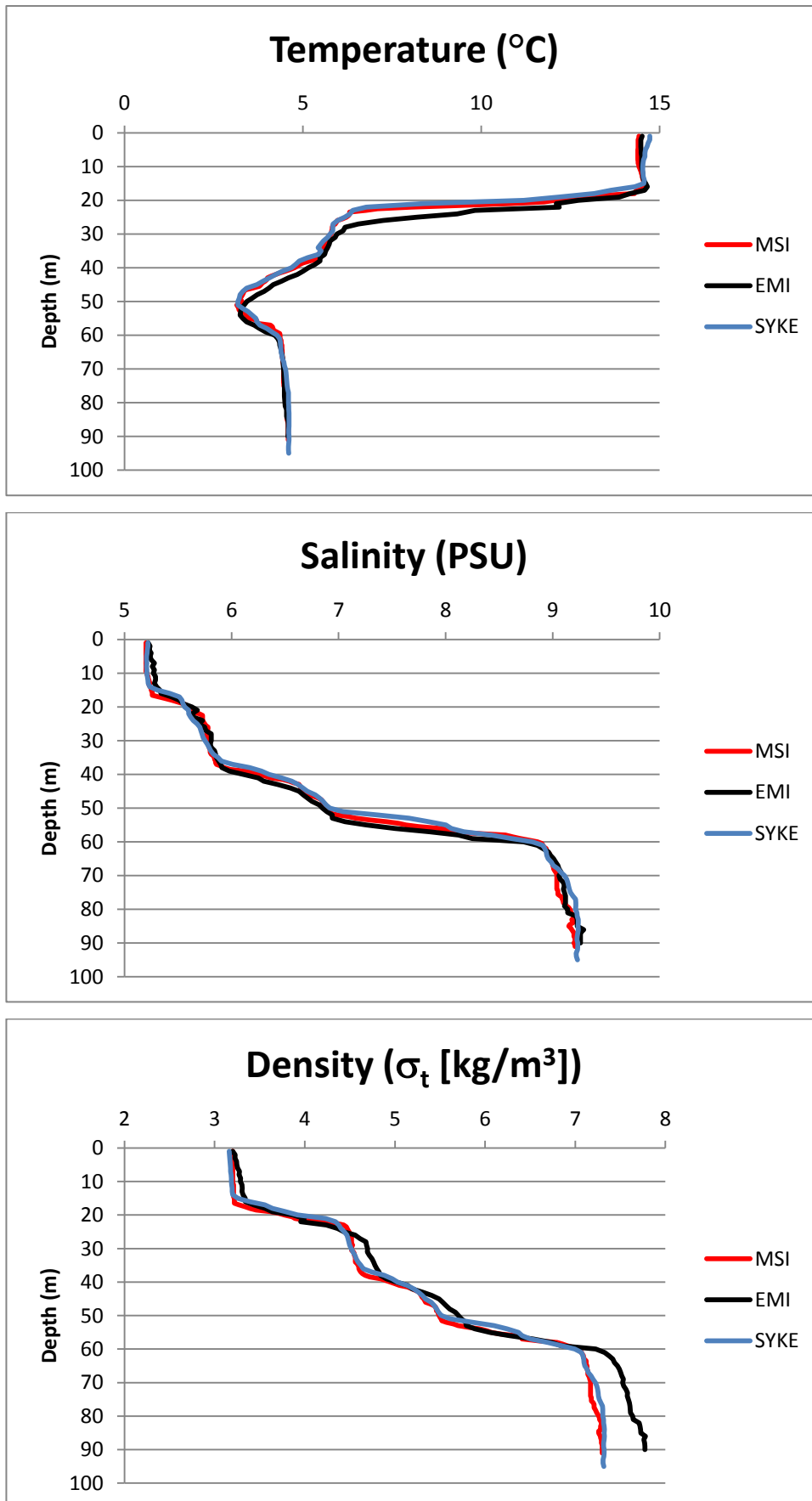
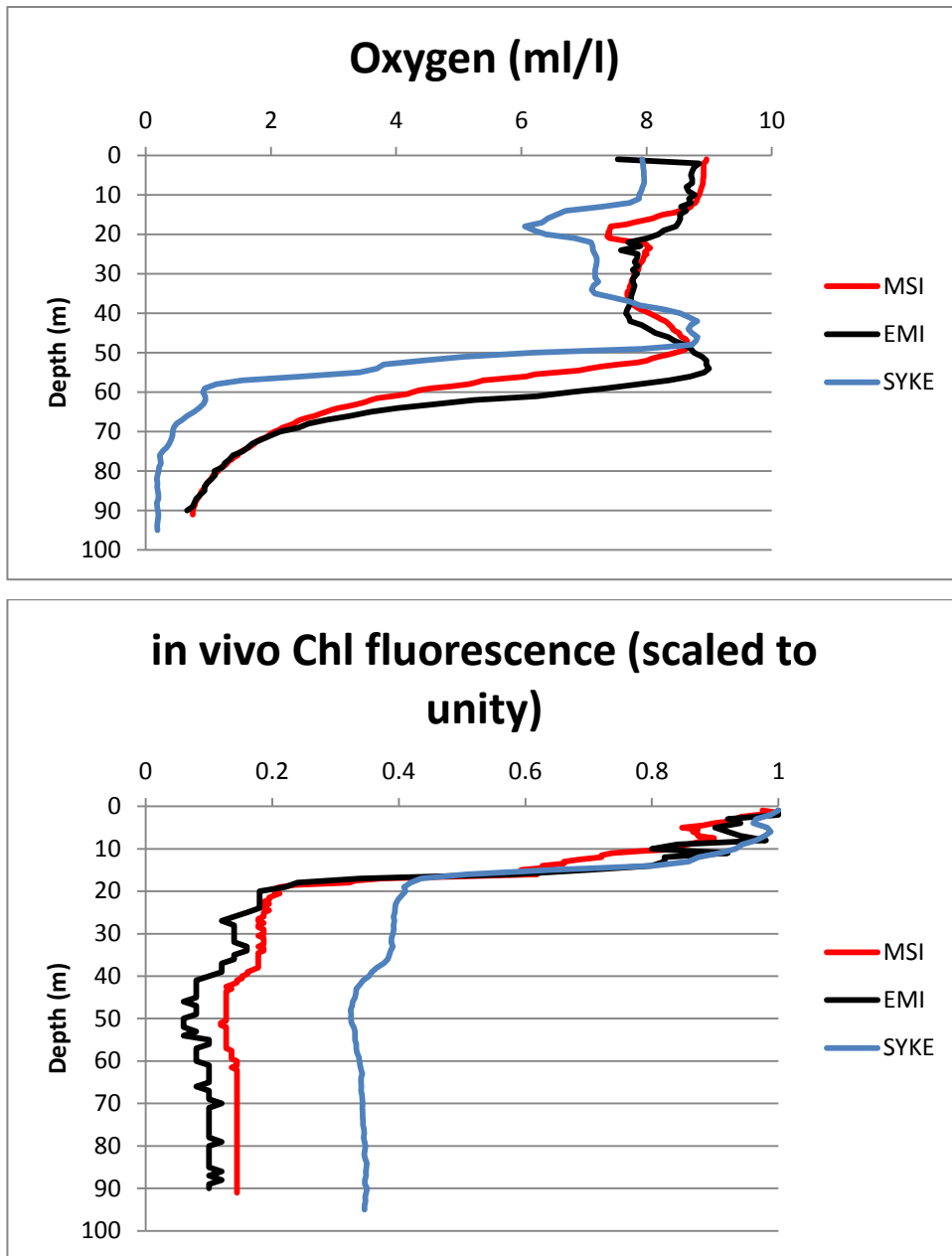


Figure 1. Continues.



## PART 2: PHYTOPLANKTON BIOMASS AND COMPOSITION

### BACKGROUND AND METHODS

For this intercalibration exercise, phytoplankton samples were taken on R/V Aranda for intercalibration between FIN and RUS on the 28<sup>th</sup> August, 2013, and on R/V Aranda and R/V Salme for intercalibration between EST and FIN on the 19<sup>th</sup> September, 2013. Samples were integrated water samples prepared by mixing the same amount of water from either 1, 5, and 10 m, or 1, 2.5, 5, 7.5, and 10 m.

The counting methods varied between the institutes (Annex 1). Russian participants used Nagoette chamber technique, while Estonian and Finnish participants used Utermöhl technique. Results from Estonian and Finnish institutes were more similar, but also most of the Russian institutes got very similar results despite of different counting methods. It should be also noted that even though Estonian and Finnish participants used Utermöhl method and followed HELCOM COMBINE guidelines, there were still differences in their counting methods (e.g. counted area, used magnifications).

Phytoplankton results are highly affected by counting methods, species identification, and formulae which are used to count species-specific biovolumes. The total biomass result may be the same even though there were differences in species identification or biomass estimates of certain taxa, for example.

Participants and samples which they analyzed are shown below:

Institute	Station LL3A 28.8.2013 Integrated sample of 0, 5, and 10 m on board R/V Aranda	Station LL3A 28.8.2013 integrated sample of 0, 2.5, 5, 7.5, and 10 m on board R/V Aranda	Station LL7 19.9.2013 Integrated sample of 0, 5, and 10 m on board R/V Aranda	Station LL7 19.9.2013 integrated sample of 0, 2.5, 5, 7.5, and 10 m on board R/V Aranda	Station LL7 19.9.2013 Integrated sample of 0, 5, and 10 m on board R/V Salme	Station LL7 19.9.2013 integrated sample of 0, 2.5, 5, 7.5, and 10 m on board R/V Salme
EMI			x	x	x	x
MSI			x	x	x	x
SYKE	x	x	x	x		
Hydromet	x	x				
RSHU	x	x				
SPbSU	x	x				

### RESULTS

Filamentous N<sub>2</sub>-fixing cyanobacterium *Aphanizomenon flos-aquae* was the dominant species (by biomass) in most of the samples (Fig. 1). Some species identifications showed differences between the participants. Spring diatom *Achnanthes taeniata*, for example, was included into the species lists by only one of the participants. This result points out the importance of workshops and training courses on species identification.

There was no consistent difference that could be connected to the sampling depths. For example, total biomass, biomass of the dominant species or species number could be either higher or lower in samples which were pooled by mixing the same volume of water from depths 0, 2.5, 5, 7.5 and 10 m, compared to the results from samples which were pooled by mixing the same volume of water from depths 0, 5 and 10 m. Also the difference in results obtained from samples taken on R/V Aranda compared to R/V Salme, did not show a consistent difference.



Fig. 1. Filamentous  $N_2$ -fixing cyanobacterium *Aphanizomenon flos-aquae* in the intercalibration sample seen with 250x magnification (SL).

Despite different counting methods between Finnish and Russian institutes, some institutes got very similar results in the intercalibration (Table 1). Generally in terms of biomass, the estimates increased in the line of SPbSU < SYKE and RSHU < Hydromet.

Table 1. Summary of phytoplankton results from the samples taken at station LL3A on R/V Aranda. Since Russian (Nagoyette chamber) and Finnish (Utermöhl method) participants used different methods, it was not appropriate to calculate means and standard deviations.

Institute	Sampling depths	Number of taxa	Total biomass	Biomass of <i>Aphanizomenon flos-aquae</i>	Dominant species
	m		mg l <sup>-1</sup>	mg l <sup>-1</sup>	(biomass dominance)
Hydromet	0, 5, 10	17	5,33	4,40	<i>Aphanizomenon flos-aquae</i> (cyanobacteria)
Hydromet	0, 2.5, 5, 7.5, 10	18	3,00	2,26	<i>A. flos-aquae</i>
SPbSU	0, 5, 10	17	-	0,13	<i>A. flos-aquae</i>
SPbSU	0, 2.5, 5, 7.5, 10	17	-	0,28	<i>A. flos-aquae</i>
RSHU	0, 5, 10	29	0,82	0,50	<i>A. flos-aquae</i>
RSHU	0, 2.5, 5, 7.5, 10	37	0,69	0,44	<i>A. flos-aquae</i>
SYKE	0, 5, 10	43	0,86	0,53	<i>A. flos-aquae</i>
SYKE	0, 2.5, 5, 7.5, 10	34	0,74	0,52	<i>A. flos-aquae</i>

With regard to total biomass in EST/FIN intercalibration, all institutes had quite similar results (Table 2).

Table 2. Summary of phytoplankton results from the samples taken at station LL7 on R/V Aranda and R/V Salme. All institutes used Utermöhl methods. Means and standard deviations are shown.

Institute	Sampling depths	Number of taxa	Total biomass	Biomass of <i>Aphanizomenon flos-aquae</i>	Dominant species (biomass dominance)
	m		mg l <sup>-1</sup>	mg l <sup>-1</sup>	
EMI	0, 5, 10 Salme	34	0,28	0,12	<i>Aphanizomenon flos-aquae</i> (cyanobacteria)
EMI	0, 2,5, 5, 7,5, 10 Salme	36	0,29	0,09	<i>A. flos-aquae</i>
EMI	0, 5, 10 Aranda	39	0,27	0,05	<i>A. flos-aquae</i>
EMI	0, 2,5, 5, 7,5, 10 Aranda	33	0,28	0,07	<i>A. flos-aquae</i>
MSI	0, 5, 10 Salme	38	0,33	0,02	<i>Coscinodiscus granii</i> (diatom)
MSI	0, 2,5, 5, 7,5, 10 Salme	40	0,21	0,02	<i>Aphanizomenon spp.</i>
MSI	0, 5, 10 Aranda	37	0,28	0,06	<i>Aphanizomenon. spp.</i>
MSI	0, 2,5, 5, 7,5, 10 Aranda	35	0,26	0,04	<i>Achnanthes taeniata</i> (diatom)
SYKE	0, 5, 10 Aranda	47	0,27	0,05	<i>Aphanizomenon. spp.</i>
SYKE	0, 2,5, 5, 7,5, 10 Aranda	44	0,33	0,04	<i>Coscinodiscus granii</i> (diatom)
Mean		38	0,28	0,06	
Standard deviation		4	0,03	0,03	

## CONCLUSIONS AND RECOMMENDATIONS FOR THE FUTURE CO-OPERATION

This intercalibration was very useful in showing how important it would be to harmonize further the phytoplankton counting methods and species identification, if we want to collect comparable results from different institutes around the Gulf of Finland.

Harmonizing methods is not just a simple task, since many of the countries may have used their own methods already decades, and so the comparability of their own long-term series would be harmed somewhat if the method would be modified. Still, in the long run, harmonization of methods would benefit the whole Baltic Sea monitoring and research.

Participating the HELCOM PEG (Phytoplankton Expert Group) work could probably be the most useful way to proceed in harmonizing methods and species identification, since in the HELCOM PEG, the work for harmonizing counting methods, species identification and biovolume calculation has been going on already since 1991 (<http://helcom.fi/helcom-at-work/projects/phytoplankton>). Nine of the Baltic Sea countries, including Estonia and Finland, are already active in the HELCOM PEG work.

All participating institutes are welcomed to join the e-mail list of the HELCOM PEG (send e-mail to [sirpa.lehtinen@environment.fi](mailto:sirpa.lehtinen@environment.fi)), and a representatives from each country are very warmly welcomed to

participate HELCOM PEG yearly meetings. Meetings include e.g. one-day training course on species identification and updating the HELCOM PEG taxa and biovolume list. This year's meeting is in Helsinki, Finland, on 12.-16.5.2014 (more information: [sirpa.lehtinen@environment.fi](mailto:sirpa.lehtinen@environment.fi)).

## ANNEX 1. THE PHYTOPLANKTON COUNTING METHODS OF THE PARTICIPANTS

### MSI / Marine Ecology Lab: Quantitative Phytoplankton Analysis

Samples are held at room temperature before settling. Sample bottles are mixed gently but efficiently by turning it upside-down for 1-2 minutes (40-50 times) before pouring it into the settling cylinder.

25 ml of sample was settled for 24 hours. Hydro-Bios tubular chambers were used. Microscope Leica DM IL Bio was used with oculars HC PLAN 10x/18, and objectives HI PLAN 20x/0.40 PH1 and HI PLAN 40x/0.65 PH2. Base magnification was 1x. We count counting units. One counting unit may be a cell, a colony of certain number of cells, or a filament of 100µm.

Analysis:

1. First we count certain counting units from 200 fields with 200x magnification (oculars 10x, objective 20x, base 1x). For example, >30 µm sized cells, such as *Dinophysis*, *Ebria*, *Mesodinium*, bigger *Gyrodinium*; larger scarce filamentous cyanobacteria, such as *Aphanizomenon*, *Nodularia*, *Dolichospermum (Anabaena)*, other scarce cells, such as *Cladopyxis*, *Oocystis*, *Planctonema*. Counting starts with 200x magnification from the central side of the quvette, and proceeds from upper side of the quvette to the lower side of the quvette until the 100 fields are counted and right side of the quvette is almost reached. Then the similar procedure is repeated to the left side. Cells that touch the right side of the field are counted. Cells that touch the left side of the field are not counted. All those parts/cells of filaments and colonies that are inside the field view are counted.
2. Then we count certain counting units from 20 to more fields (1 or more diagonals depending on the abundance of cells) with 200x magnification (oculars 10x, objective 20x, base 1x). For example, *Heterocapsa triquetra*, *Pseudanabaena*, *Snowella* and *Woronichinia*. Counting starts with 200x magnification from the central side of the quvette, and proceeds from upper side of the quvette to the lower side of the quvette until the one cross-section from edge to edge is counted and repeated if the abundance of counted cells is low. Cells that touch the right side of the field are counted. Cells that touch the left side of the field are not counted. All those parts/cells of filaments and colonies that are inside the field view are counted.
3. Finally, we count certain counting units from 40 or more fields (depending on the abundance of the cells) with 400x magnification (oculars 10x, objective 40x, base 1x). For example, <20µm cells. Counting starts with 400x magnification from the central side of the quvette, and proceeds from upper side of the quvette to the lower side of the quvette or from left side of the quvette to the right side of the quvette until the one cross-section from edge to edge is counted and repeated if the abundance of counted cells is low. Cells that touch the right side of the field are counted. Cells that touch the left side of the field are not counted. All those parts/cells of filaments and colonies that are inside the field view are counted.

Ocular micrometer is used all the time during the analysis to check the size of the cells. We use AlgaPhyto counting program which includes the HELCOM PEG phytoplankton taxa and biovolume list (<http://helcom.fi/helcom-at-work/projects/phytoplankton>). The right sized counting units are selected from the HELCOM PEG list. The AlgaPhyto counting program converts the counting results into wet biomass and carbon biomass results.

## Phytoplankton analysis information of MSI.

Institute	Settled sample ml	Used taxa and biovolumes	Microscope	Oculars	Base = obtovar magnif.	Objective	Total magnif.	Coefficient used in converting the number of counted cells into cells/liter	Examples of taxa counted with this magn.
MSI	25 ml	HELCOM PEG 2012	Leica DM IL Bio	HI PLAN 10x/18	1x	HI PLAN 20x/0.40 PH1	200x	167	<i>Dolichospermum</i> , <i>Dinophysis</i> , <i>Ebria</i> , <i>Mesodinium</i> ,
						HI PLAN 20x/0.40 PH1	200x	835	<i>Heterocapsa triquetra</i> , <i>Oscillatoriales</i> , <i>Snowella</i> , <i>Woronichinia</i>
						HI PLAN 40x/0.65 PH2	400x	3339	<20µm cells and

**SYKE: Quantitative Phytoplankton Analysis**

Samples are taken into room temperature a day before settling the sample. Sample bottles are mixed gently but efficiently by turning it upside-down for 1-2 minutes before pouring it into the settling cylinder.

50 ml of sample was settled for 24 hours. Hydro-Bios cylinders and cuvettes were used. Microscope Leica DMIRB was used with oculars HC PLAN 12.5x/16 , and objectives HC PL FLUOT 10x/0.30 PH1, HC PL FLUOTAR 20x/0.50 PH2, and HCx PL FLUOTAR 40x/0.75 PH2. Base magnification (Obtovar magnification) was 1x (Table 1). We count counting units. One counting unit may be a cell, a colony of certain number of cells, or a filament of 100µm.

Analysis:

1. First we count certain counting units from 60 squares (10x10 objective squares) with 125x magnification (oculars 12,5x, objective 10x, base 1x). For example, >30 µm sized cells and larger filamentous cyanobacteria, such as *Aphanizomenon*, *Nodularia*, *Dolichospermum* (*Anabaena*), *Dinophysis* and *Ebria* are counted with 125x magnification.
2. Then we count certain counting units from 60 squares with 250x magnification (oculars 12,5x, objective 20x, base 1x). For example, *Heterocapsa triquetra*, *Pseudanabaena*, *Mesodinium rubrum*, *Snowella* and *Woronichinia* are counted with 250x magnification.
3. Finally, we count certain counting units from 60 squares with 500x magnification (oculars 12,5x, objective 40x, base 1x). For example, <20µm cells and cyanobacterial colonies with small sized cells (<2µm) are counted with 500x magnification.

Counting starts with each magnification from the left side of the quvette, and proceeds in stripes from upper side of the quvette to the lower side of the quvette until the right side of the quvette is reached. Cells that touch the upper and right side borders of the ocular square, are counted. Cells that touch the lower or left side borders of the ocular square are not counted. All those parts/cells of filaments and colonies that are inside the square are counted. Ocular micrometer is used all the time during the analysis to check the size of the cells. We use EnvPhyto counting program which includes the HELCOM PEG phytoplankton taxa and biovolume list (<http://helcom.fi/helcom-at-work/projects/phytoplankton>). The right sized counting units are selected from the HELCOM PEG list. The EnvPhyto counting program converts the counting results into wet biomass and carbon biomass results.



## Phytoplankton analysis information of SYKE/MRC.

Institute, country	Settled sample ml	Used taxa and biovolumes	Microscope	Oculars	Base = obtovar magnif.	Objective	Total magnif.	Coefficient used in converting the number of counted cells into cells/liter	Examples of taxa counted with this magn.
SYKE/MRC, Finland	50 ml	HELCOM PEG 2012	Leica DM IRB	HC PLAN 12.5x/16	1x	HC PL FLUOT 10x/0.30 PH1	125x	184	<i>Aphanizomenon</i> , <i>Nodularia</i> , <i>Dinophysis</i> , <i>Ebria</i>
						HC PL FLUOTAR 20x/0.50 PH2	250x	725	<i>Heterocapsa triquetra</i> , <i>Oscillatoriales</i> , <i>Mesodinium rubrum</i> , <i>Snowella</i> , <i>Woronichinia</i>
						HCx PL FLUOTAR 40x/0.75 PH2	500x	2878	<20µm cells and cyanobacterial colonies with small sized cells (<2µm)

**EMI: Quantitative Phytoplankton Analysis**

In general, quantitative analysis of phytoplankton follows HELCOM COMBINE Manual and international standard „Water quality – Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)“ EVS-EN 15204:2006. Phytoplankton samples are preserved with acid Lugol solution (0.5-1 ml per 200 ml sample) and analyzed using inverted microscopes (Olympus CKX-41, IMT-2 and IX-51) and sedimentation chambers 3-50 ml (HydroBios). The sedimentation time varies between 4-24 h, respectively. We count counting units from fields of view. One counting unit may be cell, a colony of certain number of cells or a filament of 100µm. Sample bottles are mixed by turning it upside-down 25-30 times before pouring it into the settling cylinder. A minimum of 500 units should be counted per sample (if the sample is too sparse, we consider lower number, 300-400 units, also sufficient).

## Analysis:

- 200-times magnification is used for larger cells and cells along one or two diagonals (20 to 50 objective fields) are counted, depending on the density of the sample (colonial and filamentous cyanobacteria, dinophytes except *Heterocapsa rotundata*, diatoms, most chlorophytes etc.).
- The abundance of smaller cells is estimated by examining 20 to 50 objective fields using 400-times magnification (nanoplanktonic flagellates (<20 µm), small centric diatoms (<10 µm), *Monoraphidium minutum*).

Counting starts from the upper side of the cuvette to the lower side. All cells inside the square are counted and also big cells, if more than half of cell is visible. For phytoplankton counting we use the PhytoWin counting program which includes the HELCOM PEG phytoplankton taxa and biovolume list (PEG BVOL\_2013.xlsx). Cell sizes are measured using an ocular scale.

## Information on microscopes and coefficients used in quantitative analysis of EMI.

Institute, country	Settled sample ml	Microscope	Oculars	Base = obtovar magnif.	Objective	Total magnif.	Coefficient used in converting the number of counted cells from 1 field into cells/liter
EMI	2,973	IX51	WHN 10x/22	1x	LUCPlanFLN 20X0.45 Ph1	200	190428
	10		WHN 10x/22	1x	LUCPlanFLN 20X0.45 Ph1	200	56614
	25		WHN 10x/22	1x	LUCPlanFLN 20X0.45 Ph1	200	22646
	50		WHN 10x/22	1x	LUCPlanFLN 20X0.45 Ph1	200	11323
EMI	2,973	IX51	WHN 10x/22	1x	LUCPlanFLN 40X0.60 Ph2	400	746587
	10		WHN 10x/22	1x	LUCPlanFLN 40X0.60 Ph2	400	221960
	25		WHN 10x/22	1x	LUCPlanFLN 40X0.60 Ph2	400	88784
	50		WHN 10x/22	1x	LUCPlanFLN 40X0.60 Ph2	400	44392
EMI	2,973	CKX-41	WHB10x-H/20	1x	LCach N 20x/0.40 PhP	200	239300
	10		WHB10x-H/20	1x	LCach N 20x/0.40 PhP	200	71150
	25		WHB10x-H/20	1x	LCach N 20x/0.40 PhP	200	28450
	50		WHB10x-H/20	1x	LCach N 20x/0.40 PhP	200	14230
EMI	2,973	CKX-41	WHB10x-H/20	1x	LCach N 40x/0.55 PhP	400	947400
	10		WHB10x-H/20	1x	LCach N 40x/0.55 PhP	400	281670
	25		WHB10x-H/20	1x	LCach N 40x/0.55 PhP	400	112660
	50		WHB10x-H/20	1x	LCach N 40x/0.55 PhP	400	56332
EMI	2,973	IMT-2	WKH10x/20 L	1x	LWDCDPlan20 PL/0.40	200	227379
	10		WKH10x/20 L	1x	LWDCDPlan20 PL/0.40	200	67600
	25		WKH10x/20 L	1x	LWDCDPlan20 PL/0.40	200	27040
	50		WKH10x/20 L	1x	LWDCDPlan20 PL/0.40	200	13520
EMI	2,973	IMT-2	WKH10x/20 L	1x	LWDCDPlan40 PL/0.60	400	909749
	10		WKH10x/20 L	1x	LWDCDPlan40 PL/0.60	400	270468
	25		WKH10x/20 L	1x	LWDCDPlan40 PL/0.60	400	108187
	50		WKH10x/20 L	1x	LWDCDPlan40 PL/0.60	400	54094
EMI	2,973	IMT-2	WKH10x/20 L	1.5x	LWDCDPlan20 PL/0.40	300	506477
	10		WKH10x/20 L	1.5x	LWDCDPlan20 PL/0.40	300	150575
	25		WKH10x/20 L	1.5x	LWDCDPlan20 PL/0.40	300	60230
	50		WKH10x/20 L	1.5x	LWDCDPlan20 PL/0.40	300	30115
EMI	2,973	IMT-2	WKH10x/20 L	1.5x	LWDCDPlan40 PL/0.60	600	2027056
	10		WKH10x/20 L	1.5x	LWDCDPlan40 PL/0.60	600	602644
	25		WKH10x/20 L	1.5x	LWDCDPlan40 PL/0.60	600	241058
	50		WKH10x/20 L	1.5x	LWDCDPlan40 PL/0.60	600	120529

**Hydromet: Quantitative Phytoplankton Analysis**

Nagoette chamber method was used. Sedimentation occurs in bottles during 10-14 days in a dark place. After sedimentation, sample concentrates naturally on the bottom of the bottle to 30-80 ml from the first

volume. The part of concentrated sample is transported into Nageotte chamber by pipet. All cells that are on the Nageotte chamber are counted (0,02 ml).

#### Phytoplankton analysis information of Hydromet.

Institute, country	Settled sample ml	Used taxa and biovolumes	Microscope	Oculars	Objective	Total magnif.	Coefficient used in converting the number of counted cells into cells/liter	Examples of taxa counted with this magn.
FSBI "North-West AHM" (Hydromet)	0,02 Nageotte chamber	Checklist of Baltic Sea Phytoplankton Species (Hällfors 2004). Biovolumes and size-classes of phytoplankton in the Baltic Sea. Baltic Sea Environment Proceedings No. 106.	Transmitted light microscope ЛОМО Микмед -1, МБИ-6	С 15 x	10x0,30	375		<i>Ceratium</i> , <i>Surirella</i> , <i>Closterium</i> , <i>Pleurosigma</i> , big sized cells and colonies (>200 µm)
					20x0,40	750		<200µm cells and cyanobacterial colonies

#### **RSHU: Quantitative Phytoplankton Analysis**

Phytoplankton samples (volume 0.2 l) was sedimented to volume 4 ml. Calculation of the organisms was made in the Nageotte chamber (0.02 ml) under the light microscope (Ergaval Karl Zeiss, Jena) with magnification of x 256 and x 640. Wet weight biomass of phytoplankton was calculated from cell geometry (HELCOM, 1988) and using cell biovolume table (HELCOM, 2006).

## PART 3: ZOOPLANKTON BIOMASS AND COMPOSITION

### BACKGROUND AND SAMPLING

For this intercalibration exercise between EST, FIN, and RUS, zooplankton samples were taken on board R/V Aranda and R/V Salme at the station LL7 on the 19<sup>th</sup> September, 2013. On R/V Aranda, four samples were taken which were analysed by EMI (EST), Hydromet (RUS), SPbSU (RUS), and SYKE (FIN). On board R/V Salme, two samples were taken which were analysed by EMI and SYKE.

On R/V Aranda, the samples were taken with a WP-2 net (100  $\mu\text{m}$ ; 0.25  $\text{m}^2$ ) with vertical hauls from 96 m to surface, concentrated using a 100 $\mu\text{m}$  net and preserved in hexamine buffered formaline (final formaldehyde concentration 4 %). On R/V Salme, the samples were taken with a Juday net (100  $\mu\text{m}$ ; 0.1  $\text{m}^2$ ) with vertical hauls from 85 m to surface and preserved in unbuffered formaline (final formaldehyde concentration 4 %). A separate tow was taken for each sample to avoid difficulties in subsampling, as samples contained *Cercopagis pengoi*, which form large aggregations in the samples. The detailed sample analysis, see Annex 1.

### RESULTS

The zooplankton samples taken onboard R/V Aranda were analysed by all institutes. Participants used different methods for subsampling and microscoping, but in all analyses copepods were the most numerous group (Fig 1). Variation in the total abundance of all taxa (Aranda: CV 15%; Salme: CV 7%) or copepods excluding nauplii (Aranda: CV 11 %; Salme: CV 12%) was surprisingly low as the methods differed so much (Annex 2). For rarer and smaller-sized specimen (cladocerans, copepod nauplii, rotifers, others), the variation was higher.

Within copepods, *Eurytemora affinis*/sp was found the most abundant taxa by three laboratories and *Acartia* spp. by one (Fig 2). All analyses agreed on *Pseudocalanus elongatus/acuspes* being the third abundant copepod taxa. Copepods were assigned to stages (cop1-3 and cop4-6) similarly by the three laboratories that reported stages (all species put together; Appendix 3).

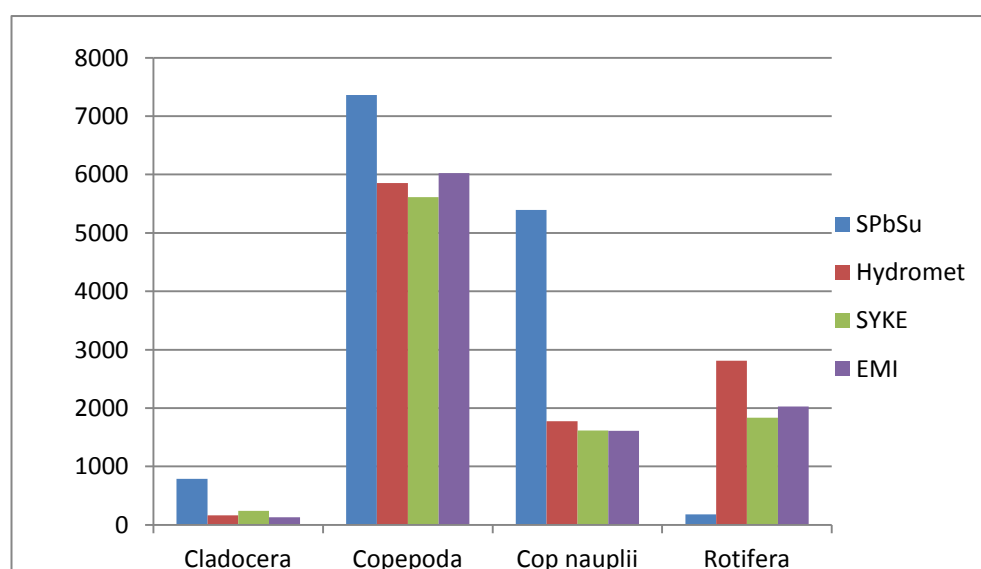


Figure 1. Zooplankton abundances ( $\text{ind m}^{-3}$ ) grouped to higher taxonomic levels counted by different laboratories from samples taken onboard R/V Aranda.

The samples were very dense and other laboratories except SPbSU subsampled only small parts of the samples (see Table 1 in Annex 1 for method comparison). Thus, abundance of species with low

abundance is easily under- or overestimated. It is only possible to check large or otherwise conspicuous specimen from the whole sample.

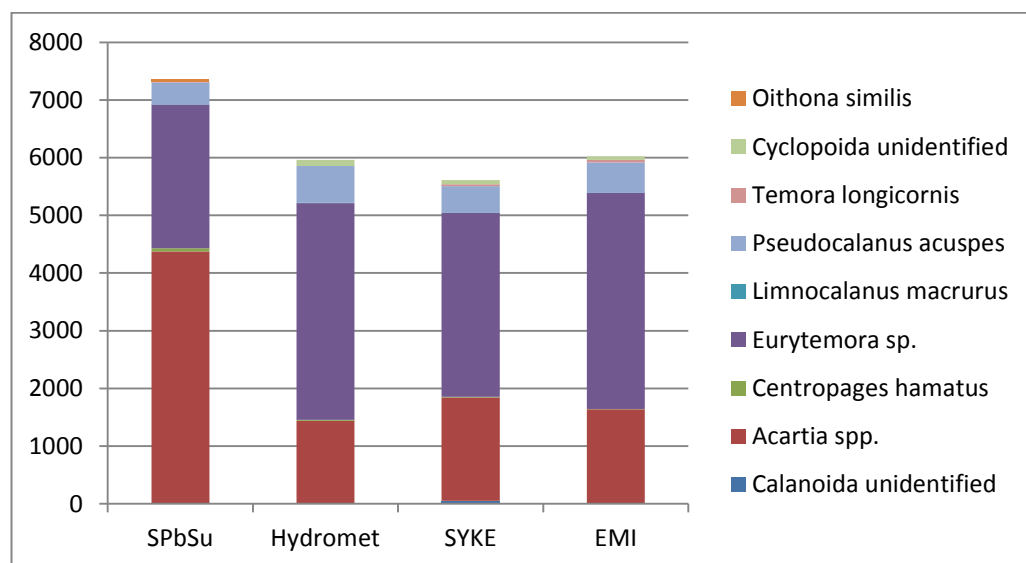


Figure 2. Copepod abundances (ind m<sup>-3</sup>) shown at species level counted by different laboratories from samples taken onboard R/V Aranda.

The zooplankton samples taken onboard R/V Salme were only analysed by SYKE and EMI. The results show that there are some differences in the counts, which may be due to different subsampling procedures (Fig. 3 and Annex 3). The largest differences were observed in counts of rare taxa as well as in species which may be difficult to subsample as they tend to attach to each others and surfaces or break easily (*Zoothamnium* spp., *Bosmina* spp., *Synchaeta monopus*, *Fritillaria borealis*). The samples taken onboard the two vessels differed also from each other (Fig. 4). Both EMI and SYKE counted more *Eurytemora affinis*/sp. and *Pseudocalanus elongatus* from samples taken onboard R/V Aranda than from those taken onboard R/V Salme while numbers of copepod nauplii and rotifers were higher in the R/V Salme samples. The differences may be accounted for by patchiness of zooplankton but possibly also by differences in the sampling. On Salme Juday net is used which has a smaller mouth area (0.1 m<sup>2</sup>) than R/V Aranda's WP-2 (0.25m<sup>2</sup>).

The taxonomy of zooplankton is not clear, and this is seen in that the laboratories used different names for probably the same species (Annex 2). In addition, the laboratories identified taxa (genus, species, sub-species) and copepodite stages and sexes to different levels. Hydromet classified the specimen according to their size and used this information to calculate biomass but as the other laboratories did not report biomass, this information cannot be compared between the participants. Copepod nauplii were identified to genus or species levels by SPbSu and SYKE, while EMI and Hydromet counted them as one group: copepod nauplii.

Laboratories found mostly the same species in the samples. Some rare species were only observed in part of the samples (*Evadne nordmanni*, *E. anonyx*, *P. polyphemoides*, Mysidacea, *Centropages hamatus*, *Temora longicornis*, Harpacticoida, *Amphibalanus improvisus*, Bivalvia larvae, Gastropoda larvae, Ctenophora, Polychaeta larvae, Nematoda, Protista). *Keratella* spp. was not counted by one laboratory. *Fritillaria borealis* was only counted by two laboratories, possibly because the specimen are mostly in bad shape after processing the samples, thus the animals may be disregarded as dead. Three species of *Acartia* spp. may be found in the Gulf of Finland, and the laboratories disagreed on which of these there were in the samples. All laboratories had identified *A. bifilosa*, one also found *A. longiremis* and one *A.*

*tonsa* in the samples; two of the laboratories assigned also younger stages to species while two counted those as *Acartia* spp. One laboratory found *Parasagitta elegans* and *Oithona similis*, whose known distribution range in the Baltic Sea has this far been restricted from the southern parts of the sea to the central and northern Baltic Proper, respectively.

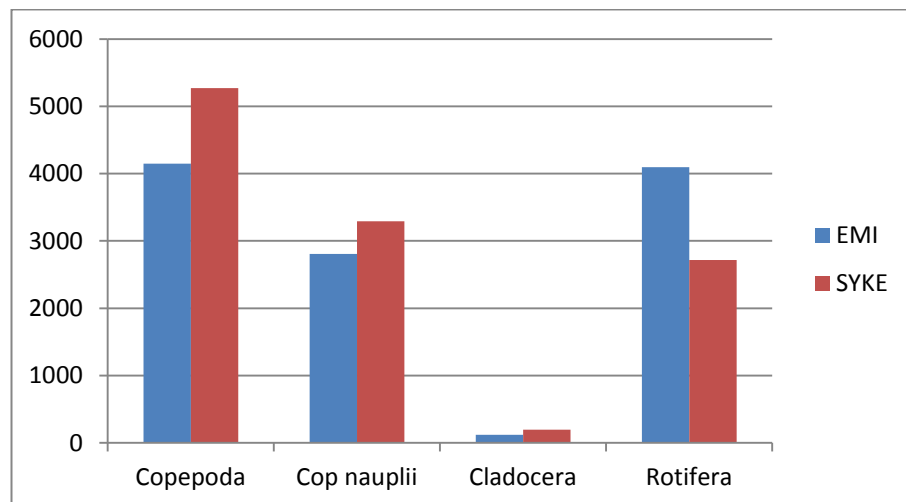


Figure 3. Zooplankton abundances (ind m<sup>-3</sup>) grouped to higher taxonomic levels counted by SYKE and EMI from samples taken onboard R/V Salme.

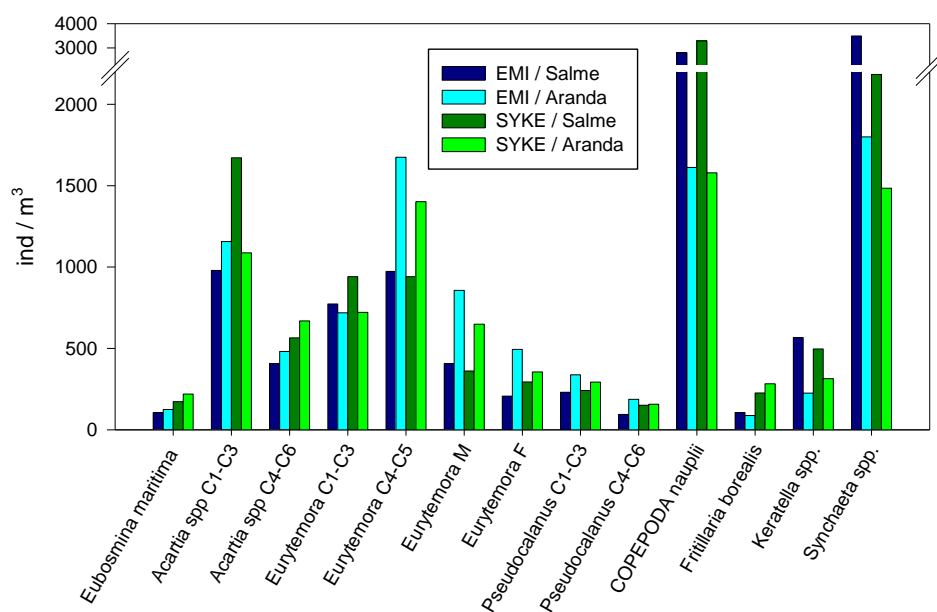


Figure 4. Comparison of samples taken with Juday-net (R/V Salme) and WP-2 net (R/V Aranda) and analysed by EMI and SYKE. Abundance of the most abundant taxa/life stages counted are presented.

## CONCLUSIONS AND RECOMMENDATIONS

The results of the intercalibration show that the most abundant taxa or groups are counted and identified quite well at the same level by all institutes. However, the problem arises from the identification to species level. Not all institutes use the same scientific names, which hampers the joint usability of data collected and analysed by different institutes. In the intercalibration the samples were taken from one location only and still there were large differences between the taxa identified.

There is a lot of work done in HELCOM Zooplankton Expert Network (ZEN) and by its present project (Quality Assurance and Integration of Zooplankton Monitoring in the Baltic Sea, ZEN QAI) in order to increase the knowledge of identification and proper scientific names to be used for species and subspecies. ZEN also organizes identification tests (Ring tests), which show to the participating laboratories and taxonomists the level of expertise in their zooplankton identification. The results of the Ring tests are analysed together in the group and discussed in order to learn from the test and to intercalibrate the taxonomic nomenclature used and the identification of species. It would be extremely useful for all Baltic Sea laboratories analyzing zooplankton samples to take part to the HELCOM ZEN work to increase the similarity in sample analysis and nomenclature used by different experts. This would ease the use of different data sets analysed by different laboratories.

It would also be good to increase discussion on the methods used by different laboratories for subsampling and microscoping, which most probably also influences the results. This would be good to initiate through HELCOM as well in order to reach all laboratories in the Baltic Sea and to increase the similarity of procedures and shareability of data.

In the future, the intercalibration tests should use subsamples of the same sample (net tow) to decrease the effects of patchiness in zooplankton communities on the end results or use web-based identification programs with shared photos of species and microscopic views to count as the latest ZEN Ring test did.

## ANNEX 1. THE DETAILED SAMPLE ANALYSES OF THE PARTICIPANTS

The institutes used different protocols for analyses of zooplankton in the samples (Table 1). Large and/or rare animals were counted from the whole samples but most taxa were analysed from subsamples. Subsampling was conducted with an ordinary or a Hensen-Stempel pipette, or with a Folsom splitter. The volume of the subsamples analysed varied a lot: from 0.03 % to 30 % of the original sample. Stereomicroscope or inverted microscope was used in the analysis.

### SYKE

The whole sample was investigated with a stereomicroscope, and *Cercopagis pengoi* and mysids were counted and removed. The remaining sample was divided with a Folsom splitter to 1/256 (Aranda sample) or 1/64 (Salme sample). Subsamples were counted with an inverted microscope (Leica DMIL Led). In addition, rare and easily distinguishable animals (*Limnocalanus macrurus* copepodites 4–6 and *Evadne anonyx*) were counted with a stereomicroscope (Leica MZ 7.5) from 1/4 divisions.

### SPbSU

Three subsamples were taken with a Hensen-Stempel pipette. Altogether 30 % of the sample was analysed for all taxa. In addition, large and/or rare specimen (large Cladocera, *Parasagitta*, etc.) were analysed from the whole sample. Microscope MS-2 Zoom was used.

### Hydromet

The sample was concentrated to a volume of 80 ml, and two subsamples of 1 ml were taken with a pipette to a Bogorov counting chamber and counted (according to species and size groups) with a stereomicroscope. Large and rare organisms were counted and measured from the whole sample.

### EMI

The samples were diluted to 500 ml (Salme) and 1500 ml (Aranda) and subsamples were taken with a Hensen-Stempel pipette. Subsamples for copepods and cladocerans were 10 ml and for rotifers, copepod nauplii and meroplankton larvae 5 ml. All specimens of *Cercopagis pengoi* and some other rare species (*Evadne anonyx*, Mysidae, *Mertensia ovum*) were counted from the whole sample. Samples were counted with a Leica M125 stereomicroscope.

Table 1. Methods used for processing the zooplankton samples by participating institutes.

Institute	Sample	Subsampling	Part analysed <sup>1)</sup>	Microscope
SPbSU	Aranda	Hensen-Stempel	30 %	MS-2 Zoom microscope
Hydromet	Aranda	Pipette	2,5 %	Stereomicroscope
EMI	Aranda Salme	Hensen-Stempel	0.3–0.7 % <sup>2)</sup> 1–2 % <sup>2)</sup>	Leica M125 stereomicroscope
SYKE	Aranda Salme	Folsom	0.4 % <sup>3)</sup> 1.6 % <sup>3)</sup>	Leica DMIL Led inverted; Leica MZ 7.5 stereomicroscope <sup>1) 3)</sup>

<sup>1)</sup> *Cercopagis pengoi*, Mysidae and other large /rare animals were counted from whole samples.

<sup>2)</sup> Larger subsample for copepods and cladocerans, smaller subsample for rotifers, copepod nauplii and meroplankton larvae.

<sup>3)</sup> In addition, *Limnocalanus* (and *Evadne anonyx*) were counted from a 25 % subsample.



**ANNEX 2. TAXA NAMED BY DIFFERENT INSTITUTES.**

Names referring to apparently the same species listed together.

Group	Taxon identified	Institute	
Protista	<i>Helicostomella subulata</i>	SYKE	
	<i>Zoothamnium</i> spp.	SYKE	
Cladocera	<i>Eubosmina maritima</i> / <i>Bosmina coregoni maritima</i>	EMI, SYKE / SPbSU, Hydromet	
	<i>Bosmina</i> spp.	SYKE, SPbSU	
	<i>Cercopagis pengoi</i>	all	
	<i>Evadne anonyx</i>	EMI, SYKE	
	<i>Evadne nordmanni</i>	all	
	<i>Pleopis</i> / <i>Podon polyphemoides</i>	SPbSU / Hydromet	
Mysidacea	<i>Mysidae</i>	EMI	
	<i>Neomysis integer</i>	SYKE	
Copepoda	<i>Acartia</i> spp.	EMI, SYKE	
	<i>Acartia tonsa</i>	SYKE	
	<i>Acartia longiremis</i>	SPbSU	
	<i>Acartia bifilosa</i>	all	
	<i>Centropages hamatus</i>	all	
	<i>Eurytemora affinis</i> / sp / spp / <i>hirundoides</i>	EMI / SPbSU / SYKE / Hydromet	
	<i>Limnocalanus macrurus</i> / <i>grimaldii</i>	EMI, SYKE, SPbSU / Hydromet	
	<i>Pseudocalanus acuspes/elongatus/minusus elongatus</i>	EMI, SPbSU / SYKE / Hydromet	
	<i>Temora longicornis</i>	EMI, SYKE, SPbSU	
	<i>Calanoida</i> spp. (nauplii)	SYKE, Hydromet	
	<i>Oithona similis</i>	SPbSU	
	<i>Cyclopidae</i>	EMI	
	<i>Cyclops</i> sp.	Hydromet	
	<i>Cyclopoida</i> spp.	SYKE	
	<i>Harpacticoida</i> spp.	EMI (A)	
	<i>Copepoda</i> spp. (nauplii)	EMI, SYKE	
	Cirripedia	<i>(Amphi)balanus improvisus</i> (nauplii)	
	Ostracoda	<i>Cypridina</i> (observed, not counted)	Hydromet
	Chordata	<i>Fritillaria borealis</i>	EMI, SYKE
Mollusca	<i>Mollusca</i>	Hydromet	
	<i>Bivalvia larvae</i>	EMI, SYKE, SPbSU	
	<i>Gastropoda larvae</i>	SPbSU, SYKE	
Nematoda	Nematoda unidentified	SYKE	
Rotifera	<i>Keratella cochlearis</i>	EMI, SYKE	
	<i>Keratella cochlearis baltica</i>	Hydromet	
	<i>Keratella cochlearis recurvispina</i>	Hydromet	
	<i>Keratella cruciformis</i>	EMI	
	<i>Keratella cruciformis eichwaldii</i>	SYKE	
	<i>Keratella quadrata platei</i>	SYKE, Hydromet	
	<i>Keratella quadrata quadrata</i>	SYKE	
	<i>Keratella quadrata frenzeli</i>	Hydromet	
	<i>Keratella quadrata</i>	EMI	
	<i>Keratella</i> spp.	SYKE	
	<i>Synchaeta baltica</i>	EMI, Hydromet, SPbSU	
	<i>Synchaeta monopus</i>	EMI, Hydromet, SYKE	

	<i>Synchaeta</i> spp.	SYKE
Ctenophora	<i>Mertensia ovum: cydippe</i>	EMI
Chaetognatha	<i>Parasagitta elegans</i>	SPbSU
Annelida	<i>Polychaeta</i> (larvae – observed, not counted)	SYKE, Hydromet
Cnidaria	<i>Aurelia aurita</i> (planula – observed, not counted)	SYKE

**ANNEX 3. ABUNDANCE OF ZOOPLANKTON TAXA IN THE SAMPLES**

Hyd: Hydromet; std: standard deviation; CV: coefficient of variation = std/mean.

Ind m <sup>-3</sup>	Aranda-sample							Salme-sample				
	EMI	SPbsU	SYKE	Hyd.	mean	std	CV%	EMI	SYKE	av.	std	CV%
Protista	0	0	11	0	3	5	173	0	1137	568	568	100
Cladocera	130	791	241	161	331	269	81	119	198	158	39	25
<i>Bosmina</i> spp.	125	761	224	152	316	260	82	106	181	143	37	26
<i>Cercopagis pengoi</i>	4	3	6	5	5	1	25	6	8	7	1	16
<i>Evadne anonyx</i>	0,28		10,7		5	5	95	1	0,9	1	0	6
<i>Evadne nordmanni</i>		27	obs	2	15	13	86	6	7,5	7	1	12
<i>P. polyphemoides</i>	0,04			2	1	1	96	0,1		0	0	0
Mysidacea	0,1	0	0,04	0	0,03	0,03	109	0,1	0,0	0	0	100
Copepoda (copepodites)	6021	7360	5612	5961	6239	666	11	4148	5272	4710	562	12
Calanoida	5959	7319	5538	5853	6167	683	11	4095	5242	4669	574	12
Calanoida cop i-iii	2219	2883	2208		2437	316	13	1994	2929	2462	467	19
Calanoida cop iv-v	3740	3982	3330		3684	269	7	2101	2313	2207	106	5
<i>Acartia</i> spp.	1638	4372	1792	1439	2310	1197	52	1387	2236	1811	425	23
<i>Centropages hamatus</i>	6	56	11	14	22	20	92	0	8	4	4	100
<i>Eurytemora</i>	3744	2491	3189	3761	3296	519	16	2360	2537	2449	89	4
<i>Limnocalanus</i>	2	2	2	3	2	0,5	22	1	2	1	1	45
<i>Pseudocalanus</i>	525	384	459	636	501	93	18	325	392	358	34	9
<i>Temora longicornis</i>	44	14	32	0	22	17	75	24	15	19	4	22
Calanoida unidentified	0	0	53	0	13	23	173	0	53	26	26	100
Cyclopoida	63	41	75	108	72	24	34	47	30	39	9	22
Harpacticoida								6		6	0	0
Copepoda nauplii	1613	5394	1611	1777	2599	1615	62	2808	3290	3049	241	8
Cirripedia				obs				12	obs			
Ostracoda				obs								
Chordata	88	0	288	0	94	118	125	106	226	166	60	36
Mollusca	13	17	0	obs	10	7	73	24	8	16	8	52
Nematoda	0	0	11	0	3	5	173	0	obs			
Rotifera	2025	179	1835	2813	1713	959	56	4095	2718	3406	688	20
<i>Keratella</i> spp.	225	0	320	342	222	135	61	602	535	568	34	6
<i>Synchaeta</i> spp.	1800	179	1515	2471	1491	833	56	3493	2184	2838	655	23
Ctenophora								0,5	0	0	0	100
Chaetognatha	0	3	0	0	1	1	173					
TOTAL	9988	13761	9907	10712	11092	1573	14	11455	13082	12269	814	7

## PART 4: ANALYTICAL INTERCALIBRATION 2014

Here follows the results from the analytical intercalibration performed by the SYKE proficiency testing service ProfTest in February, 2014. Data retrieved and presented by the written consent from the participating laboratories from Leivuori et al. (2014) Interlaboratory proficiency test NW/14/02. Natural waters I. Reports of the Finnish Environment Institute.

General information: 1 assigned value, 2 standard deviation amongst the participating laboratories, and 3 the number of participating laboratories. \*sample filtered through 0,4 µm filter.

z-value = (lab's result - assigned value) / samplewise-varying error term. Evaluation:

- $|z| < 2$       satisfactory
- $2 < |z| < 3$     questionable
- $|z| > 3$       unsatisfactory

Analyte	General information			SYKE		Hydromet		EMI		RSHU		MSI	
	1	2	3	z-value	Lab's result	z-value	Lab's result	z-value	Lab's result	z-value	Lab's result	z-value	Lab's result
NH <sub>4</sub> <sup>+</sup> (µg/l)	73,3	3,9	26	1,32	80,6	-2,42	60,0	-1,24	66,5			0,07	73,7
NO <sub>2</sub> <sup>-</sup> +NO <sub>3</sub> <sup>-</sup> (µg/l)	154	5,4	25	0,84	161	2,08	170	-0,46	151	-6,25	106	0,52	158
TOTN (µg/l)	452	25,7	26	0,59	472	3,78	580	-0,75	427			-0,10	449
pH	7,97	0,1	30	-0,93	7,88			-0,30	7,94	-1,10	7,86	0,10	7,98
PO <sub>4</sub> <sup>3-</sup> (µg/l)	21,6	0,8	24	-0,50	21,1			8,24	30,5	3,75	25,7	0,09	21,7
*PO <sub>4</sub> <sup>3-</sup> (diss, µg/l)	21,1	1,1	21	0,26	21,4	-2,94	18,0						
TOTP (µg/l)	26,6	2,0	24	-1,60	24,5					27,61	63,3	-1,76	24,3
*TOTP (diss, µg/l)	25,2	1,9	19	-2,06	22,6	-1,75	23,0						