

International Phytoplankton Intercomparison proficiency test in the abundance and composition of marine microalgae 2016 report PHY-ICN-16-MI1 VR 1.0



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1. Summary of results

• 82 analysts from 43 laboratories took part in this intercomparison exercise. 81 analysts returned sample results and 79 completed the online Hab quiz. There were 69 participants from laboratories across Europe, 5 from South America, 2 in Australia, 1 in New Zealand and 5 in Africa.

• Ten species were used in this test. These were the dinoflagellates *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen, *Prorocentrum triestinum* J.Schiller, *Karenia selliformis* A.J.Haywood, K.A.Steidinger & L.MacKenzie, *Karlodinium veneficum* (D.Ballantine) J.Larsen, *Dinophysis acuta* Ehrenberg and the diatoms *Pseudo-nitzschia australis* Frenguelli, *Guinardia delicatula* (Cleve) Hasle, *Chaetoceros didymus* Ehrenberg, *Coscinodiscus wailesii* Gran & Angst and *Thalassiosira gravida* Cleve.

• The cell counts of the species *Karlodinium veneficum* which did not past the minimum requirements for homogenization and stability were discounted for statistical purposes and also *Karenia selliformis* which did not preserve well in the samples was not used here. All the other species counts were used.

• The average and confidence limit for each test item was calculated using the robust algorithm in annex C of ISO13528 which takes into account the heterogeneity of the samples and the between samples standard deviation from the homogeneity and stability test. ISO 13528 is only valid for quantitative data. We have used the consensus values from the participants.

• All measurands passed the F-test except for *K.veneficum*. Only *A.ostenfeldii* passed the homogeneity test according to ISO13528 but they all passed the expanded criterion except for *K.veneficum*. The stability test was passed by 5 out of the 9 measurands but failed *K.veneficum*, *D.acuta*, *T.gravida* and *P.australis*. All measurands passed the stability test according to the expanded 13528:2015 except for *K.veneficum*.

• The consensus values new Standard deviation (STD) was used for all measurands regardless of the Pass/Fail flags from the homogeneity test.

• There were a small number of action signals across all measurands. 9 Red flags in total (1.4% of results), 22 (3.4%) yellow flags and 6 (0.93%) orange flags (Non-Ids) from 648 scores is evidence of good performance overall. Eight analysts did not pass the full test with a below 80% score. There is evidence of method bias on low cell density measurands due to the volume analysed.

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• The Ocean teacher online HAB quiz results suggests a high rate of proficiency. 68% of analysts achieved a score over 90% (Proficient). Another 21.5% of analysts above 80%, 8% between 70 and 80% and 2.5% needs improvement.

• There was good consensus on the various identifications of diatom species from images in questions 1 to 3. Although the images of *T.mobiliensis* and *C.densus* were the most difficult organisms to identify from these images, results suggest a good performance overall. In Questions 4 to 6, there were good overall marks on flagellate identification based on depictions. Q7-9 Good scores on Peridinioid terminology but difficulties with the lesser known Suessiaceae group. Q10-12 Problems identifying T.macroceros group (Q10) worst score(68.8% correct). Q12-15 Theory based on 1' and 2a plate for identification of Protoperidinium is understood but difficult to execute using images.

2. Introduction

The Intenational Phytoplankton Intercomparison or IPI (formerly known as Bequalm) study in 2016 was designed to test the ability of analysts to identify and enumerate correctly marine phytoplankton species in lugol's preserved water samples. As in previous years, samples have been spiked using laboratory cultures. Initially, there were ten species of interest in this intercomparison exercise.

These were; the dinoflagellates *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen, *Prorocentrum triestinum* J.Schiller, *Karenia selliformis* A.J.Haywood, K.A.Steidinger & L.MacKenzie, *Karlodinium veneficum* (D.Ballantine) J.Larsen, *Dinophysis acuta* Ehrenberg and the diatoms *Pseudo-nitzschia australis* Frenguelli, *Guinardia delicatula* (Cleve) Hasle, *Chaetoceros didymus* Ehrenberg, *Coscinodiscus wailesii* Gran & Angst and *Thalassiosira gravida* Cleve.

The collaboration between the Marine Institute in Ireland and the IOC UNESCO Centre for Science and Communication of Harmful algae in Denmark on the IPI exercise commenced in 2011. This collaboration involves the use of algal cultures from the Scandinavian Culture Collection of Algae and Protozoa in Copenhagen, the elaboration of a marine phytoplankton taxonomy quiz using the online platform 'Ocean Teacher' Global academy hosted by the IODE (International Oceanographic Data and information Exchange) office based in Oostende, Belgium, a project office of the IOC and the organization of a training workshop which is held annually to discuss the results of the intercomparison exercise and to provide training on phytoplankton taxonomy.

This workshop has become an important forum for phytoplankton taxonomists working on phytoplankton monitoring programmes from around the world to convene and be able to discuss taxonomical matters related to monitoring, new advances and finds, taxonomical nomenclature changes, looking at samples from different geographical areas and listen to relevant stories from other laboratories about harmful algal events in their regions of relevant ecological importance.

This workshop has been held in various locations in previous years but over the last 3 years, it has taken the format of a 3 days training workshop with at least 2 days dedicated to lectures on algal groups in rooms equipped with microscopes and using live cultures and preserved samples from participants and from locations across the globe (See Workshop agenda: Annex IV).

This year, 82 analysts from 43 laboratories took part in this intercomparison exercise. 81 analysts returned sample results and 79 completed the online Hab quiz. There were 69 participants from laboratories across Europe, 5 from South America, 2 in Australia, 1 in New Zealand and 5 in Africa. The list of participating laboratories can be found in Annex V and a breakdown of participation from each country in figure 1 below.



Figure 1: Breakdown participation per country of the Phytoplankton intercomparison exercise IPI 2016

This intercomparison exercise has been coded in accordance with defined protocols in the Marine Institute, for the purposes of quality traceability and auditing. The code assigned to the current study is PHY-ICN-16-MI1. PHY standing for phytoplankton, ICN for intercomparison, 16 refers to the year 2016, MI refers to the Marine Institute and 1 is a sequential number of intercomparisons for the year. So, 1 indicates the first intercomparison for the year 2016. As figure 2 indicates the number of IPI participants has increased appreciably since 2005 and the influence of the test has also been widened to all continents. In the last two years the number of participants have plateau out in and around the 80 plus mark and while the majority of laboratories come from European countries (84%), a sizeable 16% is made up from laboratories in Africa, South America and Oceania.



IPI Participants 2005-2016

Figure 2: IPI participation in the last 10 years

3. Materials and Methods

3.1 Sample preparation, homogenization and spiking

All samples were prepared following this protocol: The seawater used in this experiment was natural field water collected at Ballyvaughan pier, Galway bay, Ireland, filtered through 47mm GF/C Whatmann filters (Whatmann[™], Kent, UK), autoclaved (Systec V100, Wettenberg, Germany) and preserved using neutral Lugol's iodine solution (Clin-tech, Dublin, Ireland). The centrifuge tubes (50ml volume) were made up to the required volume with sterile filtered seawater containing neutral lugol's iodine. This was carried out using an automatic eppendorf multipipette Xstream (0-50ml) (Eppendorf, Hamburg, Germany) and the volume weighted in a calibrated balance (ME414S Sartorius, AG Gottingen, Germany). The density of seawater was considered for this purpose to be 1.025g/ml. The final volume of each sample was 45 ml approximately before spiking.

A stock solution for each of the ten species was prepared using 50ml glass screw top bottles (Duran®, Mainz, Germany). Then, a working stock containing the ten species to the required cell concentration was prepared using a measured aliquot from each stock solution into a 2l Schott glass bottle. Then, the working stock was homogenized and sub-divided into five replicate working stocks containing 400ml each. These working stocks were then inverted 100 times to homogenise the samples and 5ml aliquots were pipetted out after each 100 times inversion using a calibrated 5ml pipette (Gilson, Middleton, USA) with 1-10ml pipette tips (Eppendorf, Hamburg, Germany) The 5ml aliquots were dispensed into the 50ml centrifuge tubes (Sardstedt, Nümbrech, Germany) containing 45ml seawater.

Samples were capped and labeled. Parafilm was used around the neck of the centrifuge tube to avoid water loss through evaporation or leaking, placed in padded envelopes and couriered via TNT or DHL couriers for a one day delivery across the world, in order for all the laboratories to have approximately the same arrival time.

3.2 Culture material, treatments and replicates.

Most of the laboratory cultures used in the 2016 exercise have been collected in Galway bay and Bantry bay during the months of February and May 2016 except for the *A.ostenfeldii* culture from the CCMP culture collection in Scotland, the *Karlodinium veneficum* culture from the SCCAP culture collection in Denmark and *Dinophysis acuta* culture from the IEO, Vigo, Spain. The diatom cultures were isolated from samples collected using the micro-pipette technique into unialgal cultures. Most species were identified through light microscopy techniques using an inverted microscope Olympus IX-51 and a compound research Olympus microscope BX-53 (Olympus, UK) except for *Pseudo-nitzschia australis* which was confirmed to species level using qPCR (Roche Lightcycler) species specific gene probes.

A total of 500 samples were produced for the enumeration and identification study. Each participant was sent a set of four samples, three for analysis plus one spare for a total of 328 samples to 43 laboratories. Another 15 samples were used by the expert laboratory to carry out the homogeneity and stability test. The data generated by this laboratory was used to test the homogeneity and stability of the samples. A minimum of 10 samples (50ml volume) were necessary for the homogeneity test and a minimum of 3 samples for the stability test. Samples had to be divided in two portions of 25ml each.

A time delay between the homogeneity test and the stability test is required. ISO 13528 indicates that this delay should be similar to that experienced by the participants in the test. As analysts have a month to return results from sample receipt, it was decided that this time delayed should be of one month as well.

3.3 Cell concentrations

Preliminary cell counts from the original stock solutions were made to establish the cell concentration of each species and this was carried out using a glass Sedgewick-Rafter cell counting chamber (Pyser-SGI, Kent, UK) to ascertain an approximation of the cell concentration of each species in the samples.

3.4 Sample randomization

All samples were allocated randomly to the participants using Minitab® Statistical Software Vr16.0 randomization tool.

3.5 Forms and instructions

A set of instructions and forms required were sent via e-mail to all the analysts to complete the exercise including their unique identifiable laboratory and analyst code. Form 1 (Annex I) to confirm the receipt of materials; number and condition of samples and correct sample code. Form 2 (Annex II) in an Excel spreadsheet format to input species composition and calculate abundance for each species. Form 2 was used for the identification and enumeration part of the exercise. All analysts were asked to read and follow the instructions (Annex III) before commencing the test.

At the end of the exercise and with the publication of this report, analysts will be issued with a statement of performance certificate (See Annex VI) which is tailored specifically for each test. This is an important document for auditing purposes and ongoing competency.

3.6 Statistical analysis

Statistical analysis was carried out using PROlab Plus version 2.14, dedicated software for the statistical analysis of intercalibration and proficiency testing exercises from Quodata, Minitab® Statistical Software Vr16.0 and Microsoft office Excel 2007.

We followed the standard ISO normative 13528 which describes the statistical methods to be used in proficiency testing by interlaboratory comparisons. Here, we use this standard to determine and assess the homogeneity and stability of the samples, what to do with outliers, determining assigned values and calculating their standard uncertainty. Comparing these values with their standard uncertainty and calculating the performance statistics for the test through graphical representation and the combination of performance scores.

The statistical analysis of the data and final scores generated from this exercise has been carried out using the consensus values from the participants. The main difference with previous years is that by using ISO13528, the consensus values from the participants must undergo several transformations before they can be used to generate Z-scores.

The main transformation is the use of iteration to arrive at robust averages and standard deviations for each test item. This process allows for outliers and missing values to be dealt with, and it also allows for the heterogeneity of the samples to be taken into consideration when calculating these values.

3.7 IPI Ocean teacher online HAB quiz.

The online HAB quiz was organized and set up by Jacob Larsen (IOC UNESCO, Centre for Science and Communication on Harmful Algae, Denmark) and Rafael Salas (Marine Institute, Ireland). The exercise was prepared in the web platform 'Ocean teacher'. The Ocean teacher training facility is run by the IODE (International Oceanographic Data and information Exchange) office based in Oostende, Belgium. The IODE and IOC organize some collaborative activities among them, the IOC training courses on toxic algae and the IPI online HAB quiz. The online quiz uses the open source software Moodle Vr2.0 (https://moodle.org).

First time participants had to register in the following web address: <u>http://classroom.oceanteacher.org/</u> before allowed to access the quiz content, while analysts already registered from previous years, could go directly to the login page. Once registered, participants could login into the site and using a password, able to access the quiz. Three months time was given to analysts to register, complete and submit the online quiz. The course itself was found under the courses tab in the main menu page. Analysts could link to the International Phytoplankton Intercomparison and quiz IPI 2016 HAB quiz content from here.

The test itself consisted of 15 questions (see Annex XVII). Most questions used in this quiz this year were 'matching type' Q1 to 15 except for Q9 which was Multiple choice. Matching questions have dropdown

menus including an array of answers which analysts must choose from, while in multiple choice type questions the participant must fill in the right choices. All questions have equal value and the quiz have a maximum grade of 100% for a perfect score.

The online quiz can only be submitted once. After that, no changes can be made. However, analysts can login and out as many times as they wish throughout the period of time allocated and changes to the quiz can be saved and accessed at a later stage, so the quiz doesn't have to be completed in one sitting.

4. Results

4.1 Homogeneity and stability study

The procedure for a homogeneity and stability test is recorded in annex b (pg 60) of ISO13528. The assessment criteria for suitability, is also explained here. See Annex VII to see all the results from the homogeneity and stability test for each measurand.

The calculations have been carried out using ProLab Plus version 2.14 and the reports for homogeneity and stability are given separately for each measurand. The top of the report gives you information on the measurand, mean and analytical standard deviation for the homogeneity analysis and the homogeneity and stability mean comparison in the stability analysis. The reports also show the target standard deviation for each measurand which in this case was calculated manually using the consensus results of the participants and taking into consideration the heterogeneity of the samples as will be explained later.

The middle part of the report gives you the results of the different tests. ProLab Plus calculates whether the data has passed the criteria for the F-test, and ISO13528. The bottom part of the report is the actual graphical representation of the sample results as box plots. The homogeneity test shows the 10 samples analysed for this test and calculates the heterogeneity standard deviation (SD between samples) and the analytical standard deviation (SD within samples). The stability test graph show the 10 samples of the homogeneity test plus the 3 samples of the stability test, thirteen in total and compare their mean values. This is done for each measurand.

Table 1 above shows the pass/fail flags for each measurand. All measurands passed the F-test except for *K.veneficum*. Only *A.ostenfeldii* passed the homogeneity test according to ISO13528 but they all passed the expanded criterion except for *K.veneficum*. The stability test was passed by 5 out of the 9 measurands but

failed *K.veneficum*, *D.acuta*, *T.gravida* and *P.australis*. All measurands passed the stability test according to the expanded 13528:2015 except for *K.veneficum*.

According to ISO 13528:2015, the heterogeneity standard deviation s(sample) between the proficiency test items should not exceed 30 % of the standard deviation for proficiency assessment. If the homogeneity test fails, the heterogeneity standard deviation has to be taken into account when calculating the standard deviation for the measurand. The consensus values new heterogeneity standard deviation (STD) was used for all measurands regardless of the Pass/Fail on the homogeneity test.

ISO13528	F-test	Homogeneity test ISO 13528	ISO 13528:2015 test for adequate homogeneity	ISO 13528:2015 test for adequate heterogeneity	Stability test 13528:2015	Stability test expanded 13528:2015
Dinophysis acuta	ok	not ok	ok	ok	not ok	ok
Prorocentrum triestinum	ok	not ok	not ok	ok	ok	ok
Alexandrium ostenfeldii	ok	ok	not ok	ok	ok	ok
Karlodinium veneficum	not ok	not ok	not ok	not ok	not ok	not ok
Guinardia delicatula	ok	not ok	not ok	ok	ok	ok
Thalassiosira gravida	ok	not ok	not ok	ok	not ok	ok
Chaetoceros didymus	ok	not ok	not ok	ok	Pass	ok
Coscinodiscus wallesii	ok	not ok	not ok	ok	Pass	ok
Pseudo-nitzschia australis	ok	not ok	not ok	ok	not ok	ok

Table 1: Homogeneity and stability pass/fail test

For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.

4.2 Outliers and missing values

Outliers in the data have been addressed by using the robust analysis as set out in Annex C algorithm A + S of ISO 13528. The robust estimates for this exercise have been derived by iterative calculation, that is, by convergence of the modified data (Annex IX) for each measurand.

In relation to missing values, the standard proposes that participants must report 0.59 n replicate measurements, so in the case of three replicates, at least two replicate results from each measurand must be obtained from each participant for the data to be included in the statistical calculations. If this rule is not

fulfilled results from these participants won't be included in the calculation of statistics that affect other laboratories but they may be used for the calculation of their own, for example z-scores.

4.3 Analysts' Data

The results of the participants were collated using Excel spreadsheets. 81 analysts from 43 laboratories returned results for this exercise. There were ten measurands in the samples but only eight of these measurands were used for statistical analysis as explained earlier *Karenia selliformis* did not preserve well and *Karlodinium veneficum* did not homogenize well. The dinoflagellates *Alexandrium ostenfeldii, Prorocentrum triestinum, Dinophysis acuta* and the diatoms *Pseudo-nitzschia australis, Guinardia delicatula, Chaetoceros didymus, Coscinodiscus wailesii* and *Thalassiosira gravida* were included in our calculations.

The table of results from all participants can be found in Annex VIII at the end of this report. The average of the participant replicate results for each measurand were used to calculate the robust averages and standard deviations first by iteration, which then were used to calculate the confidence limits for the Z-scores (See Annex X).

For the purpose of this exercise we have used the consensus standard deviation from the participants and we have calculated the new standard deviation for each test item by adding the between samples standard deviation from the homogeneity test according to the formula below (A) from ISO13528.

$$\sigma_{r1} = \sqrt{\sigma_r^2 + s_s^2}$$

(A)

Where;

 σ_{r1} = the new SD for the homogeneity test σ_r = between samples Standard deviation and

Ss = the robust standard deviation for the test

Table 2 below show the results which are used to generate the confidence limits of this test for each measurand. These values are calculated using the robust analysis using algorithm A +S from annex C of the standard ISO13528. The calculations are generated by iteration and can be found for each measurand in this report in annex IX.

	Dinophysis	Prorocentrum	Alexandrium	Karlodinium	Guinardia
Species	acuta	triestinum	ostenfeldii	veneficum	delicatula
Consensus SD	328	1509	309	1720	115
Consensus SD + Between SD	421	1639	318	2846	129
	Thalassiosir	Chaetoceros	Coscinodiscus		
Species	a gravida	didymus	wallesii	Pseudo-nitzschia australis	
Consensus SD	1121	488	25	1442	2
Consensus SD + Between SD	1328	555	37	1680)

Table 2: Standard deviations for each measurand based on consensus values (SD) and consensus values plus the between sample standard deviation (new SD) calculated using Excel.

4.4 Assigned value and its standard uncertainty

The assigned values (robust mean and standard deviation) for a test material is calculated as explained before using algorithm A in annex C from the consensus values of the participants (Annex IX). The standard uncertainty of the assigned value can then be calculated using the equation (B) below;

$$u_X = 1,25 \times s^* / \sqrt{p}$$

Where;

B)

 \mathcal{U}_{x} = Standard uncertainty of the assigned value,

 s^* = robust standard deviation for the test

p = number of analysts

									Pseudo-
	Dinophysis	Prorocentrum	Alexandrium	Karlodinium	Guinardia	Thalassiosira	Chaetoceros	Coscinodiscus	nitzschia
	acuta	triestinum	ostenfeldii	veneficum	delicatula	gravida	didymus	wailesii	australis
Robust mean x*	2834	5111	1632	3377	324	5570	903	50	5406
Robust Stdev s*	328	1509	309	1720	115	1121	488	25	1442
Standard Ux	46	211	43	250	16	156	69	4	200
n=	81	80	80	74	78	81	79	71	81
if Ux < 0.3xSTdev	98	453	93	516	35	336	146	8	433
then Ux is negligible	neg	neg	neg	neg	neg	neg	neg	neg	neg
The equation is satisfied in all cases									

Table 3: Assigned values and standard uncertainties for the test.

If U_x is less than 0.3 times the standard deviation for the test, then this uncertainty is negligible for the test material. In our case, all our test materials satisfy the equation (Table 3).

4.5 Comparison of the assigned value

When the consensus values from the participants are used to calculate the standard uncertainty of the assigned values, the values can then be compared against a reference value from an expert laboratory. As we don't have a reference value as such, we used the homogeneity test results to compare these values against the values calculated by the participants using equation (C) below:

$$\sqrt{\frac{(1,25s^*)^2}{p} + u_X^2}$$

Where;

 u_x = Standard uncertainty of the assigned value, s^* = robust standard deviation for the test p = number of analysts

ISO13528 says that if the difference between the consensus values and the reference values (homogeneity test values in our case) is more than twice its uncertainty, then possible reasons need to be sought regarding bias. In our comparison, three cell counts out of nine satisfy the equation (Table 4- green bottom).

									Pseudo-
	Dinophysis	Prorocentrum	Alexandrium	Karlodinium	Guinardia	Thalassiosira	Chaetoceros	Coscinodiscus	nitzschia
	acuta	triestinum	ostenfeldii	veneficum	delicatula	gravida	didymus	wailesii	australis
Robust mean x*	2834	5111	1632	3377	324	5570	903	50	5406
Robust Stdev s*	328	1509	309	1720	115	1121	488	25	1442
Standard Ux	46	211	43	250	16	156	69	4	200
n=	81	80	80	74	78	81	79	71	81
if Ux < 0.3xSTdev	98	453	93	516	35	336	146	8	433
then Ux is negligible	neg	neg	neg	neg	neg	neg	neg	neg	neg
The equation is satisfied	d in all cases								
Cumulative distribution	function cut	off points for nor	rmal distributio	on					
x *-1.5s*	2342	2848	1168	797	152	3889	171	13	3242
x *+1.5s*	3326	7374	2095	5957	497	7251	1635	88	7569
									Pseudo-
	Dinophysis	Prorocentrum	Alexandrium	Karlodinium	Guinardia	Thalassiosira	Chaetoceros	Coscinodiscus	nitzschia
Homogeneity test	acuta	triestinum	ostenfeldii	veneficum	delicatula	gravida	didymus	wailesii	australis
Reference value mean	2756	5956	1786	12404	230	4804	928	46	4284
Reference value stdev	418	864	186	2662	101	1033	446	38	1338
	Comparison	with assigned va	lue						
									Pseudo-
	Dinophysis	Prorocentrum	Alexandrium	Karlodinium	Guinardia	Thalassiosira	Chaetoceros	Coscinodiscus	nitzschia
	acuta	triestinum	ostenfeldii	veneficum	delicatula	gravida	didymus	wallesii	australis
x *-X	78	845	154	9027	94	766	25	4	1122
Uncertainty of diff.	64	298	61	353	23	220	97	5	283
2* Uncertainty of diff.	129	596	122	707	46	440	194	10	567

Table 4: Comparison of the assigned value.

4.6 Calculation of performance statistics

The performance statistics for the exercise have been calculated using ProLab Plus software version 2.14. The summary table of all the Z-scores can be found in Annex X of this report. The summary of laboratory means and statistical parameters (Annex XI) show the results by measurand and analyst of all the results for the test including the Z-scores and outliers, the statistical method used for the data (Q Huber), means and standard deviations, measures of repeatability and reproducibility for each measurand, number of participants and other relevant information on the test. The graphical summary for each measurand by analyst can be found in Annex XII of this report.

4.6.1 Z-scores

The z-scores derived using the robust averages and standard deviations can be found in annex X. Any results in blue are within the specification of the test (2SD). The yellow triangles indicate warning signals (outside 2SD), red triangles indicate action signals (outside 3SD) and orange triangles indicate non-identifications. Correct identification of measurands are an important part of the test and will give rise to orange flags (Non-identified) and failed items.

There were a small number of action signals across all measurands. 9 Red flags in total (1.4% of results), 22 (3.4%) yellow flags and 6 (0.93%) orange flags (Non-Ids) from 648 scores is evidence of good performance overall. Eight analysts did not pass the full test with a below 80% score There is evidence of method bias on low cell density measurands due to the volume analysed. Please note, do not use small sample aliquots for measurands spiked at the limit of detection of the method.

Overall, all analysts passed the test except for eight analysts which failed some items and are below the 80% of results necessary to pass. Analysts 20, 8 and 12 have 75% (first 2) and 71% correct answers and are just below the threshold for the test. Analyst 60 (2 yellow and 1 orange flag) 62% correct and analysts 19 (4 yellow flags), 31 (2 red and 1 yellow flag) and 51 (4 yellow flags) have a correct rate of 50% need improvement in the next round. Analyst 91 with 25% correct answers only (2 red flags and 4 yellow flags) will need substantial improvement in the next round. The results of this analyst suggest a systematic positive bias or overestimation of measurands and will need to improve their analytical technique. This has to be seen within the contest of performance over several rounds and while improvement is necessary it is also important to remark that some of these analysts were participating in the scheme for the first time. See Annex XI: Performance statistics of the test.

4.7 Combined performance scores

Mandel's h and k statistic present measures for graphically surveying the consistency of the data for all measurands in the test (Annex XIV). Mandel's h statistics determines the differences between the mean values of all the laboratories and measurand combinations and it may point out at particular patterns for specific laboratories. In this graph, laboratories may have positive or negative values. Laboratories with large all-positive values or all-negative values for all measurands may indicate laboratory bias.

The k statistics only produce positive results, zero is the baseline and it looks at repeatability precision between measurands. Generally analysts with larger values tend to have poorer repeatability precision between replicates than the consensus mean values.

4.7.1 Relative Laboratory Performance (RLP) and Rescaled Sum of Z-scores (RSZ)

The chart of RLP against RSZ (Annex XV) for all measurands combined shows systematic laboratory bias. Laboratories dotted within the green colored area in the graph are within the consensus values shown by the analysts. Those outside it are showing a systematic bias towards over or under-estimating their counts in the samples, suggesting some kind of methodology bias.

4.7.2 Plots of repeatability standard deviation

The plots of repeatability standard deviations are used to identify analysts whose average and standard deviations are unusual from the consensus. They assume that the data is normally distributed and the null hypothesis is that there are no differences between the analyst means and standard deviations using the van Nuland circle technique (Annex XVI) for each measurand. The correlation between means and standard deviations from the consensus is reasonable for most measurands with a small number of outlier results but not discernible bias. There is however poor repeatability for the *P.triestinum* and *T.gravida* cell counts across the mean in both directions (over- and underestimation) and also a large positive bias for *C.didymus*.

4.8 Qualitative data

Table 5 shows the answers given by analysts on the identification of the measurands in the samples. Analysts were asked to give their answers to species level but for the purpose of the exercise and final marks, a correct answer at genus level is sufficient.

Species id	Number	%	Species id	Number	%
Dinophysis acuta	81	100	Guinardia delicatula	69	85.19
			Guinardia sp.	6	7.41
Species id	Number	%	Rhizosolenia delicatula	2	2.47
Prorocentrum triestinum	77	95.06	Rhizosolenia fragilissima	1	1.23
Prorocentrum gracile	1	1.23	NR	3	3.70
Prorocentrum micans	2	2.47			
NR	1	1.23	Species id	Number	%
Species id	Number	%	Chaetoceros didymus	63	77.78
Alexandrium ostenfeldii	43	53.09	Chaetoceros diadema	6	7.41
Alexandrium tamutum	16	19.75	Chaetoceros decipiens	3	3.70
Alexandrium minutum	12	14.81	Chaetoceros brevis	2	2.47
Alexandrium tamarense	5	6.17	Chaetoceros ceratosporus	1	1.23
Heterocapsa sp.	2	2.47	Chaetoceros constrictus	1	1.23
Scrippsiella hangoei	1	1.23	Chaetoceros debilis	2	2.47
Scrippsiella sp.	1	1.23	Chaetoceros lorenzianus	1	1.23
Pentapharsodinium dalei	1	1.23	NR	2	2.47
Species id	Number	%	Species id	Number	%
Karlodinium veneficum	58	71.60	Coscinodiscus wailesii	63	77.78
Karlodinium armiger	8	9.88	NR	9	11.11
Karlodinium micrum	3	3.70	C. concinnus	4	4.94
Karlodinium sp.	1	1.23	C. granii	4	4.94
Karenia digitata	1	1.23	Coscinodiscus sp.	1	1.23
Small flagellate	1	1.23			
Heterosigma akashiwo	1	1.23	Species id	Number	%
Naked	1	1.23	Pseudo-nitzschia seriata group	60	74.07
Scripsiella sp.	1	1.23	Pseudo-nitzschia australis	14	17.28
NR	6	7.41	P. multiseries	1	1.23
Species id	Number	%	P. seriata	4	4.94
Thalassiosira gravida/rotul	51	62.96	P. fraudulenta	1	1.23
Thalassiosira sp.	30	37.04	P. Pungens	1	1.23

Table 5: Qualitative data by measurand

4.9 Ocean Teacher online HAB quiz

The online HAB quiz consisted of 15 questions; annex XVII shows the questions and right answers for the online HAB quiz and annex XVIII show the final grades. 79 of 82 analysts submitted this quiz. Most questions in this quiz were matching types except for question 9 that was a multiple choice question. Questions 1-3 tested analysts on their identification ability of diatom species. Tables 6 show the actual response given to these questions, the analyst count for a particular answer and the percentage frequency of that answer.

There were no difficulties on identifying the phytoplankton species depicted in Q1 and Q2. In Q3 images of *T.mobiliensis* and *C.densus* caused most problems. *T.mobiliensis* and *T.regia* are very similar species but the former is smaller in size than the latter. The scale bar is the clue here. *C.densus* is markedly different to *C.convolutus*. In *C.densus* the valves are flat and the foramina, if present it is narrow with tightly packed cells, it can be confused with *C.eibenii* but not with *C.convolutus*. In *C.convolutus*, the chains are twisted and the cells are heterovalvate with one highly vaulted and the other flat. Also, the setae originate near the valve center and not to the corners as in *C.densus*.

Q1	Model response	Actual response	Partial credit	Count	Frequency
631	Image 1: Chaetoceros didymus	Chaetoceros didymus	25.00%	76	96.20%
631	Image 1: Chaetoceros didymus	Pleurosigma sp.	0.00%	1	1.27%
631	Image 1: Chaetoceros didymus	Chaetoceros lauderii	0.00%	1	1.27%
631	Image 1: Chaetoceros didymus	Chaetoceros lorenzianus	0.00%	1	1.27%
632	lmage 2: Dictyocha fibula	Dictyocha fibula	25.00%	77	97.47%
632	lmage 2: Dictyocha fibula	Dictyocha speculum	0.00%	2	2.53%
633	Image 3: Mediopyxis sp.	Mediopyxis sp.	25.00%	76	96.20%
633	Image 3: Mediopyxis sp.	Bellerochea malleus	0.00%	2	2.53%
633	Image 3: Mediopyxis sp.	Lithodesmium undulatum	0.00%	1	1.27%
634	Image 4: Pleurosigma sp.	Pleurosigma sp.	25.00%	79	100.00%
Q2	Model response	Actual response	Partial credit	Count	Frequency
619	Image 1: Chaetoceros danicus	Chaetoceros danicus	25.00%	79	100.00%
620	Image 2: Grammatophora marina	Grammatophora marina	25.00%	79	100.00%
621	Image 3: Licmophora gracilis	Licmophora gracilis	25.00%	78	98.73%
621	Image 3: Licmophora gracilis	Gomphonema sp.	0.00%	1	1.27%
622	Image 4: Chaetoceros peruvianus	Chaetoceros peruvianus	25.00%	78	98.73%
622	Image 4: Chaetoceros peruvianus	Chaetoceros densus	0.00%	1	1.27%
Q3	Model response	Actual response	Partial credit	Count	Frequency
643	Image 1: Meuniera membranacea	Meuniera membranacea	25.00%	79	100.00%
644	Image 2: Trieres mobiliensis	Trieres mobiliensis	25.00%	47	59.49%
644	Image 2: Trieres mobiliensis	Odontella aurita	0.00%	15	18.99%
644	Image 2: Trieres mobiliensis	Trieres regia	0.00%	10	12.66%
644	Image 2: Trieres mobiliensis	Odontella sinensis	0.00%	7	8.86%
645	Image 3: Chaetoceros densus	Chaetoceros densus	25.00%	52	65.82%
645	Image 3: Chaetoceros densus	Chaetoceros convolutus	0.00%	22	27.85%
645	Image 3: Chaetoceros densus	Chaetoceros lauderii	0.00%	3	3.80%
645	Image 3: Chaetoceros densus	Neocalyptrella robusta	0.00%	1	1.27%
645	Image 3: Chaetoceros densus	Chaetoceros lorenzianus	0.00%	1	1.27%
646	Image 4: Neocalyptrella robusta	Neocalyptrella robusta	25.00%	77	97.47%
646	Image 4: Chaetoceros convolutus	Chaetoceros convolutus	0.00%	2	2.53%

Table 6: Questions 1-3 answers

Questions 4 to 6 (Table 7) depicted small flagellates of diverse families and analysts were asked to identify them. In Q4 three organisms of the class Chlorophyceae were depicted. Image 2 Brachiomonas was easily

identifiable because of is characteristic shape but images 1 and 3 were confused by 5 analysts. Chlamydomonas however differs from Dunaliella on having a 'Papilla'. In Q5 on euglenophyte genera there were no difficulties here. *Euglena* has one flagellum only and *Eutreptiella* and *Eutriepta* can be separated by the way the flagella wraps around the cell in *Eutreptiella*. In Q6 on prasinophytes answers were mainly correct. These very small organisms can only be recognised by looking at the way the swim, their flagellar differences, eyespot presence, chloroplasts number and storage products.

Q4	Model response	Actual response	Partial credit	Count	Frequency
656	Image 1 belongs to the genus: Chlamydomonas	Chlamydomonas	33.33%	74	93.67%
656	Image 1 belongs to the genus: Chlamydomonas	Dunaliella	0.00%	5	6.33%
657	Image 2 belongs to the genus: Brachiomonas	Brachiomonas	33.33%	79	100.00%
658	Image 3 belongs to the genus: Dunaliella	Dunaliella	33.33%	74	93.67%
658	Image 3 belongs to the genus: Dunaliella	Chlamydomonas	0.00%	5	6.33%
Q5	Model response	Actual response	Partial credit	Count	Frequency
675	Image A belongs to the genus: Eutreptiella	Eutreptiella	33.33%	79	100.00%
676	Image B belongs to the genus: Eutreptia	Eutreptia	33.33%	78	98.73%
676	Image B belongs to the genus: Eutreptiella	Eutreptiella	0.00%	1	1.27%
677	Image C belongs to the genus: Euglena	Euglena	33.33%	79	100.00%
Q6	Model response	Actual response	Partial credit	Count	Frequency
681	Image A belongs to the genus: Pyramimonas	Pyramimonas	11.11%	79	100.00%
682	Image B belongs to the genus: Nephroselmis	Nephroselmis	11.11%	77	97.47%
682	Image B belongs to the genus: Nephroselmis	Mantoniella	0.00%	1	1.27%
682	Image B belongs to the genus: Nephroselmis	Mamiella	0.00%	1	1.27%
683	Image C belongs to the genus: Pterosperma	Pterosperma	11.11%	78	98.73%
683	Image C belongs to the genus: Pterosperma	Pyramimonas	0.00%	1	1.27%
683	Image C belongs to the genus: Pterosperma	Pyramimonas	0.00%	1	1.27%
684	Image D belongs to the genus: Mantoniella	Mantoniella	11.11%	76	96.20%
684	Image D belongs to the genus: Mantoniella	Nephroselmis	0.00%	2	2.53%
684	Image D belongs to the genus: Mantoniella	Micromonas	0.00%	1	1.27%
685	Image E belongs to the genus: Mamiella	Mamiella	11.11%	79	100.00%
686	Image F belongs to the genus: Micromonas	Micromonas	11.11%	77	97.47%
686	Image F belongs to the genus: Micromonas	Mantoniella	0.00%	2	2.53%
688	Image H belongs to the genus: Tetraselmis	Tetraselmis	11.11%	79	100.00%
689	Image I belongs to the genus: Pseudoscourfieldia	Pseudoscourfieldia	11.11%	79	100.00%

Table 7: Questions 4-6 model response table.

Q7 on Peridinioid terminology (table 7) and Q8 (table 8) on kofoidean tabulation of armoured

dinoflagellates analysts had near perfect scores. Q9 (table 10) the only multiple choice question in the quiz caused more problems. The Suessiaceae are a lesser known group of the dinoflagellates and they are similar to naked dinoflagellates, however they do possess a series of plates which are revealed under SEM analysis. These plates are dissimilar to those of armoured dinoflagellates. There were 76% of correct answers for this question. It was the second most difficult of all the questions in the quiz.

Q7	Model response	Actual response	Partial credit	Count Frequency
91	The apical plates	The apical plates	20.00%	78 98.73%
91	The apical plates	The anterior intercalary plates	0.00%	1 1.27%
92	2 The anterior intercalary plates	The anterior intercalary plates	20.00%	78 98.73%
92	2 The anterior intercalary plates	The postcingular plates	0.00%	1 1.27%
93	3 The precingular plates	The precingular plates	20.00%	78 98.73%
93	3 The precingular plates	The apical plates	0.00%	1 1.27%
94	1 The postcingular plates	The postcingular plates	20.00%	78 98.73%
94	1 The postcingular plates	The precingular plates	0.00%	1 1.27%
95	5 The antapical plates	The antapical plates	20.00%	79 100.00%

Table 8. Model responses to numerical question 7

Q8	Model response	Actual response	Partial credit	Count	Frequency
593	Alexandrium	Alexandrium	12.50%	79	100.00%
594	Protoperidinium	Protoperidinium	12.50%	78	98.73%
594	Protoperidinium	Scrippsiella	0.00%	1	1.27%
595	Podolampas	Podolampas	12.50%	75	94.94%
595	Podolampas	Diplopsalis	0.00%	2	2.53%
595	Podolampas	Dinophysis	0.00%	1	1.27%
595	[No response]	[No response]	0.00%	1	1.27%
596	Gonyaulax	Gonyaulax	12.50%	77	97.47%
596	Gonyaulax	Gambierdiscus	0.00%	1	1.27%
596	[No response]	[No response]	0.00%	1	1.27%
597	Amphidoma	Amphidoma	12.50%	77	97.47%
597	Amphidoma	Goniodoma	0.00%	1	1.27%
597	[No response]	[No response]	0.00%	1	1.27%
598	Scrippsiella	Scrippsiella	12.50%	78	98.73%
598	Scrippsiella	Protoperidinium	0.00%	1	1.27%
599	Lingulodinium	Lingulodinium	12.50%	78	98.73%
599	Lingulodinium	Gonyaulax	0.00%	1	1.27%
600	Azadinium	Azadinium	12.50%	78	98.73%
600	Azadinium	Dinophysis	0.00%	1	1.27%

Table 9. Model answers for question 8

Q9	Response	Partial credit	Count	Frequency
714	Elongated Apical vesicles	25.00%	71	89.87%
715	Thecal pores	0.00%	3	3.80%
726	sulcal plates	0.00%	3	3.80%
713	Eyespot	0.00%	2	2.53%
717	Latitudinal series	25.00%	46	58.23%
712	Amphiesmal vesicles	0.00%	24	30.38%
719	longitudinal series	0.00%	6	7.59%
718	Thecal series	0.00%	3	3.80%
720	Suessiaceae	25.00%	67	84.81%
722	Kareniaceae	0.00%	8	10.13%
721	Gymnodiniaceae	0.00%	2	2.53%
728	Peridiniaceae	0.00%	2	2.53%
723	x plate	25.00%	57	72.15%
716	Apical groove	0.00%	12	15.19%
724	1' apical	0.00%	7	8.86%
725	1 cingular	0.00%	2	2.53%
727	3 antapical plate	0.00%	1	1.27%

Table 10. Model responses for question 9

Q10-12 on the identification of species belonging to the genus *Tripos* caused some problems. In Q10 *T.macroceros* and *T.massiliensis* were confused by 38% of participants. The notable difference between these two is that *T.massiliensis* antapical horns diverge from the apical horn forming a 'W' shape between then, whereas in *T.macroceros* antapical horns, these run more or less parallel to the apical horn. Also, the way the antapical horns appear and bend from the hypotheca are different in both. This was the worst scored question in the quiz with 69% correct answers only. This is however normal as the macroceros group is the most difficult to identify group of the *Tripos* genera.

In Q11 they were also identification issues between *T.brevis* and *T.pulchellus* but *T.pulchellus* has very short antapical horns with the right horn very close the main body, very short antapical horns and very straight apical one compared to *T.brevis. T.mullerii* has pointed antapical horns and *T.paradoxides* is quite conspicuous. Q12 did not caused major issues as *T.furca* and *T.lineatus* are very common and easily recognizable members of the furca group. The fusus group are also quite distinct and easily recognizable.

Q10	Model response	Actual response	Partial credit	Count	Frequency
607	Species 1: Tripos massiliensis	Tripos massiliensis	33.33%	45	56.96%
607	Species 1: Tripos massiliensis	Tripos macroceros	0.00%	30	37.97%
607	Species 1: Tripos massiliensis	Tripos trichoceros	0.00%	4	5.06%
608	Species 2: Tripos macroceros	Tripos macroceros	33.33%	45	56.96%
608	Species 2: Tripos macroceros	Tripos massiliensis	0.00%	27	34.18%
608	Species 2: Tripos macroceros	Tripos trichoceros	0.00%	4	5.06%
608	Species 2: Tripos macroceros	Tripos brevis	0.00%	2	2.53%
608	Species 2: Tripos macroceros	Tripos longirostrus	0.00%	1	1.27%
609	Species 3: Tripos trichoceros	Tripos trichoceros	33.33%	72	91.14%
609	Species 3: Tripos trichoceros	Tripos macroceros	0.00%	4	5.06%
609	Species 3: Tripos trichoceros	Tripos massiliensis	0.00%	3	3.80%
Q11	Model response	Actual response	Partial credit	Count	Frequency
662	Species 4: Tripos brevis	Tripos brevis	33.33%	61	77.22%
662	Species 4: Tripos brevis	Tripos pulchellus	0.00%	10	12.66%
662	Species 4: Tripos brevis	Tripos muellerii	0.00%	8	10.13%
663	Species 5: Tripos muellerii	Tripos muellerii	33.33%	67	84.81%
663	Species 5: Tripos muellerii	Tripos pulchellus	0.00%	6	7.59%
663	Species 5: Tripos muellerii	Tripos brevis	0.00%	5	6.33%
663	Species 5: Tripos muellerii	Tripos hexacanthus	0.00%	1	1.27%
664	Species 6: Tripos paradoxides	Tripos paradoxides	33.33%	79	100.00%
Q12	Model response	Actual response	Partial credit	Count	Frequency
717	Species 7: Tripos extensus	Tripos extensus	16.67%	77	97.47%
717	Species 7: Tripos extensus	Tripos longirostrus	0.00%	1	1.27%
717	Species 7: Tripos extensus	Tripos fusus	0.00%	1	1.27%
718	Species 8: Tripos longirostrus	Tripos longirostrus	16.67%	73	92.41%
718	Species 8: Tripos longirostrus	Tripos fusus	0.00%	4	5.06%
718	Species 8: Tripos longirostrus	Tripos extensus	0.00%	2	2.53%
719	Species 9: Tripos fusus	Tripos fusus	16.67%	78	98.73%
719	Species 9: Tripos fusus	Tripos longirostrus	0.00%	1	1.27%
720	Species 10: Tripos hexacanthus	Tripos hexacanthus	16.67%	77	97.47%
720	Species 10: Tripos hexacanthus	Tripos massiliensis	0.00%	2	2.53%
721	Species 11: Tripos furca	Tripos furca	16.67%	79	100.00%
722	Species 12: Tripos lineatus	Tripos lineatus	16.67%	79	100.00%

Table 11. Model answers for questions 10-12 on the genus Tripos.

Q12-15 on the *Protoperidinium* genus were largely well answered. In Q12 a series of pictures show some of the indicative plates for identification to species level, this is based on the shape of the 1' and 2a plates. The answers suggest that participants understand well how this theory works.

Q13	Model response	Actual response	Partial credit	Count	Frequency
113	Fig.1 shows: ortho configuration	ortho configuration	16.67%	73	92.41%
113	Fig.1 shows: ortho configuration	1a ortho configuration	0.00%	4	5.06%
113	Fig.1 shows: ortho configuration	para configuration	0.00%	1	1.27%
113	Fig.1 shows: ortho configuration	quadra configuration	0.00%	1	1.27%
114	Fig.2 shows: meta configuration	meta configuration	16.67%	76	96.20%
114	Fig.2 shows: meta configuration	hexa configuration	0.00%	2	2.53%
114	Fig.2 shows: meta configuration	2a meta configuration	0.00%	1	1.27%
115	Fig.3 shows: para configuration	para configuration	16.67%	76	96.20%
115	Fig.3 shows: para configuration	hexa configuration	0.00%	2	2.53%
115	Fig.3 shows: para configuration	ortho configuration	0.00%	1	1.27%
116	Fig.4 shows: 2a quadra configuration	2a quadra configuration	16.67%	75	94.94%
116	Fig.4 shows: 2a quadra configuration	2a hexa configuration	0.00%	2	2.53%
116	Fig.4 shows: 2a quadra configuration	2a para configuration	0.00%	2	2.53%
117	Fig.5 shows: 2a hexa configuration	2a hexa configuration	16.67%	75	94.94%
117	Fig.5 shows: 2a hexa configuration	2a penta configuration	0.00%	2	2.53%
117	Fig.5 shows: 2a hexa configuration	2a quadra configuration	0.00%	2	2.53%
118	Fig.6 shows: 2a penta configuration	2a penta configuration	16.67%	78	98.73%
118	Fig.6 shows: 2a penta configuration	2a quadra configuration	0.00%	1	1.27%

Table 12. Model answers for question 13: Protoperidinium terminology

However, it is difficult to transfer this skill to practice as the answers to Q13 and Q14 on identification of *Protoperidinium* species indicates. The percentage of correct answers drops from 95% on Q13 to 85% for Q14 and 15 that is a 10% drop. In Q13 image 1 is *P.claudicans* which has an ortho-penta (1' + 2a) tabulation with unequal hollow spines while *P.oblongum* which is very similar in shape has an ortho-quadra/hexa arrangement. In image 2, *P.curtipes* is the right answer with a ortho-quadra arrangement *P.depressum* which is quite large compare to *P.curtipes* is wrong. Also, *P.divergens* which has an equal plate arrangement to *P.curtipes* and is similar to it in shape, but its antapical horns are diverging.

In Q14 image 1 *P.leonis* has an ortho-hexa arrangement. *P.conicum* differs from *P.leonis* on the typical inverted raised 'V' shape in ventral view and the antapical spines are different compare to *P.leonis*. Both can be confused as they are ortho-hexa. In image 2, *P.pellucidum* the right answer is a meta-hexa. The pellucida group are generally 2a hexa. *P.stenii* is a meta-penta and the antapical spines are winged and longer than in *P.pellucidum*. *P.pallidum* is para-hexa and generally larger in size but also very similar.

Table 14 shows the statistics of percentage of correct answers by question and question type. Generally, scores are over 90% or high 80% for most questions. Questions 9 and 10 with 68.35% and 76.27% of correct answers appear to have been the most difficult ones for analysts.

Q14	Model response	Actual response	Partial credit	Count	Frequency
119	Species 1 is: P. claudicans	P. claudicans	50.00%	68	86.08%
119	Species 1 is: P. claudicans	P. oblongum	0.00%	11	13.92%
120	Species 2 is: P. curtipes	P. curtipes	50.00%	71	89.87%
120	Species 2 is: P. curtipes	P. depressum	0.00%	3	3.80%
120	Species 2 is: P. curtipes	P. divergens	0.00%	3	3.80%
120	Species 2 is: P. curtipes	P. curvipes	0.00%	1	1.27%
120	Species 2 is: P. curtipes	P. pentagonum	0.00%	1	1.27%
Q15	Model response	Actual response	Partial credit	Count	Frequency
131	Species 1 is: P. leonis	P. leonis	50.00%	67	84.81%
131	Species 1 is: P. leonis	P. conicum	0.00%	9	11.39%
131	Species 1 is: P. leonis	P. claudicans	0.00%	2	2.53%
131	Species 1 is: P. leonis	P. oblongum	0.00%	1	1.27%
132	Species 2 is: P. pellucidum	P. pellucidum	50.00%	62	78.48%
132	Species 2 is: P. pellucidum	P. steinii	0.00%	9	11.39%
132	Species 2 is: P. pellucidum	P. pallidum	0.00%	7	8.86%
100	Species 2 is: P. pellucidum	P. curvipes	0.00%	1	1.27%

Table 13. Model answers for questions 14-15 on Protoperidinium identifications.

Q#	Question type	Question name	Attempts	Facility index
1	Matching	Diatoms identification IPI16 2	79	97.47%
2	Matching	Diatoms identification IPI16 1	79	99.37%
3	Matching	Diatoms identification IPI16 3	79	80.70%
4	Matching	Chlorophytes IPI 2016	79	95.78%
5	Matching	Euglenophytes IPI16	79	99.58%
6	Matching	Prasinophytes IPI16	79	98.87%
7	Matching	Peridinioid terminology,2015	79	98.99%
8	Matching	Kofoidean tabulation IPI16	79	98.10%
9	Multiple choice	Dinoflagellate terminology IPI16	79	76.27%
10	Matching	Tripos 1	79	68.35%
11	Matching	Tripos 2	79	87.34%
12	Matching	Tripos 3	79	97.68%
13	Matching	Protoperidinium identification 1, 2014,2015	79	95.57%
14	Matching	Protoperidinium identification 2, 2014	79	87.97%
15	Matching	Protoperidinium identification 3, 2014	79	81.65%

Table 14: Overall statistics by question and type

5. Discussion

The BEQUALM phytoplankton intercomparison has changed its name to the International Phytoplankton Intercomparison (IPI) from 2016 onwards. The BEQUALM office closed its doors in 2014 and we have now become an independent PT scheme provider.

The format of this intercomparison exercise has evolved over the years but its present format is in operation since 2011 and appears to be a successful working model. This test is divided into two clearly defined sections; an online HAB quiz test set up in a remote platform accessed via the web and the analysis of lugol's preserved water samples for abundance and composition of marine phytoplankton. These samples are generally spiked with algal cultures, which allows for a better control of the spiked material in relation to their cell concentration and their identity.

The identification and enumeration exercise has been prepared in a similar fashion to previous years but a number of changes have taken place since 2013 in relation to the use of statistics. We are following the statistical methods laid out in ISO13528:2015 to calculate the performance statistics for the test. Also, some of the forms used to fill the test results have been revamped. The enumeration and identification logsheet (See Annex II) is set up as an Excel spreadsheet. The Excel spreadsheet contains an embedded reduced marine phytoplankton species list which is linked to the identification log sheet table and appears as a dropdown menu list, where analysts must choose the right entries for each sample.

The advantages of using these forms set up in this way to include the analysts' results are various but primarily, the results are always readable, numerical transcription errors are avoided and no interpretation of the results are needed as it avoids most of the time identifications like e.g. unidentified armoured dinoflagellate, centric diatom, naked dinoflagellates, etc. There are also some disadvantages, as the reduced list can be construed to be an aid to the identification of the species and a deviation to the method.

The results of the exercise have been processed similarly as in previous years particularly in relation to using the consensus values of all the analysts to form the basis of the final Z-scores. However, there are definite and important changes to the way we arrive at these averages and confidence interval values.

The new way of calculating these values using the robust averages and standard deviations from ISO 13528:2015 is a definitive departure from previous years. ISO 13528:2015 is the standard used for statistical methods in proficiency testing by interlaboratory comparisons. It describes sound statistical methods and

recommendations of their use which can be applied to demonstrate unacceptable levels of laboratory bias. It gives the statistical guidelines for the interpretation of tests and it is to be used as the reference document in future exercises. This standard is only applicable to quantitative data only.

Since 2014, we are using the statistical software programme ProLab Plus version 2.14 to calculate the descriptive statistics for the test and the performance characteristics including the graphical representation of all the results.

Homogeneity and stability test

A homogeneity and stability test is carried out each year since 2013 with a set of samples by an expert laboratory and the statistic parameters are calculated using ProLab Plus (Annex VII) and summarized in table 1. This test shows whether our samples are fully homogeneous and stable according to different statistical parameters or whether there is sample heterogeneity and lack of stability over time. ISO 17043 sets the rules in relation to how these tests must be carried out.

Our experience since 2013 from running these homogeneity tests is that our samples are never quite fully homogeneous or fully heterogeneous. This is related to the way we homogenize our samples manually using the 'Paul-Schatz' figure of eight rotation method by 100 times, which is the best manual method known for carrying this type of work.

At the beginning of the test, we try not to impose too many demands on homogenization. We run the F-test first, this tells us where our values are different from '0' if they are not, then, we can assume homogeneity under this criterium. Generally, all items usually pass this test. This year one item failed (*Karlodinium* count) the F-test and was deemed not homogeneous enough and discarded from statistical analysis. Secondly, we run the ISO13528:2015 test for adequate homogeneity. This test says that the between samples standard deviation should not exceed 30% of the standard deviation for the proficiency assessment, when this happens which is the case for most of our items, we run the expanded criterion under ISO13528:2015 for significant heterogeneity. The expanded criterion allows us even if we exceed that 30% that not significant heterogeneity can be found. Generally, the expanded criterion is met by all of our items but if this expanded criterion was failed, then we make a decision not to use the data for that item or items. This year this happened with the *Karlodinium* count which did not pass the minimum criteria needed.

The test for stability is slightly different in that samples from the homogeneity and stability are compared across the board with a time delay enforced for the stability samples. A t-test is run first to see if the mean values differ significantly. The criterion for stability is that the difference between mean values of the homogeneity and stability test items should not exceed 30%. Othewise, the expanded criterion which takes into account the uncertainty of the standard deviation for the proficiency test is used. Generally, our items appear to be stable over a month time delay which is the time allowed for participants to return their results. Most items pass the ISO13528:2015 criterion the rest pass the expanded one except for the one already mentioned.

The solution to this lack of homogeneity but not significant heterogeneity is given in ISO17043. ISO 17043 in note 3 says: "In some cases, materials that are not sufficiently homogeneous or stable are the best available; in such cases, they can still be useful as proficiency test items, provided that the uncertainties of the assigned values or the evaluation of results take due account of this". We have calculated the standard uncertainty of the assigned values (table 3) from the consensus values by the participants and we have found that in all the test items used in this round the standard uncertainty is negligible.

Also, ISO13528 indicates that when the consensus values form the participants are used, the assigned value can be compared with a reference value in order to ascertain that there is no bias in the method. We have used the data generated in the homogeneity test by an expert laboratory (table 4) as reference data for comparison purposes and we found that the differences between the consensus values and the reference values by the expert laboratory are more than twice its uncertainty for most test items.

This suggests some level of bias in the measurement method either by the participants, by the expert laboratory or both. This is not critical but it demonstrates that certified reference materials are essential to investigate further where this bias lies. Also a repeatability study would be necessary to investigate how much of this variation is due to the analysts and how much is due to the analytical method.

ISO 17043 gives another option when the materials are not sufficiently homogeneous or stable which is to include the between sample standard deviation from the homogeneity test values to the assigned standard deviation calculated from the consensus values for each test item. This is usually sufficient to take into account the heterogeneity of the samples.

In this test, although not all the test items have failed the homogeneity test we have decided to include the between sample standard deviation from the homogeneity test to all the measurands (see table 2). In any

case, the addition of the in between sample SD effect is to widen the confidence limits for each test item allowing more participants to be within the set limits.

Calculation of performance statistics

The consensus values from the participants (Annex VIII) were used to calculate the performance statistics for the test. These values take into account the heterogeneity of the samples (between sample SD) from the homogeneity test and the assigned values for the test materials used in this round were calculated using the robust algorithm A in annex C of ISO13528 which are derived by an iterative calculation using the new modified averages and standard deviations until the process converges (Annex IX). This method deals with outliers in the dataset and missing values.

These assigned values for each measurand were then used to calculate the Z-scores (Annex X). Laboratory bias assumes a normal distribution of the data across zero and any results outside the warning signal (+/-2SD) or action signal (+/-3SD) would suggest an out of specification result. The results show that Z-scores are generally within the specification of the test for most analysts with a number of warning and action signals. A warning signal is a result between 2 and 3SD of zero and an action signal is a result outside 3SD. Two warning signals in consecutive intercomparisons give rise to an action signal. An action signal signifies that an investigation of the causes by the laboratory should be carried out.

There are a number of warning and action signals arising from this intercomparison which can be found in the table of Z-scores in annex X. Generally, the performance is good for most analysts with perfect scores in all measurands. In this exercise, we had a complete total of 9 (1.4%) red flags, 22 (3.4%) yellow flags and 6 (0.93%) orange flags (Non-Ids) for all measurands and laboratories from 648 scores is evidence of good performance overall.

Combined performance scores

It is common in any rounds of a proficiency testing exercise to obtain results from several test items or measurands, in our case each species found in the samples is a test item or measurand. As this is generally the case during monitoring work, the individual scores for each measurand is analysed individually but also can be used to calculate combined effects for a particular laboratory or analysts such as correlation between results for different measurands. Graphical methods for this include histograms, bar plots and repeatability standard deviations plots.

Mandel's h and k statistics in annex XIV present measures for graphically surveying the consistency of the data and specific patterns of laboratory performance. The h plot represents all measurand-sample combination possible and reveals that a small number of analysts have consistently over or underestimated the cell counts which indicate a common source of laboratory bias. It is up to individual laboratories to investigate the causes which may cause these anomalies.

The k plot can be interpreted as repeatability precision measure. Again, this graph represents all the measurand-sample combinations possible. Large values here indicate poor repeatability precision. Several large values indicate poor repeatability precision for some or all of the measurands.

The chart of RLP against RSZ (Annex XV) for all measurands combined indicates systematic laboratory bias. RSZ is based on the standardized sum of all the z-scores for each analyst and it can be interpreted as a single Z-score: that is an evaluation across all samples and measurands. If the RSZ value is within the tolerance limits (2SD), there are no significant systematic deviations of the measurement values for that analyst compared to the rest. The RLP is the mean length of all the Z-scores for each analyst and is derived from the sum of the squared mean length of all the Z-scores. Deviations in RLP are accepted as long as the mean deviations for the analysts don't exceed 1.5 times the average deviations of all laboratories. This is the top of the green area of the rectangle. Laboratories dotted within the green colored area in the graph are within the consensus values shown by the majority of analysts. Those outside it are showing a systematic bias towards over or under-estimating most of their counts in the samples, suggesting some kind of methodology bias.

The plot of repeatability standard deviations shown in annex XVI uses a modified approach to the circle technique of van Nuland. This plot uses the average and standard deviation of each laboratory/analyst and plots one against the other. Because of this modified approach, the critical region drawn doesn't have the shape of a circle anymore. This critical region corresponds to a significance level of 5% for the inner layer, 1% and 0.1% for the most outer layer. This plot determines which laboratories/analysts are having unusual averages and standard deviations. Plots of repeatability standard deviation assume that there is no difference between laboratories means +SD.

Qualitative data

The scope of ISO13528 does not include qualitative results, but the correct identification of the organisms in the samples is still a very important part of the exercise, as correct/incorrect/not-identified flags will be given for this. The data received from the analysts (Table 5) shows that analysts are highly skilled in the identification of marine phytoplankton and the results suggest that there is consensus among analysts on most of the species identified in the samples with near perfect scores for all identifications.

Originally, ten species have been spiked in the samples but the organism *K.selliformis* did not preserve well and *K.veneficum* could not finally be included in the statistical analysis as the cell counts did not pass the minimum homogeneity and stability criteria required, so we ended up with eight different species for identification and enumeration.

This year we had a mixture of dinoflagellates and diatoms in the sample and also a mixture of toxic and non-toxic species. We had 5 dinoflagellates (if we count *K.selliformis*) and 5 diatom species, although at the end only 8 species had to be identified. We also had 4 toxic species in the sample. However as we mentioned before lugol's preservation caused problems with *K.selliformis* and *K.veneficum* did not homogenise properly in the samples giving poor repeatability between analysts. These 2 species were disregarded for statistical analysis.

The *Chaetoceros* genus as you can glean from the table of results (table 5) always gives the largest variety of answers at species level. 8 different species were identified by analysts. This is what we call the *Chaetoceros* species complex. We have used *Chaetoceros* species in samples in these tests for many years now and we always find that it returns the largest and more varied number of answers in terms of number of species among analysts. *D.acuta, P.triestinum, C.wailessii, G.delicatula* and *P.australis* were largerly identified correctly. The hardest organisms to identify appeared to be *A.ostenfeldii and K.veneficum* with a variety of answers given and a small number of incorrect identifications (5 each) but also 6 analysts did not identify *Karlodinium* at all.

The organism *Thalassiosira gravida* was identified correctly by most participants, some use the name *T.rotula* which was given as correct answer here but please note that this name is now no longer recognized according to the taxonomic nomenclature.

Also, note that 11 analysts (NR) did not find *C.wailesii* in the samples. As this measurand was produced in the samples at the limit of detection of the test method it is possible that there would be none in some samples, rather than analysts failing to identify the species, as *Coscinodiscus* is a conspicuous organism and

largely because of its size it would be hard to miss, so the statistical analysis on this measurand was not applied to these analysts, resulting in not obtaining a Z-score or qualitative flag for this item.

Overall, from 720 possible correct identifications and discounting the NR results from *C.wailesii* identification, there were a total of 695 correct answers at genus level that is 96.5% correct, 1.7% of non identifications and 1.5% of incorrect answers only. This indicates a high level of proficiency amongst participants on identifications.

Online HAB quiz

This year, we have avoided 'short answer' type questions in the quiz which had created some problems before and we have instead concentrated more on using 'matching' and 'multiple choice' type questions. In fact most questions bar one (Q9) were 'matching' type questions. Also, we have stopped the software from 'shuffling' the questions around so that the first question asked corresponds to image 1 and so on. This has resulted already in an improvement in the number of correct answers.

The online quiz is set up in a way that urges participants to get back and study their taxonomic literature in order to answer the questions, the difficulty of some of these questions therefore can be higher and of a technical nature, we do this as a way to update participants with the most up to date taxonomical information available and also to widen their knowledge on the perhaps lesser known organisms or group of organisms. The online quiz allows us to assess participants training skills and compare those skills across laboratories and also geographical areas. The consensus is generally quite good between participants and the scores suggest a high level of proficiency among participants.

There was good consensus on the various identifications of diatom species from images in questions 1 to 3. Although the images of *T.mobiliensis* and *C.densus* were the most difficult organisms to identify from these images, results suggest a good performance overall. In Questions 4 to 6, there were good overall marks on flagellate identification based on depictions. In Q7-9 there were good scores on Peridinioid terminology but difficulties with the lesser known Suessiaceae group. In Q10-12 there were problems identifying *T.macroceros* group (Q10) which was the worst scored question (68.8% correct) and in Q12-15, the identification of *Protoperidinium* based in the theory of the shape of the 1' and 2a plates is understood but in practice is still difficult to go to species level using images.

ANNEX I: Form 1 return slip and checklist



IPI Intercomparison PHY-ICN-16-MI1 FORM 1: RETURN SLIP AND CHECKLIST

to + 353 91 387201 or scan and e-mail to rafael.salas@marine.ie Analyst Name: Laboratory Name: Analyst Code Assigned : Contact Tel. No. / e-mail CHECKLIST OF ITEMS RECEIVED answer) (Please circle the relevant answer)	Please ensure to complete the table below upon receipt of samples, then fax						
Laboratory Name: Analyst Code Assigned : Contact Tel. No. / e-mail CHECKLIST OF ITEMS RECEIVED (Please circle the relevant)							
Laboratory Name: Analyst Code Assigned : Contact Tel. No. / e-mail CHECKLIST OF ITEMS RECEIVED (Please circle the relevant)							
Laboratory Name: Analyst Code Assigned : Contact Tel. No. / e-mail CHECKLIST OF ITEMS RECEIVED (Please circle the relevant)							
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diiswer	(·····						
Please enter the sample codes YES NO	Please enter the sample co	es	VEC	NO			
here:	here:		TLS	NO			
Set of InstructionsYESNO	Set of Instructions			NO			
Enumeration and identification result log sheet (Form 2) YES NO	Enumeration and identifica	YES	NO				
		2					

I confirm that I have received the items, as detailed above.

(If any of the above items are missing, please contact Rafael.salas@marine.ie)

SIGNED:

DATE: _____

ANNEX II: Form 2 Enumeration and identification results log sheet



IPI 2016 Phytoplankton	Intercompa	rison Ex	kercise							
Analyst Name: Laboratory Code: Analyst Code :										
Settlement date:										
Volume Chamber (ml)										
Analysis date:										
Sample No:										
Organism	Cell	Cell	Cell	Mul	tiplica	tion	Number	Number	Number	A
Organishi	count	count	count		facto	r	cells/L	cells/L	cells/L	Average
										#DIV/0!
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Comments:										
Form 2: Results logsheet										

ANNEX III: Test instructions



IPI Phytoplankton Proficiency Test PHY-ICN-16-MI1 Vr1.0 Instructions

Please note that these instructions are designed strictly for use in this Intercomparison only.

- 1. Introduction
- 2. Preliminary checks, deadlines and use of forms
- 3. Test method
- 4. Equipment
- 5. Sedimentation chambers and sample preparation
- 6. Counting strategy
- 7. Samples
- 8. Conversion calculations of cell counts
- 9. Online HABs quiz
- **10.Points to remember**

1. Introduction

The Marine Institute, Galway, Ireland, has conducted a phytoplankton enumeration and identification ring trial, under the auspices of BEQUALM-NMBAQC annually since 2005. In 2011, the IOC Science and Communication Centre on Harmful Algae and the Marine Institute initiated collaboration on the design and organization of this exercise which has continued under the Marine Institute- IOC -BEQUALM-NMBAQC banner until 2015.

From 2016 onwards, the programme BEQUALM no longer exist and the intercomparison exercise has changed its name to IPI (International Phytoplankton Intercomparison) with the continued collaboration of the IOC Science and Communication Centre on Harmful Algae and in association with NMBAQC in the UK.

Information about this intercomparison exercise can be obtained in the NMBAQC website (www.nmbaqcs.org) under scheme components and Phytoplankton, you'll find information on the current timetable schedule for the exercise, the list of participants, previous reports and the workshop agenda from the previous exercises to give you an idea of the range of activities within this intercomparison exercise. There is also information on all the other NMBAQC schemes. Also, in the IOC website; http://hab.ioc-unesco.org there is information about the exercise under activities and training courses. Registration to the exercise is through the Marine institute. You need to contact our administrator Fiona Bradley at fiona.bradley@marine.ie to register.

The purpose of this exercise is to compare the performance of laboratories engaged in national official/non-official phytoplankton monitoring programmes, water framework directive, marine strategy framework directive and other laboratories (environmental agencies, consultancies, private companies) working in the area of marine phytoplankton analysis.

The Marine Institute is accredited to the ISO 17025 standard for toxic marine phytoplankton identification and enumeration since 2005 and recognises that regular quality control assessments are crucial to ensure a high quality output of phytoplankton data.

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This interlaboratory comparison exercise is conducted to determine the performance of individual laboratories on the composition and abundance of marine microalgae in preserved marine samples and to monitor the laboratories continuing performance.

Participants are asked to carry out microscopic analysis on three marine water samples spiked with cultured material and preserved with neutral lugol's iodine and return results on the composition of the samples to the highest possible taxon and the average abundance in cells per litre for each species in each sample. Each analyst will receive an envelope containing four samples (3 +1 spare) 50ml volume in plastic sterilin tubes.

Please adhere to the following instructions strictly. Please note that these instructions are specific to this ring test only.

2. Preliminary checks, deadlines and use of forms

Upon receipt of the samples, every analyst must make sure that they have received everything listed in the Return Slip and checklist form (Form 1). Make sure that all the samples are intact and sealed properly and check that you have received the enumeration and identification results log sheet (Form 2) as an Excel workbook. Please complete form 1: Return slip and checklist form and send it by fax to (+353 91 387201) or scan, pdf and send it via e-mail to <u>rafael.salas@marine.ie</u>. If you send the form via e-mail, please title the file as Form 1 followed by the exercise code and your full name **i.e. Form 1: BEQ16 Rafael Salas** A receipt of fax/e-mail is necessary for the Marine Institute to validate the test process for each analyst.

Once samples have been receipt, analysts have four weeks to complete the exercise and return the results to Rafael Salas, Marine Institute, Phytoplankton laboratory, Rinville, Oranmore, Co. Galway, Ireland by e-mail (<u>rafael.salas@marine.ie</u>), fax as above or post. If you decide to post your results, make sure first to make a copy of them and then send the originals to the address above. The enumeration and identification results log sheet (Form 2) **must be received** in the Marine Institute by **Friday, July 22nd 2016**.

Please note: Results received after this date will not be included in the final report. Also, if you are posting your results make sure to make a copy for your records before sending the originals. Just in case they never arrive.

An Excel workbook named 'Enumeration and identification logsheet' for you to input your results should be used to write in your results. In this form, first fill in your name, analyst and laboratory code at the top of the form. Fill in all the information relevant to the analysis of your samples like settlement date, settlement chamber volume used in mls, analysis date and sample number in the corresponding cells. Under the column 'organism' a drop down menu will appear with a list of possible species names. You must choose from this list your answers. The list of species is a reduced list and is designed to have more entries than species are in the samples, you must choose which ones you think have been spiked in the samples and provide a count.

If is not in the list, is not in the sample. The number of rows under the name 'organism' is fourteen but this is arbitrary. It doesn't mean you need to enter fourteen names or that there are fourteen species in the samples. The number of species spiked in the samples is a fixed number but you must decide that yourselves.

In the comments box, you can write information about the test method you used if deviates from the Utermöhl test method and how you performed your calculations if you think is necessary.

Finally, if you send your form back via e-mail, please re-name in the same way as Form 1 above.

3. Test method

The Utermöhl cell counting method (Utermöhl 1931, 1958) is the standard quantitative and qualitative test method used in the Marine Institute phytoplankton national monitoring programme in Ireland. We use 25ml volume sedimentation chambers and we are accredited under the ISO 17025 quality standard.

We advise the use of 25ml sedimentation chambers for the purpose of this intercomparison exercise if these are available. If not, other sub-sample volumes and/or chambers may be used.

If a different method is used, please state all this information in your results.

4. Equipment

The following are the equipment requirements to complete this exercise:

Sedimentation chambers (25ml volume if possible).

<u>Inverted Microscope</u>: This should be equipped with long distance working lenses up to 40 x objective or higher and condenser of Numerical Aperture (NA) of 0.3 or similar and capable for bright field microscopy. Other types of reflected or transmitted light capabilities may be helpful depending on the type of organisms in the samples and can be used if required.

Tally counters

5. Sedimentation chambers and sample preparation

Sedimentation chambers consist of a clear plastic cylinder, a metal plate, a glass disposable cover-slip base plate and a glass cover plate (Fig 1). Three sedimentation chambers are required.



Fig 1: Sedimentation counting chamber

- 5.1 All sedimentation chambers should be cleaned before start
- 5.2 Place a new not used disposable cover slip base plate inside a cleaned metal plate.
- 5.3 Screw the plastic cylinder into the metal plate. Extra care should be taken when setting up chambers. Disposable cover slip base plates are fragile and break easily causing cuts and grazes.
- 5.4 **Important:** Once the chamber is set up, it should be tested for the possibility of leaks by filling the completed chamber with sterile filtered seawater and allowing it to rest for a few minutes. If no leakage occurs, pour out the water, dry out completely and proceed with the next step.
- 5.5 To set up a sample for analysis or sub-sample. Firmly invert the sample 100 times to ensure that the contents are homogenised properly.
 - 5.5.1 Pour the sample into the counting chamber. Samples must be adapted to room temperature beforehand to reduce the risk of air bubbles in the chambers due to temperature changes.
 - 5.5.2 There should be enough sample volume in each sample to fill a 25ml sedimentation chamber. Top up the sedimentation chamber and cover with a glass cover plate to complete the vacuum and avoid air pockets.
 - 5.5.3 Label the sedimentation chamber with the sample number from the sterilin tube.
- 5.6 Use a horizontal surface to place chambers protected from vibration and strong sunlight.

- 5.6 Allow the sample to settle for a minimum of twelve hours.
- 5.7 Set the chamber on the inverted microscope and analyse.
- 5.8 Enumeration and identification results for each sample are to be entered in the Excel workbook Form 2 enumeration and identification results log sheet.
- 5.9 If using a different method to the Utermöhl test method, please send the Standard Operating Procedure for your method with your results. Explain briefly how it works and how samples are homogenized, set up, analysed, counted and how you calculate the final concentration.

6. Counting strategy

Each analyst should carry out a whole chamber cell count (WC) of all the species identified in the samples where possible. Other counting strategies can also be used where the cell density in the sample for a particular organism is high. Show your calculations if using a field of view or transect count.

7. <u>Samples</u>

Analysts will have to analyse three samples to complete this test.

The set consist of four samples. Three must be analysed and one is to be used as an additional sample in case of leaks or breaks. These are made up in sterile filtered Seawater and spiked with culture material consisting of several species. Participants are asked to carry out a whole sedimentation chamber cell count (where possible ; see 6.) on each organism and sample.

The cultures come from the Marine Institute Phytoplankton culture collection and the IOC Science and communication centre for Harmful Algae culture collection in Denmark. All the materials have been preserved using neutral lugol's iodine and must be homogenized following the IOC Manual on Harmful Marine Algae technique of 100 times sample inversion before settlement.

Each analyst must **count and identify all phytoplankton species** found in the three samples.

It is very important to spend some time becoming familiar with the samples and how the cells appear on the base plate before any count is carried out. The reason for this is that cultured cells could be undergoing division or fusion and look different to the known standard vegetative cell types. See figure 1.



Figure 1: Two Cells fusing

Also note that cells' emptied thecae of dinoflagellates may appear in the samples (see figure 2), or silica frustules in diatoms.



Figure 2: Empty theca

Cells may also vary in size, some cells will appear smaller than others, this is normal in culture conditions (see figure 3). Sometimes Plasmolysis may occur and the cells appear

naked and rounded (see figure 4). Aberration of cell morphology can occur also in culture conditions and upon preservation of samples with lugol's iodine.



Figure 3: Big versus small cells

Figure 4: Plasmolised cell

When counting diatom cell chains, only count fully intact cells on the chains (fig.5).



Figure 5Figure 6Sometimes cells may not be in the same focus plane (fig.6) but you still need to count them.

The following rules should be applied for cell counting and identifying in this exercise:

a) Empty theca/ silica frustules should not be counted.

b) Cells should be counted regardless of size, different sizes doesn't necessarily mean different species

c) Plasmolised cells should not be counted

e) When counting cell chains, do not count half or broken cells which are part of the chain

f) if you find single diatom cells in the samples and these are partially broken, do count them as one cell.

f) Identify to the highest taxonomic level possible all species in the samples

g) Participants should name phytoplankton species according to the current literature and scientific name for that species. Where species have been named using a synonym to the current name and if this synonym is still valid or recognized the answer will be accepted as correct. Use <u>http://www.marinespecies.org/</u> if in doubt.

These rules are applicable to this intercomparison exercise only.

8. Conversion calculations of cell counts

The number of cells found should be converted to cells per litre. Please show the calculation step in Form 2: enumeration and identification results log sheet.

9. Online HABs quiz

A HAB taxonomic quiz will be developed in the web platform 'Ocean teacher' and it should be ready by the end of June 2016. All participants will need access to the internet to complete this part of the exercise. More information on when participants will be able to access this exercise will be sent to you by e-mail later on.

In order the to access exercise you need to go to the webpage http://classroom.oceanteacher.org/ and login. Analysts which took part in the exercise in any of the last four years will already have a username and password which is still active, those using this facility for the first time need to register first.

When you go to the page <u>http://classroom.oceanteacher.org/</u> in the top right hand corner of this page, you'll see a link to login. Press login and in the next page if you have already registered between2011-2015 then enter your username and password to access the course, if you forgot your password press the forgotten password link. If this is your first time using this system, then go to create new account and register your details. Once you register your details we will be able to activate your account. Participants should be able to self-enrol to this exercise, so once you are registered and logged in you must supply an enrolment key to access the exercise. This key is **IPI2016**. We will tell you the exact date the exercise is opened.

So, how do you do access the course?, Once you are all logged in, in the main page scroll down to the bottom and under interdisciplinary courses, click courses, on the next page and under categories click Harmful Algal Bloom (HAB). The Harmful algal bloom programme Bequalm 2015 link will appear, click on it, enter your key (**IPI2016**) and start your quiz. Make sure you enter the right course.

Analysts will have several months to complete the exercise once it opens (dates to be decided). Only one attempt to the exercise is allowed and once the exercise is submitted analysts won't have access to it, only to review. So, make sure you review all your answers before submitting. There are a number questions and a maximum grade of 100% for a perfect score. All questions have the same score.

There are different types of questions (true/false, numerical, matching, multiple choice short answer, etc..). Please note that if you are asked for a number as the answer do not use text, use a numerical value. Also, in questions where you are asked to write the answer, please make sure that the grammar is correct. Incorrect grammar will give an incorrect answer. Please review your work carefully before submitting.

10. Points to remember

- 1. All results must be the analysts' own work. Conferring with other analysts is not allowed.
- The Excel worksheet Form 2: Enumeration and identification results log sheet must be received by the Marine Institute, Phytoplankton unit by Friday July 22nd 2016.

ANNEX IV: Workshop agenda



Agenda 'International Phytoplankton Intercomparison' (IPI) workshop

Danhostel, Hillerød, Denmark. 27 Nov – 1 Dec 2016

	Morning 9.00-12.00	Afternoon 13.30-17.00
Sunday		Arrival to Danhostel at 16.00,
27 Nov		Light evening meal, sandwich
Monday,	International phytoplankton	Development and Improvement of Standards in support of
29 Nov	intercomparison (IPI) exercise	the Water Framework Directive.
composition of marine microalgae		CEN mandate M/424- Work package 7: Guidance on the estimation of algal biovolume
	Rafael Salas and Jacob Larsen	estimation of algal biovolume Aqua T Dr. Claus-Dieter Dürselen Coord
		Presentations by the participants:
	<u>Ocean teacher online HABs quiz</u> , exercise results	An unusual bloom of <i>Dinophysis acuta</i> in Scottish coastal waters linked to a change in diarrhetic shellfish toxin profiles
	Rafael Salas and Jacob Larsen	Sarah Swan SAMS
		Biotoxin Monitoring in England and Wales
	Elacomi & alticut Data Comi & alticut Data Com	Charlotte Mitchell
Tuesday,	Lecture and microscope	Presentations by the participants:
29 Nov	demonstration:	
29 1000	Dinoflagellates with focus on species of the <i>Tripos</i> -group	Habs Bulletin. The journey so far

	Jacob Larsen and Rafael Salas	Tara Chamberlain Marine Institute Phytoplankton Laboratory: Portuguese Institute for the Sea and Atmosphere Alexandra Silva Imaging FlowCytoBot (IFCB) Tångesund observatory. Malin Mohlin Microscopy of participants' samples / mixed samples
Wednesday	Lecture and microscope	Presentations by the participants:
30 Nov	<u>demonstration</u> : Dinoflagellates with focus on <i>Protoperidinium</i>	Analysis of the potential impact of ocean acidification on the pelagic gastropod community in the North East of Scotland
	Jacob Larsen and Rafael Salas	marinescotland Pablo Diaz
		Fish mortality: Swamps of L'Houmeau
	Marine Institute Forai na Mara	Christophe Arnaud
		Lecture and microscope demonstration:
		Dinoflagellates with focus on Protoperidinium continue.
		Jacob Larsen and Rafael Salas
Thursday 1 Dec	10 am, departure	

ANNEX V: Participating Laboratories

Company Name		Company Name	
	Marine Institute		
Narine Institute (Ireland)	roras na mara	Isle of Man Government Laboratory (UK)	
	koeman en bijkerk bv)
Koeman en Bijkerk bv (Netherlands)	ecologisch onderzoek en advies	Aristotle University of Thessaloniki (Greece)	9
micro	balgal services	AP	EM
	monitoring and identification	APEM Limited (UK)	
IDTA	9		
RTA (Spain)	NOLDGY	ARPA Puglia Dap Brindisi (Italy)	
	**		
nstitut za oceanografiju i ribarstvo (IOR) (Croatia) 🗡		ARPA Puglia - DAP BARI - U.O.S. Biologia delle Acque (Italy)	TA PUGLIA
		ARFA Fuglia - DAF BARI - 0.0.3. Biologia delle Acque (Raly)	>
	a De Arford and .		
Agri Food and Biosciences Institute (AFBI) (Northern Ireland		Polo specializzazione Biologia avanzata Acque (Italy)	UGLIA
Cefas (UK) Cefas		Biologia delle Acque - DAP Taranto - ARPA Puglia (Italy)	
	ARPA FVG		
ARPA FVG (Italy)	enzia Regionale per la Protezione ll'Ambiente del Friuli Venezia Giulia		PUGLIA
	Aco		
AGQ PERU S.A.C (Perú)	AGQ	Fondazione Centro Ricerche Marine (Italy)	ICERCHE MARINE
Cawthron Institute (New Zealand)		IFREMER (France)	emer
	ليبيد لاطنا للحث في العبيد الحرق		
	INSTITUT NATIONAL DE RECHERCHE HALIEUTIQUE	Constitute Marken and a start and the day is start in with the (Constant)	
nstitut National de Recherche Halieutique (Morocco)		Swedish Meteorological and Hydrological Institute (Sweden)	
	ANIPES		ENINGEN LI R
Organismo Nacional De Sanidad Pesquera (Perú)		IMARES (Netherlands)	
No	ERPER		$\widehat{}$
Certificaciones del Peru S.A. (Perú)	NOONES DEL PERU S.A.	Laboratorio de Control de Calidad de los Recursos Pesqueros (S	pain)
20	AMS		SEP
SAMS Research Services Ltd (SRSL) (Scotland)	AMO	Scottish Environment Protection Agency (Scotland)	Scottish Environment Protection Agency
Fondazione Centro Ricerche Marine (Italy)	ERCHE MARINE	Food Safety and Veterinary Institute FSVI (Albania)	
			a
stituto Zooprofilattico Sperimentale delle Venezie (Italy)	Latituto Zeoprefilattico Sparimentale delle Venzzie	Environmental Protection Agency (Ireland)	COO Environmental Potentian Agency
	Department of the	Sydi	nev
Northern Ireland Environment Agency (NIEA) (Northern Ire	Land)	Sydney Water (Australia)	ATER
Northern Relation Environment Agency (MEA) (Northern Re		Syuney water (Australia)	(ABA)
PML	Piymouth Mariné Laboratory		SAHFOS
Plymouth Marine Laboratory (UK)	•	Sir Alister Hardy Foundation for Ocean Science (SAHFOS) (UK)	
			marinescotland
PMA - Fitoplâncton Lab (Portugal)	da atmostere	Marine Scotland Marine Laboratory (Scotland)	<u> </u>
			Sh.
MEA-nl (Netherlands)	Ander 705	Institute of Marine Biology (IMBK) (Montenegro)	IBM
an 🤶	BICON		LIENSS
ORBICON (Denmark)		LIENSS / CNRS (France)	
li l			
aboratorio di Biotossicologia ambientale ARPAL (Italy)	O WWW		

Marin Starin	ne Institute Foras na Mara	THUTTOPLANTION		United Nations	Intergovernmenta Occeanographic Commission
	onal Marine Biolo STATEN	ity Assurance in Mor gical Analytical Qua Marine Institute IENT OF PERFORI Component of Comn Year 2016	ulity Contr MANCE	rol Scheme	
Participant details: Name of organisation Country: Participant: Year of joining: Years of participatio					
Statement Issued: Statement Number: Summary of results:	XX/XX/2016 MI-BQM-16-				
Component Name	Subcontracted	Results Z-score (+/- 2 Sigma limit	s)	identification]
IPI 2016 Phytoplankton abundance and composition PHY-ICN-16-MI1	Marine Institute	Pseudo-nitzschia australis Guinardia delicatula Dinophysis acuta Thalassiosira gravida Chaetoceros didymus Coscinodiscus walessii Prorocentrum triestinum			
	Overall Result Taxonomic quiz	Alexandrium Ostenfeldii (Pass Mark 70%, over 90% profici	ient)		-
IPI 2016 Phytoplankton Taxonomy quiz PHY-ICN-16- MI1	IOC Science and communication Centre on Harmful algae				
n/r: no data received from p	articipant	Participant not participating in the laboratory participated. Se	1		
Details certified by:	Estant G	Harden Solas			
Joe Silke Section manager	Rafael Gallardo Sa Scientific Technic				

ANNEX VI

Description of Scheme components and associated performance standards

In the table overleaf, for those components on which a standard has been set, 'Proficient', 'Good', and ' "Pass" flags indicate that the participants results met or exceeded the standards set by the Bequalm Phytoplankton scheme; 'Participated' flag indicates that the candidate participated in the exercise but did not reach these standards. The Scheme standards are under continuous review.

Component	Annual exercises	Purpose	Description	Standard
Phytoplankton Enumeration Exercise	1	To assess the performance of participants using the Utermöhl cell counting technique on the analysis of prepared sample/s of Seawater preserved in Lugol's iodine spiked using biological or synthetic materials.	Prepared marine water sample/s distributed to participants for abundance and composition of marine phytoplankton species	Participants are required to enumerate the test/s material/s and give a result to within ±2SD or sigma limits of the robust average/s. The robust average/s is/are the mean calculated from the consensus values by the participants following the assessment criteria as set out in ISO13528, Annex c robust analysis: Algorithm A. Participants are also required to identify the organisms found in the samples correctly to the required taxon. Flags will be given as correct, incorrect or not identified
Phytoplankton Oceanteacher online HAB quiz	1	To assess the accuracy of identification of a wide range of Marine phytoplankton organisms.	This is a proficiency test in the identification of marine phytoplankton The exercise tests the participant's ability to identify organisms from photographs and/or illustrations supplied.	The pass mark for the identification exercise is 70%. Results above 90% are deemed proficient, results above 80% are deemed good, results above 70% are deemed acceptable, and results below 70% are reported as "Participated". There are no standards for phytoplankton identification. These exercises are unique and made from scratch.

ANNEX VII: Homogeneity and stability test using ProLab plus

Alexandrium ostenfeldii homogeneity test

IPI2016

Survey of homogeneity test results



Sample:	Water 16		Date:	08/11/2016
Measurand:	Alexandrium			
Mean:		1786		
Analytical standa	ard deviation:	240		
Heterogeneity st	tandard deviation s(samples):	76		
Standard deviat	ion for proficiency assessment:	321 (Manual)		

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 w ere randomly selected, and the measurand Alexandrium Ostenfeldii w as analyzed 2 times. The mean across all 10 proficiency test items is 1786. The standard deviation within proficiency test items s(analytical) (=analytical precision) is 240, and the standard deviation betw een proficiency test items s(sample) is 76.

F test

According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level 5 %), therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity

According to ISO 13528:2015, the heterogeneity standard deviation s(sample) between the proficiency test items should not exceed 30 % of the standard deviation for proficiency assessment.

The heterogeneity standard deviation is less than 30 % of the standard deviation for proficiency assessment 321 (Manual), therefore the proficiency test items can be considered adequately homogeneous according to ISO 13528:2015.

ISO 13528:2015 - Test for significant heterogeneity

For the proficiency test items, no significant heterogeneity can be identified, therefore they can be considered homogeneous.





Marine Institute Phytoplankton 0 Rafael Salas

08/11/2016

ANNEX VII: Alexandrium ostenfeldii stability test

IPI2016

Survey of stability test results



Date: 08/11/2016

Sample: Measurand:	Water 16 Alexandrium	
measurand:	Alexandrium	
Mean of homogene	eity:	1786
Mean of stability:		1727
Uncertainty of mea	in for homogeneity measurement	: 59
Uncertainty of mea	an for stability measurement:	108
Standard deviation	n for proficiency assessment:	318 (Manual)

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Alexandrium Ostenfeldii has been analyzed 2 times.

The mean value across all proficiency test items of the homogeneity analysis equals 1786, the mean value across all proficiency test items of the stability analysis equals 1727.

Therefore, the mean value of the stability analysis lies 3.3 % below the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Therefore, given the standard deviation for proficiency assessment of 318, the proficiency test items may be considered as adequately stable.

By means of the t test it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance 5 %).

The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the t test.





Marine Institute Phytoplankton 08/11/2016 Rafael Salas

IPI2016

Survey of homogeneity test results



Sample:	Water 16		Date:	08/11/2016
Measurand:	Chaetoceros			
Mean:		928		
Analytical standa	ard deviation:	508		
Heterogeneity st	andard deviation s(samples):	263		
Standard deviati	on for proficiency assessment:	555 (Manual)		

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 were randomly selected, and the measurand Chaetoceros didymus was analyzed 2 times. The mean across all 10 proficiency test items is 928. The standard deviation within proficiency test items s(analytical) (=analytical precision) is 508, and the standard deviation betw een proficiency test items s(sample) is 263.

F test

According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level 5 %), therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity

According to ISO 13528:2015, the heterogeneity standard deviation s(sample) between the proficiency test items should not exceed 30 % of the standard deviation for proficiency assessment.

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 555 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity

For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.





Marine Institute Phytoplankton Rafael Salas

08/11/2016

ANNEX VII: Chaetoceros didymus stability test

IPI2016

Survey of stability test results



Sample:	Water 16		Date:	08/11/2016
Measurand:	Chaetoceros			
Mean of homoge	neity:	928		
Mean of stability		867		
Uncertainty of m	ean for homogeneity measurement	: 141		
Uncertainty of m	ean for stability measurement:	191		
Standard deviat	on for proficiency assessment:	555 (Manual)		

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Chaetoceros didymus has been analyzed 2 times.

The mean value across all proficiency test items of the homogeneity analysis equals 928, the mean value across all proficiency test items of the stability analysis equals 867.

Therefore, the mean value of the stability analysis lies 6.6 % below the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Therefore, given the standard deviation for proficiency assessment of 555, the proficiency test items may be considered as

adequately stable. By means of the t test it is checked w hether the mean values of the homogeneity analysis and the stability analysis differ

By means of the t test it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance 5 %).

The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the t test.





Marine Institute Phytoplankton Rafael Salas

08/11/2016

ANNEX VII: Coscinodiscus wailesii homogeneity test

IPI2016

Survey of homogeneity test results



Sample:	Water 16		Date:	08/11/2016
Measurand:	Coscinodiscu			
Mean:		46		
Analytical standa	ard deviation:	37		
Heterogeneity st	andard deviation s(samples):	27		
Standard deviati	on for proficiency assessment:	37 (Manual)		

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 w ere randomly selected, and the measurand Coscinodiscus w alessii w as analyzed 2 times. The mean across all 10 proficiency test items is 46. The standard deviation w ithin proficiency test items s(analytical) (=analytical precision) is 37, and the standard deviation betw een proficiency test items s(sample) is 27.

F test

According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level 5 %), therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity

According to ISO 13528:2015, the heterogeneity standard deviation s(sample) between the proficiency test items should not exceed 30 % of the standard deviation for proficiency assessment.

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 37 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity

For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.





Marine Institute Phytoplankton Rafael Salas

08/11/2016

ANNEX VII: Coscinodiscus wailesii stability test

IPI2016

Survey of stability test results



Sample:	Water 16		Date:	08/11/2016
Measurand:	Coscinodiscu			
Mean of homoge	neity:	46		
Mean of stability		47		
Uncertainty of m	ean for homogeneity measuremer	t 12		
Uncertainty of m	ean for stability measurement:	18		
Standard deviati	on for proficiency assessment:	37 (Manual)		

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Coscinodiscus walessii has been analyzed 2 times.

The mean value across all proficiency test items of the homogeneity analysis equals 48, the mean value across all proficiency test items of the stability analysis equals 47.

Therefore, the mean value of the stability analysis lies 1.4 % above the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Therefore, given the standard deviation for proficiency assessment of 37, the proficiency test items may be considered as adequately stable.

By means of the t test it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance 5 %).

The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the t test.





Marine Institute Phytoplankton 08/11/2016 Rafael Salas

ANNEX VII: Dinophysis acuta homogeneity test

IPI2016

Survey of homogeneity test results



Sample:	Water 16		Date:	08/11/2016
Measurand:	Dinophysis			
Mean:		2756		
Analytical standa	ard deviation:	460		
Heterogeneity st	andard deviation s(samples):	263		
Standard deviati	on for proficiency assessment:	421 (Manual)		

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 were randomly selected, and the measurand Dinophysis acuta was analyzed 2 times. The mean across all 10 proficiency test items is 2756. The standard deviation within proficiency test items s(analytical) (=analytical precision) is 460, and the standard deviation betw een proficiency test items s(sample) is 263.

F test

According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level 5 %), therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity

According to ISO 13528:2015, the heterogeneity standard deviation s(sample) between the proficiency test items should not exceed 30 % of the standard deviation for proficiency assessment.

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 421 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity

For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.





Marine Institute Phytoplankton Rafael Salas

08/11/2016

ANNEX VII: Dinophysis acuta stability test

IPI2016

Survey of stability test results



 Sample:
 Water 16
 Date:
 08/11/2016

 Measurand:
 Dinophysis
 Dinophysis

 Mean of homogeneity:
 2756

 Mean of stability:
 2447

 Uncertainty of mean for homogeneity measurement:
 132

 Uncertainty of mean for stability measurement:
 182

 Standard deviation for proficiency assessment:
 421 (Manual)

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Dinophysis acuta has been analyzed 2 times.

The mean value across all proficiency test items of the homogeneity analysis equals 2756, the mean value across all proficiency test items of the stability analysis equals 2447.

Therefore, the mean value of the stability analysis lies 11.2 % below the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Although for the given standard deviation for proficiency assessment of 421, the proficiency test items may not be considered as adequately stable, the expanded acceptance criterion by adding the uncertainty of the difference to the standard deviation for proficiency assessment is fulfilled. Hence, stability of the proficiency test items is given only according to the expanded criterion of ISO 13528:2015.

By means of the t test it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance 5 %).

The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the t test.







ANNEX VII: Guinardia delicatula homogeneity test

IPI2016

Survey of homogeneity test results



Sample:	Water 16		Date:	08/11/2016
Measurand:	Guinardia			
Mean:		230		
Analytical standa	ard deviation:	118		
	andard deviation s(samples): on for proficiency assessment:	58 129 (Manual)		

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 were randomly selected, and the measurand Guinardia delicatula was analyzed 2 times. The mean across all 10 proficiency test items is 230. The standard deviation within proficiency test items s(analytical) (=analytical precision) is 118, and the standard deviation betw een proficiency test items s(sample) is 58.

F test

According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level 5 %), therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity

According to ISO 13528:2015, the heterogeneity standard deviation s(sample) between the proficiency test items should not exceed 30 % of the standard deviation for proficiency assessment.

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 129 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity

For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.





Marine Institute Phytoplankton Rafael Salas

08/11/2016

ANNEX VII: Guinardia delicatula stability test

IPI2016

Survey of stability test results



Sample:	Water 16		Date:	08/11/2016
Measurand:	Guinardia			
Mean of homogeneity:		230		
Mean of stability		260		
Uncertainty of m	ean for homogeneity measuremen	t: 32		
Uncertainty of mean for stability measurement:		95		
Standard deviati	on for proficiency assessment:	129 (Manual)		

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Guinardia delicatula has been analyzed 2 times.

The mean value across all proficiency test items of the homogeneity analysis equals 230, the mean value across all proficiency test items of the stability analysis equals 260.

Therefore, the mean value of the stability analysis lies 13.0 % above the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Therefore, given the standard deviation for proficiency assessment of 129, the proficiency test items may be considered as adequately stable.

By means of the t test it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance 5 %).

The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the t test.





Marine Institute Phytoplankton Rafael Salas

08/11/2016

ANNEX VII: Pseudo-nitzschia australis homogeneity test

IPI2016

Survey of homogeneity test results



Sample: Measurand:	Water 16 Pseudo-		Date:	08/11/2016
Mean: Analytical standar Heterogeneity sta	rd deviation: Indard deviation s(samples):	4284 1448 862		
• •	on for proficiency assessment:	1680 (Manual)		

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 were randomly selected, and the measurand Pseudo-nitzschia australis was analyzed 2 times. The mean across all 10 proficiency test items is 4284. The standard deviation within proficiency test items s(analytical) (=analytical precision) is 1448, and the standard deviation betw een proficiency test items s(sample) is 862.

F test

According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level 5 %), therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity

According to ISO 13528:2015, the heterogeneity standard deviation s(sample) between the proficiency test items should not exceed 30 % of the standard deviation for proficiency assessment.

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 1680 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity

For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.





Marine Institute Phytoplankton Rafael Salas 08/11/2016

ANNEX VII: Pseudo-nitzschia australis stability test

IPI2016

Survey of stability test results



Sample:	Water 16		Date:	08/11/2016
Measurand:	Pseudo-			
Mean of homoge	neity:	4284		
Mean of stability		3047		
Uncertainty of m	ean for homogeneity measuremen	t 423		
Uncertainty of m	ean for stability measurement:	687		
Standard deviati	on for proficiency assessment:	1680 (Manual)		

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Pseudo-nitzschia australis has been analyzed 2 times.

The mean value across all proficiency test items of the homogeneity analysis equals 4284, the mean value across all proficiency test items of the stability analysis equals 3047.

Therefore, the mean value of the stability analysis lies 28.9 % below the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment.

Although for the given standard deviation for proficiency assessment of 1680, the proficiency test items may not be considered as adequately stable, the expanded acceptance criterion by adding the uncertainty of the difference to the standard deviation for proficiency assessment is fulfilled. Hence, stability of the proficiency test items is given only according to the expanded criterion of ISO 13528:2015.

By means of the t test it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance 5 %).

The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the t test.





Marine Institute Phytoplankton 08 Rafael Salas 1/2016

ANNEX VII: Prorocentrum triestinum homogeneity test

IPI2016

Survey of homogeneity test results



Sample:	Water 16		Date:	08/11/2016
Measurand:	Prorocentrum			
Mean:		5956		
Analytical standa	ard deviation:	823		
Heterogeneity st	andard deviation s(samples):	639		
Standard deviati	on for proficiency assessment:	1639 (Manual)		

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 w ere randomly selected, and the measurand Prorocentrum triestinum w as analyzed 2 times. The mean across all 10 proficiency test items is 5956. The standard deviation within proficiency test items s(analytical) (=analytical precision) is 823, and the standard deviation betw een proficiency test items s(sample) is 639.

F test

According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level 5 %), therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity

According to ISO 13528:2015, the heterogeneity standard deviation s(sample) between the proficiency test items should not exceed 30 % of the standard deviation for proficiency assessment.

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 1639 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity

For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.





ANNEX VII: Prorocentrum triestinum stability test

IPI2016

Survey of stability test results



Date: 08/11/2016

Sample:	Water 16		
Measurand:	Prorocentrum		
Mean of homoger	neity:	5956	
Mean of stability:		5487	
Uncertainty of me	an for homogeneity measurement:	273	
Uncertainty of me	an for stability measurement:	182	
Standard deviation	on for proficiency assessment:	1639 (Manual)	

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Prorocentrum triestinum has been analyzed 2 times.

The mean value across all proficiency test items of the homogeneity analysis equals 5956, the mean value across all proficiency test items of the stability analysis equals 5487.

Therefore, the mean value of the stability analysis lies 7.9 % below the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Therefore, given the standard deviation for proficiency assessment of 1639, the proficiency test items may be considered as adequately stable.

By means of the t test it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance 5 %).

The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the t test.





Marine Institute Phytoplankton Rafael Salas

08/11/2016

ANNEX VII: Thalassiosira gravida homogeneity test

IPI2016

Survey of homogeneity test results



Sample:	Water 16		Date:	08/11/2016
Measurand:	Thalassiosira			
Mean:		4804		
Analytical standa	ard deviation:	1057		
· ·	andard deviation s(samples):	713		
Standard deviati	on for proficiency assessment:	1328 (Manual)		

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Water 16 w ere randomly selected, and the measurand Thalassiosira gravida w as analyzed 2 times. The mean across all 10 proficiency test items is 4804. The standard deviation w ithin proficiency test items s(analytical) (=analytical precision) is 1057, and the standard deviation betw een proficiency test items s(sample) is 713.

F test

According to the F test, the heterogeneity standard deviation is not significantly different from 0 (significance level 5 %), therefore the proficiency test items can be considered sufficiently homogeneous according to this criterion.

ISO 13528:2015 - Test for adequate homogeneity

According to ISO 13528:2015, the heterogeneity standard deviation s(sample) between the proficiency test items should not exceed 30 % of the standard deviation for proficiency assessment.

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 1328 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity

For the proficiency test items, no significant heterogeneity can be identified, although the heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment. Hence, the proficiency test items can be considered homogeneous.





Marine Institute Phytoplankton Rafael Salas

08/11/2016

ANNEX VII: Thalassiosira gravida stability test

IPI2016

Survey of stability test results



Sample: Water	16		Date:	08/11/2016
Measurand: Thala	ssiosira			
Mean of homogeneity:		4804		
Mean of stability:		4240		
Uncertainty of mean for ho	mogeneity measurement	: 327		
Uncertainty of mean for sta	ability measurement:	431		
Standard deviation for pro	ficiency assessment:	1328 (Manual)		

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Water 16 have been selected randomly and the measurand Thalassiosira gravida has been analyzed 2 times.

The mean value across all proficiency test items of the homogeneity analysis equals 4804, the mean value across all proficiency test items of the stability analysis equals 4240.

Therefore, the mean value of the stability analysis lies 11.7 % below the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Although for the given standard deviation for proficiency assessment of 1328, the proficiency test items may not be considered as adequately stable, the expanded acceptance criterion by adding the uncertainty of the difference to the standard deviation for proficiency assessment is fulfilled. Hence, stability of the proficiency test items is given only according to the expanded criterion of ISO 13528:2015.

By means of the t test it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of significance 5 %).

The difference of the mean values is not statistically significant. Therefore the proficiency test items can be considered stable according to the t test.





Marine Institute Phytoplankton Rafael Salas



ANNEX VIII: Analysts results

Dinoph	iysis acuta (c	ells/L)	Analyst Code	Prorocentr	um triestinu	m (cells/L)	Analyst	Alexandriu	ım ostenfeld	lii(cells/L)	Analyst	Guinard	ia delicatula	(cells/L)	Analyst Code
sample 1	sample 2	sample 3	Code	sample 1	sample 2	sample 3	Code	sample 1	sample 2	sample 3	Code	sample 1	sample 2	sample 3	Code
2654	2962	3231	74	5077	3852	7704	74	2385	2038	1769	74	269	346	115	74
3400	3000	2800	73	6400	6200	6000	73	1600	2400	1600	73	400	1000	200	73
2680	2920	2720	70	4520	4720	6120	70	1440	1440	1800	70	200	440	200	70
2240	2360	1920	61	3160	4760	4320	61	1360	1240	1920	61	160	360	320	61
2520	2440	3080	69	4160	3760	5000	69	1960	2080	1480	69	240	520	240	69
3080	2280	3720	81	5280	6200	7280	81	1280	1880	1600	81	280	360	240	81
2960	3000	3480	1	5240	5320	4440	1	1480	1520	1600	1	320	200	320	1
3320	2640	2320	79	5640	4040	5160	79	2360	1560	1080	79	400		440	79
2280	3880	2360	37	3400	4720	1880	37	1960	1760	1800	37	440		400	37
2695	3550	2744	40		6850	5782	40	1470	1650	1960	40	441		245	40
2886	2701	3145	29	3922	4551	4255	29	2109	1628	2220	29	592		444	29
2080	2400	2360	38		2760	3360	38	1000	1200	1320	38	240		360	38
3000	3200	2960	60	5480	5480	7800	60	2640	1960	2360	60	280		440	60
2960	2880	2320	18		5680	6320	18	1560	1720	1880	18	480		640	18
3200	3840	2320	32		6960	5600	32	2200	1960	2040	32	280		80	32
2800	2520	2600	27		4320	5000	27	1160	1520	2040	27	280		200	27
1960	2040	1760	75		3120	3400	75	1360	1640	1320	75	480		200	75
3200	2840	3160	63		5560	6720	63	1600	1920	1680	63	400		320	63
2840	2360	2320	80		4800	3560	80	1520	1440	1280		Notid	Notid	Notid	80
1360	1600	1800	66		1640	2200	66	1360	920	1480	66	200		160	66
2870	3478	3174	14	7913	6522	7392	14	2261	1652	2131	14	304		522	14
3520	3160	3720	52		3000	5680	52	2440	1360	1560	52	160		360	52
1720	2000	2000	8		3600	3480	8	640	1280	1160	8	200		240	8
2480	1880	2560	72		4590	6426	72	1640	1880	1280	72	40		360	72
3120	2680	3200	50		3520	2680	50	4200	1000	1000	50	200		400	50
3720	3000	3080	47	5440	5360	6720	47	1280	1600	1960	47	160		280	47
3280	3160	2720	43		6520	5800	43	2240	2120	2240	43	600		520	43
2520	2560 2500	2720	53		3440 7800	3680	53 19	1680 2000	1200	1320	53 19	320 550		360 850	53
4100 2800	2500	2050 3240	19 12		5800	12250 4000	19	2000 960	3150 2040	2150 1080		Not id	Notid	Not id	19 12
3280	2480	3000	5		6360	4000	5	880	1360	1080	5	360		80	5
4514	3330	3367	51	5217	5957	6956	51	2035	2590	2923	51	407		407	51
2800	2640	2560	7		1760	2240	7	960	1080	1040	7			407	7
2300	2680	2760	58		5200	5680	58	1400	1080	1520	58	400		280	58
4039	1420	7913	31		1420	5755	31	2019	1420	2158	31	2716		0	31
3283	3547	3556	25		3211	9103	25	1283	1321	1667	25	377		259	25
2600	2520	2040	44		3400	3880	44	1600	1040	1480	44	280		0	44
2480	2320	2880	17	4000	3600	4880	17	1920	2000	1400	17	160		160	17
2913	3131	3522	67	7739	7870	8783	67	1739	1783	2217	67	261		435	67
		NR				NR				NR	-	NR		NR	13
2800	2560	2720	46		7440	7760	46	1760	1600	1400	46	360		360	46
6040	3080	2800	59		7120	6880	59	1240	1360	1320	59	480		320	59

Dinoph	ysis acuta (c	ells/L)	Analyst	Prorocentr	um triestinu	m (cells/L)	Analyst	Alexandriu	ım ostenfeldi	ii(cells/L)	Analyst	Guinard	ia delicatula	(cells/L)	Analyst
sample 1	sample 2	sample 3	Code	sample 1	sample 2	sample 3	Code	sample 1	sample 2	sample 3	Code	sample 1	sample 2	sample 3	Code
2400	2700	3400	48	6100	4100	4800	48	900	1300	1400	48	0	600	100	48
3640	2840	2440	39	5200	4360	6520	39	1400	2080	1640	39	560	360	240	39
2080	2360	2480	49	3600	5360	5240	49	1160	1560	1120	49	240	200	200	49
3000	2400	2900	15	7250	6900	4800	15	2050	1650	1400	15	450	550	200	15
2760	2240	2760	41	2760	4520	5480	41		1560	2000	41	200	160	200	41
3240	2520	3160	36	6720	6040	5360	36		1680	1560	36	360		360	36
2720	2720	2360	45	5400	5520	4960	45		1840	2040	45	440		200	45
3280	1760	3400	33	8000	2560	2800	33		800	1640	33	400	200	40	33
2200	3200	3120	68	7344	7038	7650	68		1280	1600	68	120		240	68
2720	2880	2640	24	4640	4760	5160	24		1640	2240	24	400	440	520	24
2520	2240	2800	21	4880	4800	6640	21		1240	1640	21	320		160	21
1640	3600	2640	28	3880	4480	6120	28		2560	1440	28	120		800	28
2850	2900	2950	11	7820	7557	8050	11		1400	1550	11	100	150	450	11
3400	1960	3440	57	7640	6760	5760	57		1440	2000	57	360		200	57
2040	3800	2760	2	6400	5520	6520	2		2080	1760	2	280	240	160	2
2840	2640	2360	42	3200	4160	2920	42		1040	1120	42	440		280	42
3300	3000	3120	34	7650	5120	5200	34		1880	1640	34	150		360	34
1040	980	880	91	1360	960	1520	91		960	680	91	80	40	40	91
2640	1960	2200	22	3640	2880	3840	22		1240	1120	22	200		360	22
2720	2440	2960	10	3800	5120	3680	10		840	1280	10	400	280	640	10
2960	3200	3160	26	3960	4800	5480	26		1960	2160	26	320	440	280	26
2960	3560	3880	55	5040	6080	5080	55		2240	1440	55	360		960	55
2080	2800	3960	3	4600	3640	3600	3		1480	840	3	400		200	3
3308	3231	1538	56	7592	9425	5498	56		1577	1077	56	269		615	56
2308	2096	2423	30	5666	4979	5323	30		1231	1577	30	462		423	30
2846	2846	3269	23	6000	7090	5181	23		1269	1115	23	308		462	23
3280	3360	2200	54	4920	4760	4720	54		1680	1760	54	400		560	54
2720	2600	3520	78	5240	5200	5320	78		1920	1880	78	400		400	78
2480	1920	2200	65	5000	2560	3680	65		2320	1400	65	120		240	65
2900	3200	2450	76	2300	4000	4600	76		1800	1600	76	350	100	650	76
3160	3280	3520	35	6880	7040	7000	35		1640	2200	35	160		400	35
3560	3120	3760				NN	20		1800	2040	20	800	720	680	20
2480	2640	3520	71	2960	6480	5440	71		2080	1480	71	280	440	280	71
2680	2560	2760	16	5600	5560	5840	16		1840	1920		Not id		Not id	16
3320	2400	3040	62	3720	5800	3680	62		2640	1880	62	40	280	400	62
2000	2680	3560	6	6680	5960	6840	6		1520	1640	6	520		280	6
2760	2560	2760	77	2200	1280	2360	77		880	1400	77	120	40	40	77
3600	2700	3000	4	4400	5300	6400	4		1800	2100	4	400		200	4
3080	2680	2720	64	5120	4280	5360	64 9		1600	1400	64	360		320	64
4000 Dipophysis a	2480 cuta	2840	9	5280 Prorocentru	6880 m triestinum	6720	-	1720 Alexandriun	2000 a ostanfaldii	2280	9	280 Guinardia d	240 elicatula	40	9
Dinophysis a	cuta		81			1			ostemeidi			Guinardia d Guinardia si			69
			01	Prorocentru Prorocentru	_			A. minutum A. tamutum					o. a fragilissima	-	5
				NR				A. tamarens	0			Rhizosoleni	-	a	2
								A. tamarens Scrippsiella				Not id	a uencatula		2
								Scrippsiella	-		1	NULIU			81
									sp. odinium dalei	i22	1				16
								Heterocapsa			2				
									· 5p.		81				

Thalassi	osira g	ravida/rot	ula (cells/L)	Analyst	Chaetoce	eros didymus	(cells/L)	Analyst	Coscinod	iscus wailes	ii (cells/L)) [Analyst	Pseudo-nitz	schia austra	lis (cells/L)	Analyst
sample	1 s	ample 2	sample 3	Code	sample 1	sample 2	sample 3	Code	sample 1	sample 2	sample	3	Code	sample 1	sample 2	sample 3	Code
5	231	5192	5346	74	1038	500	769	74	N/A	N/A	N/A		74	5308	4115	4654	74
5	200	5800	5400	73	800	1400	800	73	2000	600		500	73	6800	8400	4400	73
52	200	5880	5360	70	800	1480	2680	70	0	C)	40	70	5200	4520	6200	70
3	520	5240	4200	61	360	520	160	61	40	80)	40	61	3080	5400	7120	61
54	440	6040	4360	69	600	440	360	69	40	80) 1	160	69	4080	4760	6120	69
6	920	6480	4840	81	1960	1280	1040	81	0	80)	40	81	4920	3520	4520	81
5	520	5720	5840	1	960	1040	280	1	40	C)	80	1	3920	4520	4960	1
6	720	7000	6200	79	1880	960	760	79	N/A	N/A	N/A		79	4680	4560	8440	79
64	400	6600	5400	37	720	80	400	37	80	80)	0	37	4400	6480	6640	37
74	497	8900	7987	40	1372	1600	882	40	49	C)	0	40	6664	6550	7595	40
6	549	6105	7696	29	1258	1813	1295	29	37	74	Ļ	37	29	5106	4699	6142	29
40	000	4760	4040	38	480	440	360	38	40			80	38	2960	4000	3920	38
1	320	3400	3080	60	Notid	Not id	Notid	60	40	C)	40	60	3320	2480	2960	60
54	440	5720	5760	18	840	1120	920	18	40			40	18	6240	6120	6080	18
4	640	6360	5480	32	400	520	600	32	80	80)	0	32	7320	9640	8040	32
7	160	5160	6240	27	1560	400	2000	27	0)	40	27	3680	4880	6280	27
	080	4760	7720	75	840	1440	760	75	40			120	75	3360	4120	3600	75
5	960	5520	6680	63	920	680	1000	63	0	40)	40	63	7560	6160	6840	63
64	400	5800	4560	80	240	320	480	80	40	40		120	80	4240	3840	5040	80
4	840	2320	2560	66	1080	200	120	66	N/A	N/A	N/A		66	5240	2160	3560	66
4	696	5783	6565	14	522	652	783	14	87	43		43	14	3348	6261	3739	14
7	120	8480	6280	52	2080	2240	2120	52	80	40)	80	52	5640	3160	5280	52
3	800	3920	2560	8	600	720	560	8	280			160	8	3280	9320	3160	8
5	267	4809	3664	72	960	640	1360	72	80	40		40	72	4440	8280	5360	72
		4440	4840	50	240	600	320		N/A	N/A	N/A		50	7320	5720	5240	50
64	480	4920	6320	47	960	520	2680	47	40	C)	0	47	6920	9120	8160	47
6	720	7000	6080	43	960	880	1040	43	40			40	43	5840	7040	7560	43
5	920	5560	4760	53	480	800	1520	53	40			120	53	6560	5320	4840	53
	850	10550	8450	19	2000	2000	3150	19	50	200		100	19	9200	7200	8650	19
	400	5160	4800				Notid		N/A	N/A	N/A		12	4000	4640	3040	12
6	120	5080	5000	5	840	640	1160	5	80			40	5	4960	4280	7120	5
	141	8473	9176	51	3626	1665	1110	51	37	37		0	51	8621	7585	6549	51
3.	200	2960	7280	7	560	160	480	7	160	C)	80	7	4320	2880	4400	7
	640	5360	6200	58	800	160	1360	58	0	40		80	58	5880	6000	7720	58
74	405	5678	8633	31	3366	2129	2158	31	N/A	N/A	N/A		31	3366	3549	6475	31
6	113	7019	5778	25	1019	1472	1667	25	38			37	25	3925	5208	6333	25
	120	4840	2000	44	480	1320	440	44	40			0	44	2520	6360	3000	44
	000	5280	4000	17	520	360	360	17	80	200		120	17	4000	4800	6000	17
	261	4565	5392	67	217	435	609	67	87	43		87	67	3261	4348	8609	67
NR	NF		NR				NR	13		NR	NR		13			NR	13
	000	7880	5560	46	680	1000	1040	46	40			40	46	6000	7200	5800	46
6	040	5640	5720	59	1680	880	1040	59	0	C)	0	59	7920	6440	6240	59

Thalassiosira	a gravida/rot	ula (cells/L)	Analyst	Chaetoce	ros didymus	(cells/L)	Analyst	Coscinod	iscus wailesi	i (cells/L)	Analyst	Pseudo-nitz	zschia austra	lis (cells/L)	Analyst
sample 1	sample 2	sample 3	Code	sample 1	sample 2	sample 3	Code	sample 1	sample 2	sample 3	Code	sample 1	sample 2	sample 3	Code
1500	1000	1800	48		1300	1600	48	N/A	N/A	N/A	48		5400	5300	48
4720	4960	4680	39	160	1360	440	39	0			39		5240	3480	39
4200	4600	3000	49	120	0	0	49	0	120	0	49	3800	2960	3600	49
9300	3650	2150	15	1250	0	0	15	50	50	50	15	8750	2800	3550	15
4000	5160	5600	41	200	120	1520	41	40	240	80	41	4440	4560	7360	41
6920	6360	6720	36	1040	920	240	36	40	40	40	36	7160	6960	6520	36
5760	4800	6240	45	360	760	1200	45	40	80	80	45	4320	4600	7240	45
8240	7520	4240	33		680	480	33	0	40				4800	6560	33
2960	3120	3160	68	240	480	640	68	80	160	120	68	5000	4600	5640	68
4520	5120	5720	24	1240	720	1000	24	40	40	40	24	5840	6440	2640	24
5120	5560	7200	21	1040	1320	1080	21	0	40	80	21	3480	4800	6800	21
3440	4000	6840	28	640	280	40	28	80	80	120	28	3520	6400	5800	28
7500	5750	4850	11	300	500	750	11	50	100	0	11	6700	5350	4100	11
6520	5360	7880	57	920	1560	440	57	80	0	0	57	7200	2360	3760	57
7280	6080	8120	2		600	640	2	0	80				8360	7320	2
6120	5280	5800	42		3840	360	42	40	40				5520	4320	42
5300	5800	7880	34		840	1400	34	50	0				7360	5160	34
920	1720	1520	91		160	0	91	40	0				1520	840	91
6760	4320	6920	22		200	960		N/A	N/A	N/A	22		4840	2400	22
4400	4560	5760	10		840	720	10	80	40				4480	6720	10
3320	5840	7320	26		1080	840		NN	NN	NN	26		4320	5080	26
6280	6400	6000	55		320	200	55	80	80				320	5000	55
7560	3720	4080	3		360	280	3	0	40				4800	1720	3
8462	7000	5500	56		1615	1577	56	115	77	0			5692	4692	56
5769	5000	6462	30		385	1192	30	38	38				3038	2423	30
4231	5154	3808	23		1885	538	23	308	115				7038	5962	23
6680	5400	6200	54	1880	1760	1160	54	80	80				6840	6480	54
5160	6720	6920	78		1000	840	78	80	80				3680	3440	78
			65				65	0							
3480	4920 5850	4240 6050	76		720 1900	160	76	50	40				2560 7750	2640 7100	65
6600						1900			0						76
6200	7640	4920	35		1280	1240	35	40	0				5880	6360	35
5240	4720	5320	20		1120	1200	20	80	40				7000	6000	20
4120	4960	5680	71		960	320	71	80	160				12400	4080	71
4800	4680	5240	16		400	440	16	80	80				5200	5280	16
3160	5200	2480	62		1000	0	62	40	0				4840	1760	62
7360	8360	8320	6		1080	1800	6	80	80				7320	7520	6
3320	4280	2440	77		1840	360	77	0	80				5480	5880	77
7000	7100	6100	4		1200	1000	4	100	100				6000	8400	4
5800	7160	6480	64		1120	1240	64	40	40				7560	5320	64
6480	6360	7280	9		1920	1680	9	80	0	80			11720	5720	9
Thalassiosira		ula		Chaetoceros				Coscinodisc	us wailesii			Pseudo-nitz			60
Thalassiosira	a sp.			Chaetoceros				N/A				Pseudo-nitz		is	14
			81	C. decipiens	i			C. concinnu	s			P. multiserie	es		1
				C. brevis				C. granii				P. seriata			4
				C. ceratospo				Coscinodisc	us sp.		-	P. fraudulen	ita		1
				C. constrictu	IS		1				81	P. Pungens			1
				C. debilis			2								81
				C. lorenzian	us		1								
				Notid			2								
							81								

Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Dinophysis acuta iteration

						29	2911	71	2911	2911
Analyst Code		Average 🖵	X-X*	it1	it2	62	2920	80	2920	2920
	91	967	1873	2365	2365	57	2933	93	2933	2933
						3	2947	107	2947	2947
66		1587	1253			54	2947	107	2947	2947
	8	1907	933		2365	78	2947	107	2947	2947
	75 61	1920 2173	920 667	2365 2365		74	2949	109	2949	2949
	65	2173	640			39	2973	133	2973	2973
	22	2267	573			36	2973	133	2973	2973
	30	2276	564		2365	23	2987	147	2987	2987
	38	2280	560		2365	40	2996	156	2996	2996
	72	2307	533			50	3000	160	3000	3000
	49	2307	533			5	3000	160	3000	3000
	44	2387	453		2387	81	3027	187	3027	3027
	80	2507	333	2507	2507	60	3053	213	3053	3053
	21	2520	320		2520	43	3053		3053	
	58	2560	280			73		213		3053
	17	2587	253	2587	2587		3067	227	3067	3067
	41	2587	253	2587	2587	63	3067	227	3067	3067
						4	3100	260	3100	3100
						26	3107	267	3107	3107
	53	2600	240	2600	2600	9	3107	267	3107	3107
	45	2600	240	2600	2600	32	3120	280	3120	3120
	42	2613	227	2613	2613	34	3140	300	3140	3140
	28	2627	213	2627	2627	1	3147	307	3147	3147
	27	2640	200	2640	2640	14	3174	334	3174	3174
	7	2667	173	2667	2667	67	3189	349	3189	3189
	16	2667	173		2667	47	3267	427	3267	3267
	69	2680	160			35	3320	480	3315	3315
	56	2692	148			25	3462	622	3315	3315
	46	2693	147			52	3467	627	3315	3315
	77	2693	147			55	3467	627	3315	3315
	10	2707	133			20	3480	640	3315	3315
	18	2720	120			51	3737	897	3315	3315
	24 6	2747	93 93		2747 2747	59	3973	1133	3315	3315
	79	2747	80			31	4457	1617	3315	3315
	15	2760	73			Average X	2822	1017	2834	2834
	70	2707	67			SD S	476		2834	2834
	33	2813	27		2813			new <i>X*</i>	2834	2834
	64	2827	13		2827	robust average X*				
	48	2833	7			robust stdev S*		new <i>S*</i>	328	328
	37	2840	0			δ= 1.5S*	475		492	492
	12	2840	0			Χ*-δ	2365		2342	2342
	68	2840	0			Χ*+δ	3315		3326	3326
	76	2850	10			no of analysts P	81		81	81
	2	2867	27		2867					
	71	2880	40		2880	Between Samples SD	263			
	19	2883	43	2883	2883					
	11	2900	60	2900	2900	new stdev for DACUTA	421			

Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Prorocentrum triestinum iteration

						4	5367	233	5367	5367
		A				55	5400	267		
Analyst Code			X-X*	it1	it2	21	5440	307	5440	
	91	1280	3853	2924	2924	74	5544	411		
	77	1947	3187	2924	2924	43	5640	507		
	7	2107	3027	2924	2924	16	5667	533		
	66	2373	2760	2924	2924	47	5840	707		
	75	2413	2720	2924	2924	18	5867	733		
	38	2747	2387	2924	2924	58	5867	733		
	50	2813	2320	2924	2924	25	5948	815		
	31	2840	2293	2924	2924	34	5990	857		
	8	3213	1920	3213		36	6040	907		
	53	3213	1920	3213		51	6043	910		
	37	3333	1800	3333	3333	23	6090	957		
	44	3427	1707	3427	3427	2	6147	1013		
	42	3427	1707	3427	3427	63	6160	1027	6160	6160
	22	3453	1680	3453	3453	40	6171	1037		
	76	3633	1500	3633	3633	32	6187	1053	6187	6187
	65	3747	1387	3747	3747	73	6200	1067	6200	6200
	3	3947	1187	3947	3947	81	6253	1120	6253	6253
	61	4080	1053	4080	4080	60	6253	1120	6253	6253
	80	4080	1053	4080	4080	9	6293	1160	6293	6293
	17	4160	973	4160		15	6317	1183	6317	6317
	10	4200	933	4200	4200	6	6493	1360	6493	6493
	29	4243	891	4243	4243	57	6720	1587	6720	6720
	41	4253	880	4253	4253	59	6893	1760	6893	6893
	12	4293	840	4293	4293	35	6973	1840	6973	6973
	69	4307	827	4307	4307	46	7240	2107	7240	7240
	52	4400	733	4400	4400	14	7276	2142	7276	
	62	4400	733	4400	4400	72	7344	2211	7343	7343
	33	4453	680	4453	4453	68	7344	2211	7343	7343
	27	4560	573	4560	4560	56	7505	2372	7343	7343
	49	4733	400	4733	4733	11	7809	2676		
	26	4747	387	4747	4747	67	8131	2997		
	54	4800	333	4800	4800	19	8883	3750		
	28	4827	307	4827	4827	20	not id	not id	notid	not id
	24	4853	280	4853	4853	Average X	5087		5111	
	64	4920	213	4920	4920	SD S	1520		1331	1331
	79	4947	187	4947	4947	robust average X*		newX*	5111	
	71	4960	173	4960	4960	robust stdev S*		new <i>S*</i>	1509	
	1	5000	133	5000	5000	δ= 1.5S*	2210		2263	
	48	5000	133	5000	5000	Χ*- δ	2924		2848	
	70	5120	13	5120	5120	Χ*+δ	7343		7374	
	5	5147	13	5147	5147	no of analysts P	80		80	80
	78	5253	120	5253	5253					
	45	5293	160	5293	5293	Between Samples SD	639			
	30	5323	189	5323	5323					
	39	5360	227	5360	5360	new stdev for PTRIES	1639			
Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Alexandrium ostenfeldii iteration

Analyst Code		Average 🖃	X-X* i	t1 it2	it3	i	t4	24	1707	40	1707	1707	1707	1707
	91	707	960	1192	1192	1192	1192	18	1720	53	1720	1720	1720	1720
								2	1720	53	1720	1720	1720	1720
	8	1027	640	1192	1192	1192	1192	63	1733	67	1733	1733	1733	1733
	7	1027	640	1192	1192	1192	1192	54	1733	67	1733	1733	1733	1733
	42	1040	627	1192	1192	1192	1192	28	1747	80	1747	1747	1747	1747
	77	1133	533	1192	1192	1192	1192	57	1747	80	1747	1747	1747	1747
	38	1173	493	1192	1192	1192	1192	55	1760	93	1760	1760	1760	1760
	48	1200	467	1200	1200	1200	1200	17	1773	107	1773	1773	1773	1773
	5	1227 1227	440	1227 1227	1227 1227	1227 1227	1227 1227	52	1787	120	1787	1787	1787	1787
	22		440					34	1790	123	1790	1790	1790	1790
	10	1240	427	1240	1240 1253	1240	1240	26	1800	133	1800	1800	1800	1800
	66 23	1253 1269	413 397	1253 1269	1253	1253 1269	1253	35	1800	133	1800	1800	1800	1800
	49	1289	397	1289	1289	1269	1269 1280	69	1840	173	1840	1840	1840	1840
	59	1307		1307	1307	1280	1280	37	1840	173	1840	1840	1840	1840
	11	1307	360 350	1317	1317	1307	1307	31	1866	199	1866	1866	1866	1866
	3	1333	333	1333	1333	1333	1317	73	1867	200	1867	1867	1867	1867
	12	1360	307	1360	1360	1360	1355	65	1880	213	1880	1880	1880	1880
	44	1373	293	1373	1373	1373	1373	16	1880	213	1880	1880	1880	1880
	53	1400	293	1400	1400	1400	1400	67	1913	246	1913	1913	1913	1913
	80	1413	253	1413	1413	1413	1413	78	1933	267	1933	1933	1933	1933
	25	1423	243	1423	1423	1423	1423	20	1960	293	1960	1960	1960	1960
	30	1436	231	1436	1436	1436	1436	41	1973	307	1973	1973	1973	1973
	75	1440	227	1440	1440	1440	1440	45	1973	307	1973	1973	1973	1973
	56	1449	218	1449	1449	1449	1449	29	1986	319	1986	1986	1986	1986
	58	1453	213	1453	1453	1453	1453	62	2000	333	2000	2000	2000	2000
	61	1507	160	1507	1507	1507	1507	9	2000	333	2000	2000	2000	2000
	64	1520	147	1520	1520	1520	1520	14	2015	348	2015	2015	2015	2015
	1	1533	133	1533	1533	1533	1533	74	2064	397	2064	2064	2064	2064
	33	1533	133	1533	1533	1533	1533	32	2067	400	2067	2067	2067	2067
	21	1533	133	1533	1533	1533	1533	43	2200	533	2141	2105	2097	2096
	68	1547	120	1547	1547	1547	1547	60	2320	653	2141	2105	2097	2096
	70	1560	107	1560	1560	1560	1560	19	2433	767	2141	2105	2097	2096
	27	1573	93	1573	1573	1573	1573	51	2516	849	2141	2105	2097	2096
	81	1587	80	1587	1587	1587	1587	Average X	1632		1634	1632	1632	1632
	46	1587	80	1587	1587	1587	1587	SD S	327		277	273	273	273
	72	1600	67	1600	1600	1600	1600	robust average X*		new X*	1634	1632	1632	1632
	47	1613	53	1613	1613	1613	1613	robust stdev S*		new S*	314	310	309	309
	76	1617	50	1617	1617	1617	1617	$\delta = 1.5S^*$	475		470	465	464	464
	4	1633	33	1633	1633	1633	1633	X*-δ	1192		1164	1167	1168	1168
	79	1667	0	1667	1667	1667	1667	X*+δ	2141		2105	2097	2096	2095
	36	1667	0	1667	1667	1667	1667				80		2096	2093
	71	1680	13	1680	1680	1680	1680	no of analysts P	80		80	80	80	80
	6	1680	13	1680	1680	1680	1680	Detroises Complete CD	70					
	40	1693	27	1693	1693	1693	1693	Between Samples SD	76					
	15	1700	33	1700	1700	1700	1700		240					
	39	1707	40	1707	1707	1707	1707	new stdev for AOSTEN	318					

									42	347	30	347	347	347	347	347
Analyst Code		Averag	X-X*	it1	it2	it3	it4	it5	26		30	347	347	347	347	
Analyst Coue	91	53	263						67	348	31	348	348	348	348	348
	91		203	132	140	5 150	, 13.	1 151	63	360	44	360	360	360	360	360
	77	67	250	132	148	3 150) 15:	1 151	35							
	66	147	170	147	148	3 150) 15:	1 151	76						367	367
	32	187	130	187	187	7 187	/ 18	7 187	34							
	47	187	130	187	187	7 187	/ 18	7 187	23							
	41	187	130	187	187	7 187	/ 18	7 187	46							
	65	187	130	187	187	7 187	/ 18	7 187	39						387	387
	9	187	130	187	187	187	/ 18	7 187	28						387	387
	44	200	116	200	200) 200) 200	200	78						387	387
	68	200	116	200	200) 200) 200	200	78							
	8	213	103	213	213	3 213	3 21 3	3 213	15							
	72	213	103	213	213	3 213	3 21 3	3 213	51							
	58	213	103	213	213	3 213	3 21 3	3 213								407
	49	213	103	213	213	3 213	3 21 3	3 213	14							
	33	213	103	213	213	3 213	3 21 3	3 213	59							
	3	213	103	213	213	8 213	8 213	3 213	10							
	27	227	90						30							
	2	227	90						36							
	48	233	83						24							
	11	233	83						6							453
	17	240	76	240					54					467	467	467
	62	240	76	240	240) 240) 240	240	29	481	. 165	481	481	481	481	481
	74	244	73						43						493	
	50	267	50						56					498	498	
	5	267	50						73	533	217	501	500	498	498	498
	21	267	50						18	533	217	501	500	498	498	498
	57	267	50						55	547	230	501	500	498	498	498
	70	280	36						19	567	250	501	500	498	498	498
	61	280	36						20	733	417	501	500	498	498	498
	1	280	36						31	1142	826	501	500	498	498	498
	81	293	23						80	not id						
	53	293	23						12	not id						
	4	300	16						16	not id						
	37	307	10						Average X	336	5	324	324	324	324	324
	60	307	10						SD S	150)	103	102	102	102	102
	75	307	10						robust average X*	316	new X*	324	324	324	324	
	52	307	10						robust stdev S*		new S*	117				
	45	307	10						δ= 1.5S*	185		176				
	25	313	4						X*-δ	132		148				
	38	320	4						X*+δ	501		500				
	22	320	4							78		78				
	64	320	4						no of analysts P	/2	2	/8	/8	/8	/8	/8
	40	329	12						Detween Complete CD	F(
	69	333	17						Between Samples SD	58	•					
	71	333	17							4.00						
	79	347	30	347	347	7 347	34	7 347	new stdev for GDELIC	129						

Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Guinardia delicatula iteration

Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Thalassiosira gravida/rotula iteration

								59	5800	160	5800	5800	5800	5800
Analyst Code	🔽 Αν	verag🖵	X-X* it:	it.	2	it3	it4	75	5853	213	5853	5853	5853	5853
	91	1387	4253	4009	4009	4009	4009	47	5907	267	5907	5907	5907	5907
								21	5960	320	5960	5960	5960	5960
	48	1433	4207	4009	4009	4009	4009	22	6000	360	6000	6000	6000	6000
	60	2600	3040	4009	4009	4009	4009	11	6033	393	6033	6033	6033	6033
	68	3080	2560	4009	4009	4009	4009	63	6053	413	6053	6053	6053	6053
	66	3240	2400	4009	4009	4009	4009	81	6080	440	6080	6080	6080	6080
	77	3347	2293	4009	4009	4009	4009	54	6093	453	6093	6093	6093	6093
	8	3427	2213	4009	4009	4009	4009	37	6133	493	6133	6133	6133	6133
	62	3613	2027	4009	4009	4009	4009	76	6167	527	6167	6167	6167	6167
	49	3933	1707	4009	4009	4009	4009	27	6187	547	6187	6187	6187	6187
	44	3987	1653	4009	4009	4009	4009	55	6227	587	6227	6227	6227	6227
	65 38	4213 4267	1427 1373	4213 4267	4213 4267	4213 4267	4213	35	6253	613	6253	6253	6253	6253
	61	4267	1373	4267	4267	4267	4267 4320	78	6267	627	6267	6267	6267	6267
	23	4320	1320	4320	4320	4320	4320	25	6303	663	6303	6303	6303	6303
	23	4397	1160	4397	4397	4397	4480	34	6327	687	6327	6327	6327	6327
	72	4480	1060	4480	4480	4480	4480	46	6480	840	6480	6480	6480	6480
	50	4580	1000	4580	4580	4580	4640	64	6480	840	6480	6480	6480	6480
	67	4739	901	4739	4739	4739	4739	57	6587	947	6587	6587	6587	6587
	17	4760	880	4760	4760	4759	4760	43	6600	960	6600	6600	6600	6600
	28	4760	880	4760	4760	4760	4760	79	6640	1000	6640	6640	6640	6640
	12	4787	853	4787	4787	4787	4787	36	6667	1027	6667	6667	6667	6667
	39	4787	853	4787	4787	4787	4787	33	6667	1027	6667	6667	6667	6667
	10	4907	733	4907	4907	4907	4907	9	6707	1067	6707	6707	6707	6707
	16	4907	733	4907	4907	4907	4907	4	6733	1093	6733	6733	6733	6733
	41	4920	720	4920	4920	4920	4920	29	6783	1143	6783	6783	6783	6783
	71	4920	720	4920	4920	4920	4920	56	6987	1347	6987	6987	6987	6987
	15	5033	607	5033	5033	5033	5033	2	7160	1520	7160	7160	7160	7160
	20	5093	547	5093	5093	5093	5093	31	7239	1599	7239	7239	7239	7239
	24	5120	520	5120	5120	5120	5120	52	7293	1653	7271	7256	7252	7251
	3	5120	520	5120	5120	5120	5120	6	8013	2373	7271	7256	7252	7251
	74	5256	384	5256	5256	5256	5256	40	8128	2488	7271	7256	7252	7251
	69	5280	360	5280	5280	5280	5280	51	8263	2623	7271	7256	7252	7251
	5	5400	240	5400	5400	5400	5400	19	8617	2977	7271	7256	7252	7251
	53	5413	227	5413	5413	5413	5413	Average X	5496	2577	5571	5570	5570	5570
	73	5467	173	5467	5467	5467	5467	SD S	1345		990	989	988	988
	70	5480	160	5480	5480	5480	5480		5640		5571	5570	5570	5570
	32	5493	147	5493	5493	5493	5493	robust average X*	1088					
	26	5493	147	5493	5493	5493	5493	robust stdev S*		new 3*	1123	1121	1121	1121
	80	5587	53	5587	5587	5587	5587	δ= 1.5S*	1631		1684	1682	1681	1681
	45	5600	40	5600	5600	5600	5600	Χ*-δ	4009		3887	3888	3889	3889
	18	5640	О	5640	5640	5640	5640	Χ*+δ	7271		7256	7252	7251	7251
	14	5681	41	5681	5681	5681	5681	no of analysts P	81		81	81	81	81
	1	5693	53	5693	5693	5693	5693							
	58	5733	93	5733	5733	5733	5733	Between Samples SD	713					
	42	5733	93	5733	5733	5733	5733							
	30	5744	104	5744	5744	5744	5744	new stdev for TGRAVIDA	1328					

Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Chaetoceros didymus iteration

Analyst Code	-	Averag	X-X*	it1 it2	it3	it	4 it5										
,	49	40	800		156	168	170	170	43					960		960	960
									57					973		973	973
	91	80	760	39	156	168	170	170	72				987	987		987	987
	28	320	520		320	320	320	320	24				987	987		87	987
	55	333	507		333	333	333	333	73					1000		000	1000
	61	347	493		347	347	347	347	26				1000	1000		000	1000
	80	347	493		347	347	347	347	75				1013	1013		013	1013
	62	347	493		347	347	347	347	23					1038)38	1038
	50	387	453		387	387	387	387	20				1093	1093		93	1093
	16	387	453		387	387	387	387	35				1107	1107		.07	1107
	37	400	440		400	400	400	400	21				1147	1147		47	1147
	7	400	440		400	400	400	400	79				1200	1200		200	1200
	17	413	427		413	413	413	413	59				1200	1200		200	1200
	15	417	423		417	417	417	417	64				1253	1253		253	1253
	67	420	420		420	420	420	420	40				1285	1285		285	1285
	38	427	413		427	427	427	427	27				1320	1320		320	1320
	68	453	387		453	453	453	453	48				1367	1367		867	1367
	69	467	373		467	467	467	467	25				1386	1386		886	1386
	66	467	373		467	467	467	467	47				1387	1387		87	1387
	32	507	333		507	507	507	507	81				1427	1427		27	1427
	65	507	333		507	507	507	507	29				1455	1455		155	1455
	11	517	323		517	517	517	517	34				1480	1480		80	1480
	33	520	320		520	520	520	520	9				1480	1480		80	1480
	41	613	227		613	613	613	613	54				1600	1600		500	1600
	8	627	213		627	627	627	627	56				1615	1615		515	1615
	14	652	188		652	652	652	652					1641	1638		536 536	1635
	39	653	187		653	653	653	653	76				1641	1638		536	1635
	22	653	187	653	653	653	653	653	42				1641	1638		536 536	1635
	71	720	120		720	720	720	720					1641	1638			1635
	36	733	107		733	733	733	733	51				1641	1638		536 526	1635
	44	747	93		747	747	747	747	52 19				1641 1641	1638 1638		536 536	1635 1635
	2	747	93		747	747	747	747	31		1543			1638		536 536	1635
	1	760	80		760	760	760	760		not id	not id	not id	not id		not id		ot id
	74	769	71		769	769	769	769		not id	not id	notid	not id		not id		otid
	58	773	67		773	773	773	773	Average X	943		900				903	903
	45	773	67		773	773	773	773	SD S	528		438		431		31	430
	10	787	53		787	787	787	787	robust average X*		new X*	900		903		903	430 903
	30	795	45		795	795	795	795	robust stdev S*		new S*	496		489		188	488
	77	827	13		827	827	827	827		801		744				/32	732
	4	833	7		833	833	833	833	δ= 1.5S*				735	733		_	732 171
	3	840	0		840	840	840	840	X*-δ	39		156		170		70	
	63	867	27		867	867	867	867	X*+δ	1641		1644	1638	1636		35	1635
	5	880	40		880	880	880	880	no of analysts P	79		79	79	79		79	79
	46	907	67		907	907	907	907	Baturaan Camalaa CD	202						_	
	78	907	67		907	907	907	907	Between Samples SD	263						_	
	53	933	93		933	933	933	933	now atday for CDIDYA							_	
	18	960	120	960	960	960	960	960	new stdev for CDIDYMUS	555							

						34		57		7	57		57
Analyst Code		Averag	X-X*	it1	it2	14		58		8	58		58
	70	13	37	13	12	56		64		14	64		64
	47	12	27	42	13	38		67		17	67		67
	47	13	37			75		67		17	67		67
	39	13	37			80		67		17	67		67
	65 62	13	37 37			52		67		17	67		67
	40	13	37			45		67		17	67		67
	51	25	25			54		67		17	67		67
	60	23	23			78		67		17	67		67
	63	27	23			6		67		17	67		67
	44	27	23			4		67		17	67		67
	44	27	23			67		72		22	72		72
	33	27	23			7		80		30	80		80
	57	27	23			16		80		30	80		80
	42	27	23			69		93		43	87		87
	91	27	23			28		93		43	87		87
	35	27	23			71		93		43	87		87
	77	27	23			19		117		67	87		87
	76	33	17			41		120		70	87		87
	25	38	12			68		120		70	87		87
	30	38	12			17		133		83	87		87
	81	40	10			23		167	1	.17	87		87
	1	40	10			8		200	1	.50	87		87
	27	40	10			73		1067		17	87		87
	43	40	10				N/A		N/A	N/A		N/A	
	5	40	10	40	40		N/A		N/A	N/A		N/A	
	58	40	10	40	40		N/A		N/A	N/A		N/A	
	49	40	10	40	40		N/A		N/A	N/A		N/A	
	36	40	10	40	40		N/A		N/A	N/A		N/A	
	24	40	10	40	40		N/A		N/A	N/A		N/A	
	21	40	10	40	40		N/A		N/A	N/A		N/A	
	2	40	10	40	40		N/A		N/A	N/A		N/A	
	3	40	10	40	40		N/A		N/A	N/A		N/A	
	64	40	10	40	40		N/A		N/A	N/A		N/A	
	29	49	1	49	49	Average X	N/A	69		N/A	50		50
	15	50	Ο	50	50	SD S		125			22		22
	11	50	Ο	50	50								22 50
	61	53	3	53	53	robust average X*			newX*		50		
	37	53	3			robust stdev S*			new <i>S*</i>		25		25
	18	53	3			$\delta = 1.5S^*$		37			38		38
	32	53	3	53		Χ*-δ		13			12		12
	72	53	3	53		Χ*+δ		87			88		88
	53	53	3	53	53	no of analysts P		71			71		71
	10	53	3	53	53								
	55	53	3			Between Samples SD		27					
	20	53	3										
	9	53	3	53	53	new stdev for CWALL		37					

Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 Pseudo-nitzschia australis iteration

												53	5573	200	5573	5573	5573	5573	5573	5573	5573
Analyst Code	Average			it1 it.		it3	it4	it5	it6	it7		39	5600	227	5600	5600	5600	5600	5600	5600	5600
	91 1	.107 4	4267	3127	3208	323	0 32	38	3241	3242	3242	34	5640	267	5640	5640	5640	5640	5640	5640	5640
	55 2	.653	2720	3127	3208	323	n 27	38	3241	3242	3242	48	5833	460	5833	5833	5833	5833	5833	5833	5833
			2655	3127	3208	323		.50 138	3241	3242	3242	37	5840	467	5840	5840	5840	5840	5840	5840	5840
			2453	3127	3208	323		.38	3241	3242	3242	10	5880	507	5880	5880	5880	5880	5880	5880	5880
			2347	3127	3208	323		.38	3241	3242	3242	79	5893	520	5893	5893	5893	5893	5893	5893	5893
			2093	3280	3280	328		.30	3280	3280	3280	72	6027	653	6027	6027	6027	6027	6027	6027	6027
			2040	3333	3333	333		33	3333	3333	3333	50	6093	720	6093	6093	6093	6093	6093	6093	6093
			1920	3453	3453	345		53	3453	3453	3453	18	6147	773	6147	6147	6147	6147	6147	6147	6147
			1747	3627	3627	362		27	3627	3627	3627	46	6333	960	6333	6333	6333	6333	6333	6333	6333
			1720	3653	3653	365		53	3653	3653	3653	76	6383	1010	6383	6383	6383	6383	6383	6383	6383
			1680	3693	3693	3693		i93	3693	3693	3693	33	6400	1027	6400	6400	6400	6400	6400	6400	6400
			1507	3867	3867	386		67	3867	3867	3867	35	6400	1027	6400	6400	6400	6400	6400	6400	6400
			1480	3893	3893	3893		93	3893	3893	3893	64	6467	1093	6467	6467	6467	6467	6467	6467	6467
			1413	3960	3960	396		60	3960	3960	3960	23	6474	1101	6474	6474	6474	6474	6474	6474	6474
			1240	4133	4133	413		.33	4133	4133	4133	73	6533	1160	6533	6533	6533	6533	6533	6533	6533
			1227	4147	4147	414		47	4147	4147	4147	58	6533	1160	6533	6533	6533	6533	6533	6533	6533
			1053	4320	4320	432		20	4320	4320	4320	54	6733	1360	6733	6733	6733	6733	6733	6733	6733
			1000	4373	4373	437		73	4373	4373	4373	20	6787	1413	6787	6787	6787	6787	6787	6787	6787
		440	933	4440	4440	444		40	4440	4440	4440	43	6813	1440	6813	6813	6813	6813	6813	6813	6813
		440	933	4440	4440	444		40	4440	4440	4440	63	6853	1480	6853	6853	6853	6853	6853	6853	6853
		449	924	4449	4449	444		49	4449	4449	4449	59	6867	1493	6867	6867	6867	6867	6867	6867	6867
		463	910	4463	4463	446		63	4463	4463	4463	36	6880	1507	6880	6880	6880	6880	6880	6880	6880
		467	907	4467	4467	446		67	4467	4467	4467	40	6936	1563	6936	6936	6936	6936	6936	6936	6936
		547	827	4547	4547	454		47	4547	4547	4547	6	6947	1573	6947	6947	6947	6947	6947	6947	6947
		692	681	4692	4692	4693		92	4692	4692	4692	56	7026	1652	7026	7026	7026	7026	7026	7026	7026
		693	680	4693	4693	469		93	4693	4693	4693	4	7067	1693	7067	7067	7067	7067	7067	7067	7067
		933	440	4933	4933	493		33	4933	4933	4933	71	7173	1800	7173	7173	7173	7173	7173	7173	7173
	27 4	947	427	4947	4947	494	7 49	47	4947	4947	4947	51	7585	2212	7585	7585	7581	7574	7571	7570	7569
	24 4	973	400	4973	4973	497	3 49	73	4973	4973	4973	2	7707	2333	7620	7596	7581	7574	7571	7570	7569
	69 4	987	387	4987	4987	498	7 49	87	4987	4987	4987	9	8040	2667	7620	7596	7581	7574	7571	7570	7569
	21 5	027	347	5027	5027	502	7 50	27	5027	5027	5027	47	8040	2693	7620	7596	7581	7574	7571	7570	7569
		033	340	5033	5033	503		33	5033	5033	5033	32	8333	2093	7620	7596	7581	7574	7571	7570	7569
	68	080	293	5080	5080	508	0 50	80	5080	5080	5080	19	8350	2900	7620	7596	7581	7574	7571	7570	7569
		155	218	5155	5155	515		.55	5155	5155	5155	Average X	5392	2911	5402	5406	5406	5406	5406	5406	5406
	61	200	173	5200	5200	520	0 52	00	5200	5200	5200	-									
		240	133	5240	5240	524	0 52	40	5240	5240	5240	SD S	1433		1290	1279	1275	1273	1272	1272	1272
	8 5	253	120	5253	5253	525	3 52	53	5253	5253	5253	robust average X*	5373 r		5402	5406	5406	5406	5406	5406	5406
	77 5	280	93	5280	5280	528	0 52	80	5280	5280	5280	robust stdev S*	1498 r	1ew 5*	1463	1450	1445	1443	1443	1442	1442
	70 5	307	67	5307	5307	530	7 53	07	5307	5307	5307	δ= 1.5S*	2247		2194	2175	2168	2165	2164	2164	2163
	29 5	316	58	5316	5316	531	6 53	16	5316	5316	5316	Χ*-δ	3127		3208	3230	3238	3241	3242	3242	3242
	16 5	373	0	5373	5373	537	3 53	73	5373	5373	5373	Χ*+δ	7620		7596	7581	7574	7571	7570	7569	7569
	11 5	383	10	5383	5383	538	3 53	83	5383	5383	5383	no of analysts P	81		81	81	81	81	81	81	81
	45 5	387	13	5387	5387	538	7 53	87	5387	5387	5387										
	67 5	406	33	5406	5406	540	6 54	06	5406	5406	5406	Between Samples SD	862								
	5 5	453	80	5453	5453	545	3 54	53	5453	5453	5453										
	41 5	453	80	5453	5453	5453	3 54	53	5453	5453	5453	new stdev for PAUS	1680								









Analyst code	Within tolerance limits	Total	Percentage	Successful	Analyst code	Within tolerance limits	Total	Percentage	Successful
91	2	8	25 %	No	67	8	8	100 %	Yes
51	4	8	50 %	No	2	8	8	100 %	Yes
19	4	8	50 %	No	37	8	8	100 %	Yes
31	4	8	50 %	No	34	8	8	100 %	Yes
12	5	7	71 %	No	71	8	8	100 %	Yes
60	5	8	62 %	No	76	8	8	100 %	Yes
20	6	8	75 %	No	38	8	8	100 %	Yes
8	6	8	75 %	No	47	8	8	100 %	Yes
66	6	7	86 %	Yes	18	8	8	100 %	Yes
48	6	7	86 %	Yes	69	8	8	100 %	Yes
59	6	7	86 %	Yes	61	8	8	100 %	Yes
74	7	7	100 %	Yes	81	8	8	100 %	Yes
23	7	8	88 %	Yes	62	8	8	100 %	Yes
52	7	8	88 %	Yes	28	8	8	100 %	Yes
79	7	7	100 %	Yes	72	8	8	100 %	Yes
50	7	7	100 %	Yes	41	8	8	100 %	Yes
42	7	8	88 %	Yes	68	8	8	100 %	Yes
26	7	7	100 %	Yes	77	8	8	100 %	Yes
73	7	8	88 %	Yes	9	8	8	100 %	Yes
80	7	8	88 %	Yes	5	8	8	100 %	Yes
17	7	8	88 %	Yes	58	8	8	100 %	Yes
75	7	8	88 %	Yes	10	8	8	100 %	Yes
22	7	7	100 %	Yes	30	8	8	100 %	Yes
16	7	8	88 %	Yes	39	8	8	100 %	Yes
29	8	8	100 %	Yes	49	8	8	100 %	Yes
36	8	8	100 %	Yes	65	8	8	100 %	Yes
25	8	8	100 %	Yes	35	8	8	100 %	Yes
56	8	8	100 %	Yes	1	8	8	100 %	Yes
53	8	8	100 %	Yes	44	8	8	100 %	Yes
57	8	8	100 %	Yes	3	8	8	100 %	Yes
24	8	8	100 %	Yes	7	8	8	100 %	Yes
21	8	8	100 %	Yes	33	8	8	100 %	Yes
78	8	8	100 %	Yes	55	8	8	100 %	Yes
6	8	8	100 %	Yes	32	8	8	100 %	Yes
70	8	8	100 %	Yes	54	8	8	100 %	Yes
27	8	8	100 %	Yes	63	8	8	100 %	Yes
43	8	8	100 %	Yes	46	8	8	100 %	Yes
45	8	8	100 %	Yes	40	8	8	100 %	Yes
64	8	8	100 %	Yes	11	8	8	100 %	Yes
4	8	8	100 %	Yes	15	8	8	100 %	Yes
14	8	8	100 %	Yes					

ANNEX XI: Performance statistics for the test

Analyst code	Pseudo-nitz australi		Guinardia del	icatula	Dinophysis a	acuta	Thalassios gravida/ro		Chaetoceros d	lidymus
	mean (cells/L)	Z score	mean (cells/L)	Z score	mean (cells/L)	Z score	mean (cells/L)	Z score	mean (cells/L)	Z score
1	4467	-0.56	280	-0.34	3147	0.74	5693	0.09	760	-0.26
2	7707	1.37	227	-0.75	2867	0.08	7160	1.2	747	-0.28
3	4133	-0.76	213	-0.86	2947	0.27	5120	-0.34	840	-0.11
4	7067	0.99	300	-0.19	3100	0.63	6733	0.88	833	-0.13
5	5453	0.03	267	-0.44	3000	0.39	5400	-0.13	880	-0.04
6	6947	0.92	453	1	2747	-0.21	8013	1.84	1733	1.5
7	3867	-0.92	400	0.59	2667	-0.4	4480	-0.82	400	-0.91
8	5253	-0.09	213	-0.86	1907	-2.2	3427	-1.61	627	-0.5
9	8040	1.57	187	-1.06	3107	0.65	6707	0.86	1480	1.04
10	5880	0.28	440	0.9	2707	-0.3	4907	-0.5	787	-0.21
11	5383	-0.01	233	-0.7	2900	0.16	6033	0.35	517	-0.7
12	3893	-0.9	not id	-3	2840	0.01	4787	-0.59	not id	-3
14	4449	-0.57	420	0.75	3174	0.81	5681	0.08	652	-0.45
15	5033	-0.22	400	0.59	2767	-0.16	5033	-0.4	417	-0.88
16	5373	-0.02	not id	-3	2667	-0.4	4907	-0.5	387	-0.93
17	4933	-0.28	240	-0.65	2587	-0.59	4760	-0.61	413	-0.88
18	6147	0.44	533	1.62	2720	-0.27	5640	0.05	960	0.1
19	8350	1.75	567	1.88	2883	0.12	8617	2.29	2383	2.67
20	6787	0.82	733	3.17	3480	1.53	5093	-0.36	1093	0.34
21	5027	-0.23	267	-0.44	2520	-0.75	5960	0.29	1147	0.44
22	3280	-1.27	320	-0.03	2267	-1.35	6000	0.32	653	-0.45
23	6474	0.64	372	0.37	2987	0.36	4398	-0.88	1038	0.24
24	4973	-0.26	453	1	2747	-0.21	5120	-0.34	987	0.15
25	5155	-0.15	313	-0.09	3462	1.49	6303	0.55	1386	0.87
26	4547	-0.51	347	0.18	3107	0.65	5493	-0.06	1000	0.17
27	4947	-0.27	227	-0.75	2640	-0.46	6187	0.46	1320	0.75

ANNEX XII: Summary of laboratory means

Analyst code	Pseudo-nitz australi		Guinardia del	icatula	Dinophysis a	acuta	Thalassios gravida/ro		Chaetoceros d	lidymus
	mean (cells/L)	Z score	mean (cells/L)	Z score	mean (cells/L)	Z score	mean (cells/L)	Z score	mean (cells/L)	Z score
28	5240	-0.1	387	0.49	2627	-0.49	4760	-0.61	320	-1.05
29	5316	-0.05	481	1.22	2911	0.18	6783	0.91	1455	1
30	2718	-1.6	449	0.97	2276	-1.33	5744	0.13	795	-0.19
31	4463	-0.56	1142	6.34	4457	3.86	7239	1.26	2551	2.97
32	8333	1.74	187	-1.06	3120	0.68	5493	-0.06	507	-0.71
33	6400	0.59	213	-0.86	2813	-0.05	6667	0.83	520	-0.69
34	5640	0.14	370	0.36	3140	0.73	6327	0.57	1480	1.04
35	6400	0.59	360	0.28	3320	1.15	6253	0.51	1107	0.37
36	6880	0.88	453	1	2973	0.33	6667	0.83	733	-0.31
37	5840	0.26	307	-0.13	2840	0.01	6133	0.42	400	-0.91
38	3627	-1.06	320	-0.03	2280	-1.32	4267	-0.98	427	-0.86
39	5600	0.12	387	0.49	2973	0.33	4787	-0.59	653	-0.45
40	6936	0.91	329	0.04	2996	0.39	8128	1.93	1285	0.69
41	5453	0.03	187	-1.06	2587	-0.59	4920	-0.49	613	-0.52
42	4440	-0.57	347	0.18	2613	-0.52	5733	0.12	2053	2.07
43	6813	0.84	493	1.31	3053	0.52	6600	0.78	960	0.1
44	3960	-0.86	200	-0.96	2387	-1.06	3987	-1.19	747	-0.28
45	5387	-0.01	307	-0.13	2600	-0.56	5600	0.02	773	-0.23
46	6333	0.55	373	0.38	2693	-0.33	6480	0.69	907	0.01
47	8067	1.58	187	-1.06	3267	1.03	5907	0.25	1387	0.87
48	5833	0.25	233	-0.7	2833	0	1433	-3.11	1367	0.84
49	3453	-1.16	213	-0.86	2307	-1.25	3933	-1.23	40	-1.55
50	6093	0.41	267	-0.44	3000	0.39	4640	-0.7	387	-0.93
51	7585	1.3	407	0.64	3737	2.14	8263	2.03	2134	2.22
52	4693	-0.42	307	-0.13	3467	1.5	7293	1.3	2147	2.24
53	5573	0.1	293	-0.24	2600	-0.56	5413	-0.12	933	0.05
54	6733	0.79	467	1.11	2947	0.27	6093	0.39	1600	1.26

Analyst code	Pseudo-nitz australi		Guinardia del	icatula	Dinophysis a	acuta	Thalassios gravida/ro		Chaetoceros d	idymus
	mean (cells/L)	Z score	mean (cells/L)	Z score	mean (cells/L)	Z score	mean (cells/L)	Z score	mean (cells/L)	Z score
55	2653	-1.64	547	1.73	3467	1.5	6227	0.49	333	-1.03
56	7025	0.96	500	1.36	2692	-0.34	6987	1.07	1615	1.28
57	4440	-0.57	267	-0.44	2933	0.24	6587	0.77	973	0.13
58	6533	0.67	213	-0.86	2560	-0.65	5733	0.12	773	-0.23
59	6867	0.87	440	0.9	3973	2.71	5800	0.17	1200	0.54
60	2920	-1.48	307	-0.13	3053	0.52	2600	-2.24	not id	-3
61	5200	-0.12	280	-0.34	2173	-1.57	4320	-0.94	347	-1
62	4147	-0.75	240	-0.65	2920	0.2	3613	-1.47	347	-1
63	6853	0.86	360	0.28	3067	0.55	6053	0.36	867	-0.07
64	6467	0.63	320	-0.03	2827	-0.02	6480	0.69	1253	0.63
65	3333	-1.23	187	-1.06	2200	-1.51	4213	-1.02	507	-0.71
66	3653	-1.04	147	-1.37	1587	-2.96	3240	-1.75	467	-0.79
67	5406	0	348	0.19	3189	0.84	4739	-0.63	420	-0.87
68	5080	-0.19	200	-0.96	2840	0.01	3080	-1.88	453	-0.81
69	4987	-0.25	333	0.07	2680	-0.37	5280	-0.22	467	-0.79
70	5307	-0.06	280	-0.34	2773	-0.14	5480	-0.07	1653	1.35
71	7173	1.05	333	0.07	2880	0.11	4920	-0.49	720	-0.33
72	6027	0.37	213	-0.86	2307	-1.25	4580	-0.75	987	0.15
73	6533	0.67	533	1.62	3067	0.55	5467	-0.08	1000	0.17
74	4692	-0.42	243	-0.63	2949	0.27	5256	-0.24	769	-0.24
75	3693	-1.02	307	-0.13	1920	-2.17	5853	0.21	1013	0.2
76	6383	0.58	367	0.33	2850	0.04	6167	0.45	1800	1.62
77	5280	-0.07	67	-1.99	2693	-0.33	3347	-1.67	827	-0.14
78	3027	-1.42	387	0.49	2947	0.27	6267	0.52	907	0.01
79	5893	0.29	347	0.18	2760	-0.18	6640	0.81	1200	0.54
80	4373	-0.61	not id	-3	2507	-0.78	5587	0.01	347	-1
81	4320	-0.65	293	-0.24	3027	0.46	6080	0.38	1427	0.94
91	1107	-2.56	53	-2.1	967	-4.44	1387	-3.15	80	-1.48

	P.Australis	G.delicatula	D.acuta	T.grav/rotula	C.didymus	C.wailesii	P.triestinum	A.ostenfeldi
Statistical method	Q/Huber	Q/Huber	Q/Huber	Q/Huber	Q/Huber	Q/Huber	Q/Huber	Q/Huber
Assessment	Z <=2.00	Z <=2.00	Z <=2.00	Z <=2.00	Z <=2.00	Z <=2.00	Z <=2.00	Z <=2.00
No. of laboratories that submitted results	81	81	81	81	81	72	81	81
No. of participants (according to design)	81	81	81	81	81	81	81	81
No. of laboratories with quantitative values	81	78	81	81	79	71	80	81
Median of No of measurement repetitions	3	3	3	3	3	3	3	3
Arithmetical mean	5204	322	2809	5468	883	60	4988	1619
Median	5200	320	2800	5720	840	40	5140	1600
Assigned value	5406	324	2834	5570	903	50	5111	1626
Mean	5324	321	2819	5500	884	50	5054	1618
Reference value	5406	324	2834	5570	903	50	5111	1626
Target s.d.	1680	129	421	1328	555	37	1639	321
Reproducibility s.d.	1876	178	537	1509	580	42	1772	443
Repeatability s.d.	1282	128	362	890	316	42	966	330
Reprod. s.d. / Repeatability s.d. ratio	1.46	1.39	1.48	1.7	1.83	1	1.83	1.34
Rel. SDPA	31.08 %	39.81 %	14.86 %	23.84 %	61.46 %	74.00 %	32.07 %	19.74 %
Rel. reproducibility s.d.	34.71 %	54.80 %	18.95 %	27.09 %	64.19 %	84.24 %	34.68 %	27.21 %
Rel. repeatability s.d.	23.72 %	39.53 %	12.76 %	15.97 %	34.98 %	84.24 %	18.90 %	20.27 %
Reference s.d.	1680	129	421	1328	555	37	1639	321
Rel. limit of reproducibility	104.13 %	164.40 %	56.85 %	81.27 %	192.58 %	252.72 %	104.03 %	81.64 %
Rel. limit of repeatability	71.16 %	118.59 %	38.29 %	47.92 %	104.95 %	252.72 %	56.70 %	60.81 %
imit of reference value (3.00 X Ref. s.d.)	5040	387	1263	3984	1665	111	4917	963
Measurand name	PAUS	GDELIC	DACUTA	TGRAVIDA	CDIDYMUS	CWALL	PTRIES	AOSTEN
No. of measurement values outside of	14	31	33	26	13	14	14	28
tolerance limits								
No. of laboratories after elimination of	81	78	81	81	79	71	80	81
outliers type A-L except E (without laboratori	es that only gave st	ates but no mea	asured values)					
No of labs with replicates out. of tol. limits	12	23	26	17	9	10	11	24
No of labs with mean outside tol.limits	1	3	7	5	5	4	2	4
No. of measurement values and states	81	81	81	81	81	72	81	81
No. of measurement values	243	234	243	242	237	213	240	241
No. of measurement values without outliers	243	234	243	242	237	213	240	241

Explanation of outlier types: A: Single outlier (Grubbs); B: Differing laboratory mean (Grubbs); C: Excessive laboratory s.d. (Cochran); D: Excluded manually; E: mean outside tolerance limits; F: |Z-Score|>3.5; L: Differing laboratory mean (Grubbs II)











ANNEX XIII: Graphical summary of *G.delicatula* results by analyst







ANNEX XIV: Mandel's h statistics



ANNEX XIV Mandel's k statistics





















ANNEX XVII: Ocean Teacher HAB Quiz



ANNEX XVII: Ocean Teacher HAB Quiz












Question 7

Correct

1.00 Flagged

The illustrations show schematic drawings in ventral, dorsal, apical, and antapical views of the main series of plates in a peridinioid dinoflagellate. Assign the right names to the plate series.

Question 8	Match the following kofoidean tabulations to the	right genus name:		
Correct				
Mark 1.00 out of 1.00	1. Po cp 4' 0a 6" 6c 9-10s 5" 2"" 2. Po x 4' 2-3a 7" 4c 6s 5" 2""			
rlagged	3. Po cp x 3' 1a 5" 3c 5s 5"" 1""			
🗱 Edit question	4. Po 3' 2a 6" 6c 7s 6" 2"" 5. Po 6' 0a 6" 6c 4?s 6" 2""			
	6. Po x 4' 3a 7" 6c 4-5s 5" 2""			
	7. Po 3' 3a 6" 6c 7s 6" 2""			
	8. Po cp x 4' 3a 6'' 6c 5s 6''' 2''''			
	Kofoidean tabulation 1 correspond to the genus:	Alexandrium	*	~
	Kofoidean tabulation 2 correspond to the genus:	Protoperidinium	•	~
	Kofoidean tabulation 3 correspond to the genus:	Podolampas	¥	~
	Kofoidean tabulation 4 correspond to the genus:	Gonyaulax	¥	~
	Kofoidean tabulation 5 correspond to the genus:	Amphidoma	•	~
	Kofoidean tabulation 6 correspond to the genus:	Scrippsiella	•	~
	Kofoidean tabulation 7 correspond to the genus:	Lingulodinium	•	~
	Kofoidean tabulation 8 correspond to the genus:	Azadinium	•	~

Question 9	Answer the following questions:
Correct	1. What family does this organism belongs to?
Mark 1.00 out of	2. what characteristic feature is the arrow pointing at ?
1.00	3. What is the name of the plate in the circle? (1' apical, intercalary, Po, etc)
Flagged	4. The plate series in this family of organisms are known as
Edit question	Choose the correct answers from the list. There are 4 answers, each correct answer is a 25% of the total mark.
	AND AND A REAL PROPERTY AND AND AND AND A REAL PROPERTY AND



Select one or more:

- Amphiesmal vesicles
- Eyespot
- 🖉 Elongated Apical vesicles 🗸
- Thecal pores
- Apical groove
- 🖉 Latitudinal series 🗸
- Thecal series
- Iongitudinal series
- 🖉 Suessiaceae 🗸
- 📄 Gymnodiniaceae
- Kareniaceae
- 🖉 🗴 plate 🗸
- 📄 1' apical
- 📄 1 cingular
- sulcal plates
- 3 antapical plate
- Peridiniaceae





Question 12 Members of the genus Tripos are common in marine habitats. Many species are extremely variable and difficult to identfy. The following questions show a number of illustrations of common and characteristic species. Identify the speces using the list of names provided. Correct Mark 1.00 out of 1.00 Species Flagged Edit question Species 10 Species Species 8 9 100 µm 50 µm Species 12 **Species** 11 50 µm 50 µm 50 µm-50 µm Species 7 Tripos extensus 🔹 🗸 Species 8 Tripos longirostrus 🔹 🗸 Species 9 Tripos fusus + 4 Species 10 Tripos hexacanthus 🔹 🖌 Species 11 Tripos furca + 1 Species 12 Tripos lineatus 🔹 🗸







ANNEX XVIII: HABs	Oceanteacher q	uiz results
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Analyst code	Final Grade	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15
63	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
29	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
60	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
38	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
58	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
67	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
41	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
45	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
56	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
6	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
64	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
32	98.33	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00
54	98.33	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00
35	98.33	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
25	97.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	66.57	100.00	100.00	100.00	100.00
36	96.67	74.96	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
22	96.67	100.00	100.00	49.93	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
30	96.67	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00
23	96.67	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	49.93

Analyst code	Final Grade	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15
16	96.67	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	49.93	100.00	100.00	100.00	100.00	100.00	100.00
9	96.67	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00
39	95.83	100.00	100.00	74.96	100.00	100.00	100.00	100.00	87.41	74.96	100.00	100.00	100.00	100.00	100.00	100.00
43	95.56	100.00	100.00	100.00	33.28	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
21	95.56	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	33.28	100.00	100.00	100.00	100.00	100.00
24	95.56	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	33.28	100.00	100.00	100.00	100.00	100.00
57	95.56	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	33.28	100.00	100.00	100.00	100.00	100.00
61	95	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	49.93
1	95	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	49.93	100.00	100.00	100.00	100.00	100.00	100.00
12	95	100.00	74.96	74.96	100.00	100.00	100.00	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00
81	95	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	49.93
28	95	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	49.93
62	95	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	49.93
74	94.44	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	74.96	100.00	66.57	100.00	100.00	100.00	100.00
7	94.44	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	74.96	100.00	66.57	100.00	100.00	100.00	100.00
14	93.89	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	74.96	33.28	100.00	100.00	100.00	100.00	100.00
53	93.89	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00	33.28	100.00	100.00	100.00	100.00	100.00
19	93.89	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	74.96	33.28	100.00	100.00	100.00	100.00	100.00
5	93.89	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	74.96	33.28	100.00	100.00	100.00	100.00	100.00
10	93.89	74.96	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	33.28	100.00	100.00	100.00	100.00	100.00

ANNEX XVIII: HABs Oceanteacher quiz results

Analyst code	Final Grade	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15
65	93.33	100.00	100.00	100.00	33.28	100.00	100.00	100.00	100.00	100.00	100.00	66.57	100.00	100.00	100.00	100.00
8	92.22	74.96	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00	33.28	100.00	100.00	100.00	100.00	100.00
68	92.22	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	74.96	33.28	100.00	100.00	100.00	100.00	100.00
2	92.22	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	74.96	100.00	33.28	100.00	100.00	100.00	100.00
91	92.22	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	74.96	100.00	33.28	100.00	100.00	100.00	100.00
27	91.67	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00
66	91.67	100.00	100.00	49.93	100.00	100.00	100.00	100.00	100.00	74.96	66.57	100.00	100.00	83.36	100.00	100.00
69	91.67	100.00	100.00	49.93	100.00	100.00	100.00	100.00	100.00	74.96	100.00	66.57	100.00	83.36	100.00	100.00
78	91.67	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	74.96	33.28	100.00	66.57	100.00	100.00	100.00
4	91.67	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00	33.28	66.57	100.00	100.00	100.00	100.00
42	91.11	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	74.96	33.28	100.00	100.00	83.36	100.00	100.00
72	90.56	100.00	100.00	49.93	100.00	100.00	100.00	100.00	100.00	74.96	33.28	100.00	100.00	100.00	100.00	100.00
55	90.56	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	74.96	100.00	100.00	100.00	83.36	49.93	49.93
77	90.56	100.00	100.00	49.93	100.00	100.00	100.00	100.00	100.00	74.96	33.28	100.00	100.00	100.00	100.00	100.00
15	90	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	49.93	100.00	66.57	100.00	83.36	49.93	100.00
73	88.89	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	33.28	100.00	100.00	100.00	49.93	49.93
40	88.89	74.96	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00	33.28	100.00	100.00	100.00	49.93	100.00
52	88.61	74.96	100.00	74.96	100.00	100.00	100.00	100.00	87.41	74.96	100.00	100.00	100.00	66.57	100.00	49.93

ANNEX XVIII: HABs Oceanteacher quiz results

Analyst code	Final Grade	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15
37	87.78	100.00	100.00	49.93	100.00	100.00	100.00	100.00	100.00	49.93	33.28	100.00	100.00	83.36	100.00	100.00
46	87.22	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	0.00	66.57	100.00	100.00	66.57	100.00	100.00
59	87.22	100.00	100.00	49.93	100.00	100.00	100.00	100.00	100.00	74.96	33.28	100.00	100.00	100.00	49.93	100.00
34	86.11	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	74.96	33.28	33.28	100.00	100.00	100.00	49.93
18	85	100.00	100.00	49.93	100.00	100.00	100.00	100.00	100.00	25.04	33.28	66.57	100.00	100.00	100.00	100.00
17	85	100.00	100.00	49.93	100.00	100.00	100.00	100.00	100.00	25.04	33.28	66.57	100.00	100.00	100.00	100.00
11	85	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00	33.28	33.28	83.36	100.00	100.00	49.93
47	83.33	100.00	100.00	74.96	100.00	100.00	100.00	100.00	74.96	49.93	100.00	66.57	100.00	83.36	100.00	0.00
44	83.33	100.00	100.00	25.04	100.00	100.00	100.00	100.00	100.00	25.04	33.28	66.57	100.00	100.00	100.00	100.00
3	83.33	74.96	74.96	49.93	100.00	100.00	100.00	100.00	100.00	49.93	100.00	66.57	100.00	83.36	49.93	100.00
51	82.78	100.00	100.00	74.96	100.00	100.00	100.00	100.00	100.00	100.00	33.28	33.28	100.00	100.00	49.93	49.93
31	82.22	100.00	100.00	49.93	100.00	100.00	100.00	100.00	100.00	100.00	33.28	100.00	100.00	100.00	0.00	49.93
49	81.39	100.00	100.00	74.96	100.00	100.00	100.00	100.00	87.41	25.04	0.00	100.00	100.00	83.36	49.93	100.00
76	81.11	100.00	100.00	49.93	100.00	100.00	100.00	100.00	100.00	100.00	33.28	33.28	100.00	100.00	49.93	49.93
75	79.44	100.00	100.00	100.00	33.28	66.57	100.00	100.00	100.00	25.04	100.00	66.57	100.00	100.00	49.93	49.93
71	78.89	74.96	100.00	49.93	100.00	100.00	100.00	100.00	100.00	74.96	33.28	66.57	83.36	100.00	49.93	49.93
70	77.22	100.00	100.00	74.96	33.28	100.00	100.00	100.00	100.00	49.93	0.00	100.00	100.00	100.00	49.93	49.93
50	76.94	100.00	100.00	49.93	100.00	100.00	100.00	100.00	87.41	49.93	33.28	100.00	100.00	83.36	49.93	0.00
20	74.72	100.00	100.00	74.96	100.00	100.00	100.00	100.00	87.41	25.04	0.00	100.00	66.57	66.57	49.93	49.93
79	71.11	100.00	100.00	74.96	100.00	100.00	66.57	100.00	74.96	0.00	33.28	33.28	83.36	100.00	100.00	0.00
80	69.91	100.00	100.00	49.93	33.28	100.00	77.81	100.00	62.52	74.96	33.28	100.00	66.57	100.00	49.93	0.00
26	54.11	74.96	100.00	74.96	100.00	100.00	66.57	19.94	100.00	25.04	33.28	33.28	66.57	16.64	0.00	0.00
	Total	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15
Overall	90.91	97.451	99.25	80.66	95.802	99.55	98.801	98.951	98.051	76.162	68.366	87.256	97.601	95.502	87.856	81.559