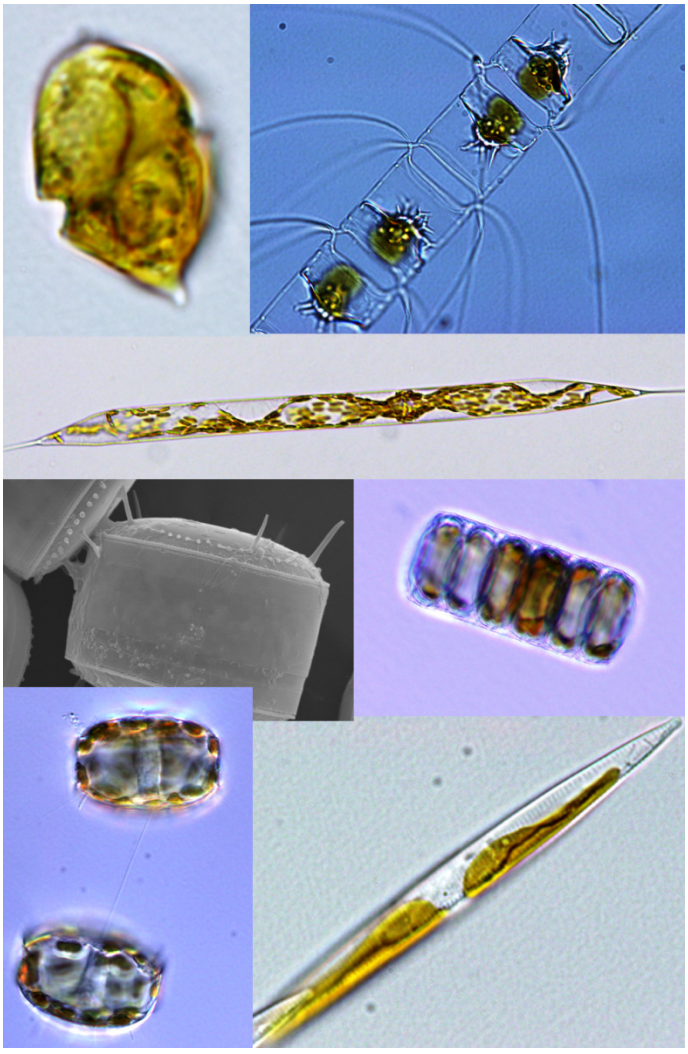
BEQUALM Phytoplankton proficiency test in the abundance and composition of marine microalgae 2014 report.

PHY-ICN-14-MI1 VR 1.0



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1. Summary of results

- 64 analysts from 40 laboratories took part in this intercomparison exercise. All analysts returned sample results and completed the online Hab quiz. This year, a new laboratory joined the scheme from New Zealand.

- Laboratories from Europe (31): Ireland (3), Northern Ireland (1), Scotland (3), England (5), France (12), Netherlands (2), Sweden (1), Spain (2), Croatia (1) and Greece (1). Laboratories outside Europe (9): Morocco (6), Tunisia (1), New Zealand (1) and Peru (1).

- There were six species of interest in this intercomparison exercise. These were: *Chaetoceros diadema* (Ehrenberg) Gran*, Rhizosolenia setigera* Brightwell*, Paralia sulcata* (Ehrenberg) Cleve, *Pseudo-nitzschia australis* Frenguelli, *Heterocapsa triquetra* (Ehrenberg) F.Stein and *Thalassiosira punctigera* (Castracane) Hasle.

- 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.

- The homogeneity test was passed for 4 out 6 measurands and the stability test passed for four out 6 measurands. *R.setigera* and *H.triquetra* failed the homogeneity test and *H.triquetra* and *P.sulcata* failed the stability test.

- The assigned values standard uncertainty was found to be negligible for all test items. The comparison of the assigned value appear not to be negligible, however, the comparison is not equal between the homogeneity test and the analysts results as the volume analysed is different.

- Z-scores show four warning signals for the *C.diadema* count for analysts 16, 28, 57 and not identified by 60, five warning signals for the *H.triquetra* count, one for analyst 43 and not identified by analysts 16, 34, 56 and 57. Six warning (analysts 21, 27, 31, 32, 33, 45) and two action signals (analysts 38, 54) for *P.australis.* Two warning (55, 57) and two action signals (37, 56) for *P.sulcata* count. Six warning (23, 31, 34, 37, 45, and 54) and two action signals (43, 56) for *R.setigera* and five warning (15, 19, 27, 31, and 32) and two action signals (16, 50) for the *T.punctigera* count.

- Mandel's h shows that analysts 16, 37 and 56 exhibit significantly higher or lower mean values across all measurands compared to the rest. This may suggest some source of bias. Mandel's k statistics shows that analyst; 7, 33, 43 and 50, exhibit poorer repeatability precision across all measurands.

- RLP versus RSZ plot indicates significant systematic underestimation deviations of the measurement values of several analysts. Analysts 56, 37 and 57 shows systematic underestimation on all test items and poor mean deviation suggesting some kind of methodology bias.

- The repeatability standard deviation plots show poor repeatability for *P.australis, R.setigera and T.punctigera* cell counts. There is good correlation, however with *C.diadema, H.triquetra* and *P.sulcata* counts for most analysts.

- The diatoms *P.sulcata* and *R.setigera* appear to be the easiest species to identify in the samples. *H.triquetra* was also easy to identify. Four analysts did not identify the species in the sample, possibly because it had the lowest cell density in the samples of all the measurands. *C.diadema* gave the widest variability of answers of all the measurands at species level. All participants, identified correctly to genus level except for one 'not id'. Most analysts identified *Pseudo-nitzschia* to genus level only as 'seriata complex'. *Thalassiosira* appeared to be the most difficult species to identify in the samples even at genus level.

- The Ocean teacher online HAB quiz results suggests a high rate of proficiency. 32 analysts (50%) scored above the 90% mark, 18 analysts (29%) scored above the 80% mark, 6 analysts (10%) over 70% and the rest (7 analysts (11%)) below 70% needing improvement. Overall, 88% was the mean overall grade for all analysts.

- The video question was the worst answered. Short answer questions created problems and analysts committed some spelling and grammar errors which cost them some points. There was consensus on numerical questions indicating that we all have a similar approach to enumeration. Theoretical knowledge of algal groups doesn't seem to translate into better answers to identification questions on the same algal groups, as with *Pseudo-nitzschia* and *Protoperidinium* questions.

2. Introduction

The Phytoplankton Bequalm intercomparison study in 2014 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. There were six species of interest in this intercomparison exercise. These were: *Chaetoceros diadema* (Ehrenberg) Gran*, Rhizosolenia setigera* Brightwell*, Paralia sulcata* (Ehrenberg) Cleve, *Pseudo-nitzschia australis* Frenguelli, *Heterocapsa triquetra* (Ehrenberg) F.Stein and *Thalassiosira punctigera* (Castracane) Hasle.

Collaboration between the Marine Institute in Ireland and the IOC UNESCO Centre for Science and Communication of Harmful algae in Denmark on the Bequalm intercomparison exercise commenced in 2011. This collaboration involves the use of algal cultures from the Scandinavian Culture Collection of Algae and Protozoa in Copenhagen, cultures isolated from field samples and from the Marine Institute culture collection. This collaboration also includes the elaboration of a marine phytoplankton taxonomy quiz using an online platform called 'Ocean Teacher'. This online HAB quiz was designed by Jacob Larsen (IOC) and Rafael Salas (MI).

This year, 64 analysts from 40 laboratories took part in this intercomparison. All analysts returned sample and online Hab quiz results. A laboratory from New Zealand participated in this exercise for the first time. Most laboratories are based in Europe (32): Ireland (3), Northern Ireland (1), Scotland (3), England (5), France (12), Netherlands (2), Sweden (1), Spain (2), Croatia (1) and Greece (1). Laboratories outside Europe (9): Morocco (6), Tunisia (1), New Zealand (1) and Peru (1). The list of participating laboratories can be found in Annex V.

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-14-MI1. PHY standing for phytoplankton, ICN for intercomparison, 14 refers to the year 2014, 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 2014.

Also, as part of this intercomparison exercise, a training workshop is held annually to discuss the results of the intercomparison exercise and to provide training in some areas of interest on phytoplankton taxonomy to the participants. This workshop has been held in various places over the years and it has taken the format of a 2 ½ days training workshop with at least 1 ½ days dedicated to lectures on algal groups in rooms equipped with microscopes and using live cultures (see workshop agenda: Annex IV).

This workshop has become an important forum for scientists 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 issues with harmful algal events in their regions and of high ecological importance.

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 GF/C Whatmann filters (Whatmann™, Kent, UK), autoclaved (Systec V100, Wettenberg , Germany) and preserved using Lugol's iodine solution (Clin-tech, Dublin, Ireland). The sterilin tubes were made up to the required volume with sterile filtered seawater containing neutral lugol's iodine. This was carried out using 25ml serological pipettes (Sardstedt, Nümbrech, 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 29 ml approximately before spiking the samples.

A stock solution for each of the six species was prepared using 50ml glass screw top bottles (Duran®, Mainz, Germany). Then, a working stock containing the six species to the required cell concentration was prepared using a measured aliquot from each stock solution into a 2l Schott glass bottle. Then, each working stock was inverted 100 times to homogenate the samples and 1ml aliquots were pipetted out after each 100 times inversion using a calibrated 1ml pipette (Gilson, Middleton, USA) with 1ml pipette tips (Eppendorf, Cambridge, UK). The 1ml aliquots were dispensed into the 30ml plastic sterilin tubes (Sardstedt, Nümbrech, Germany) containing 29ml.

Samples were capped and labeled. Parafilm was used around the neck of the sterilin tube to avoid water loss through evaporation or leaking, placed in padded envelopes and couriered via TNT 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.

The laboratory cultures used in this exercise were collected in Galway bay South during the months of February and March 2014. All the cultures were isolated using the micro-pipette technique as unialgal cultures. Scanning Electron Microscopy (Hitachi S-4700) was used to identify to species level two of the

cultures; *H.triquetra* and *T.punctigera*. The other four cultures used were identified using light microscopy techniques only except for *Pseudo-nitzschia australis* which was confirmed to species level using molecular species specific gene probes.

A total of 300 samples for the enumeration and identification study were produced. Each participant was sent a set of four samples, three for analysis and one spare sample that is a total of 256 samples. Another 15 samples were sent to an 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 (30ml 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 10ml each.

A time delay between the homogeneity test and the stability test was 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 it 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.

Generally cell concentrations were low to medium and ranging from concentrations of 800 cells/Litre for *H.triquetra*, 1400 cells/L for *C.diadema*, 6000 cells/L for *P.sulcata, 10000* cells/L for *T.punctigera,* 15000 cells/L for *R.setigera* and 22000 cells/L for *P.australis*. The highest concentration (22000) would correspond to a count of 550 cells in a 25ml sedimentation chamber.

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, how to deal 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 Bequalm 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 for example: the IOC training courses on toxic algae and the Bequalm 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: http://classroom.oceanteacher.org/ 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. Twelve weeks were 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 Harmful Algal Bloom programme BEQUALM 2014 and quiz content from here.

The test itself consisted of 13 questions (see Annex XVI). There were different question types used in this quiz;' matching', 'numerical' and 'short answer'. 'Matching' questions have dropdown menus including an array of answers which analysts must choose from, 'numerical' questions need numerical values as answers and 'short answer' type questions need the correct answer to be written in the space provided. All questions have equal value and the quiz have a maximum grade of 100% for a perfect score.

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

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. 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.

In two cases, the heterogeneity standard deviation (s sample) for *P.sulcata* and *T.punctigera* appears to be 0. This is because the ISO 13528 model of homogeneity is based on ANOVA. For the s (sample), the underlying variance was probably calculated to be less than 0. It is a convention to then set the SD to 0 (as in ProLab Plus), even though from a mathematical point of view it is not defined at all (variances have to be 0 or larger) if calculated with Excel for example. In practical terms the s (sample) = 0 means that the standard deviation between replicates (thus in the same test portion) is larger than the standard deviation between test portions.

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, ISO13528 and the harmonized protocol. 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.

ISO13528	Homogeneity test	Stability test
Chaetoceros diadema	Pass	Pass
Rhizosolenia setigera	Fail	Pass
Pseudo-nitzschia australis	Pass	Pass
Heterocapsa triquetra	Fail	Fail
paralia sulcata	Pass	Fail
Thalassiosira punctigera	Pass	Pass

Table 1: Homogeneity and stability pass/fail test

Table 1 above shows the pass/fail flag for each measurand. The homogeneity test seemed to have failed the criteria for *R.setigera* and *H.triquetra* counts and passed for the rest. The stability test was passed for all the measurands except *H.triquetra* and *P.sulcata*. According to ISO13528, if the homogeneity test fails, the heterogeneity standard deviation has to be taken into account when calculating the standard deviation for the measurand.

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. However, there are no missing values on the data received for Bequalm 2014.

4.3 Analysts' Data

The results of the participants were collated using Excel spreadsheets. 64 analysts from 41 laboratories returned results for this exercise. There were six species of interest in the samples: *Chaetoceros diadema* (Ehrenberg) Gran*, Rhizosolenia setigera* Brightwell*, Paralia sulcata* (Ehrenberg) Cleve, *Pseudo-nitzschia australis* Frenguelli, *Heterocapsa triquetra* (Ehrenberg) F.Stein and *Thalassiosira punctigera* (Castracane) Hasle. 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

S_s= 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.

Species	Chaetoceros diadema (cells/L)	Rhizosolenia setigera (cells/L)	Pseudo-nitzschia australis (cells/L)	Heterocapsa triquetra (cells/L)	paralia sulcata (cells/L)	Thalassiosira punctigera (cells/L)
SD	741	2402	9669	426	2093	1256
new SD	745	2767	9669	461	2243	1390

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.

The new standard deviation (new SD) will be used to set the 2 and 3 sigma limits of the robust averages for each test item.

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;

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

Where;

u_x= Standard uncertainty of the assigned value,

s^*= robust standard deviation for the test

p= number of analysts

	C. diadema	R. setigera	P. asutralis	P.sulcata	H.triquetra	T.punctigera
Robust mean x*	1804	13644	19291	9135	728	2997
Robust Stdev s*	741	2402	9669	2093	426	1256
Standard Ux	117	381	1511	327	69	196
n=	63	62	64	64	60	64
if Ux < 0.3xSTdev	222	721	2901	628	128	377
then Ux is negligible	neg	neg	neg	neg	neg	neg
The equation is satisfied in all cases						

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

If *Ux* 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.

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:

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

C)

Where;

u_x= Standard uncertainty of the assigned value,

s^*= robust standard deviation for the test

p= number of analysts

	C. diadema	R. setigera	P. asutralis	P.sulcata	H.triquetra	T.punctigera
Robust mean x*	1804	13644	19291	9135	728	2997
Robust Stdev s*	741	2402	9669	2093	426	1256
Standard Ux	117	381	1511	327	69	196
n=	63	62	64	64	60	64
if Ux < 0.3xSTdev	222	721	2901	628	128	377
then Ux is negligible	neg	neg	neg	neg	neg	neg
The equation is satisfied in all cases						
Cumulative distribution function cut off points for normal distribution						
*x *-1.5s**	693	10041	4788	5996	89	1113
*x *+1.5s**	2916	17247	33795	12275	1367	4881
Homogeneity test	CDIA	RSET	PAUS	PSUI	HTRIQ	TPUNCT
Reference value mean	1430	15750	22565	5910	715	10190
Reference value stdev	199	1500	955	974	292	898
	Comparison with assigned value					
	CDIA	RSET	PAUS	PSUI	HTRIQ	TPUNCT
*x *-X*	374	2106	3274	3225	13	7193
Uncertainty of diff.	165	539	2137	462	97	278
2* Uncertainty of diff.	330	1079	4273	925	194	555
If diff. Is more than twice its Uncertainty then rule is not satisfied						

Table 4: Comparison of the assigned value.

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, only *P.australis* and *H.triquetra* counts satisfy the equation.

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 and red triangles indicate action signals. Where an organism wasn't identified in the samples, this was given a -3.0 result but they appear as yellow triangles. An 'x' indicates that this component is not applicable to the analyst.

There are four warning signals for the *C.diadema* count for analysts 16, 28, 57 and 60(not identified), five warning signals for the *H.triquetra* count for analysts 43 and 16, 34, 56, 57 (not identified). Six warning signals for analysts 21, 27, 31, 32, 33, 45 and two action signals for analysts 38, 54 for *P.australis,* two warning (55, 57) and two action signals (37, 56) for *P.sulcata* count. Six warning (23, 31, 34, 37, 45, 54) and two action signals (43, 56) for *R.setigera* and five warning (15, 19, 27, 31,32) and two action signals (16, 50) for the *T.punctigera* count.

4.7 Combined performance scores

Mandel's h and k statistic present measures for graphically surveying the consistency of the data (Annex XIII). Mandel's h statistics determines the differences between the mean values of all the laboratories plus 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 high all-positive values or all-negative values for all measurands may indicate laboratory bias.

For example, analysts 37, 56 and 57 exhibit significantly lower values for all their cell counts compared to the rest of participants. This may suggest some source of bias.

The k statistics only produce positive results, zero is the baseline and it looks at repeatability precision between measurands. Generally laboratories with larger values tend to have poorer repeatability precision between replicates than the rest. Analysts 7, 33, 43 and 50 exhibit a larger variability between replicates than the rest in some or all of their counts.

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

The chart of RLP against RSZ (Annex XIV) 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 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.

4.7.2 Plots of repeatability standard deviation

The plots of repeatability standard deviations are used to identify analysts whose average and standard deviation are unusual. 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 XV) for each measurand. The graphs show poor repeatability for *P.australis, R.setigera and T.punctigera* cell counts. There is good correlation, however with *C.diadema, H.triquetra* and *P.sulcata* counts for most analysts.

4.8 Qualitative data

Table 5 below shows how analysts identified the species in the samples. Analysts were asked to give their answers to species level but for the purpose of the exercise and final marks, it was only necessary an answer to genus level. Therefore, we allowed the participants to identify the measurands to the highest taxonomical level to obtain more information on how analysts go about identifying species and whether particular patterns of thinking exist between laboratories around the world.

The diatoms *P.sulcata* and *R.setigera* appeared to be the easiest species to identify. 71.9% of analysts identify *paralia* as *P.sulcata* and only 3.1% identified as *P.fenestrata.* 25% identified to genus level only. In the case of *R.setigera,* there was a division between *R.setigera* 54.7%, *R.hebetata* 17.2% and *R.styliformis* 10.9%. Another 17.2% only identified the organism to genus level.

H.triquetra was also easy to identify with 54.7% of analysts identifying to species level and 31.3% to genus only. Four analysts did not identify these species, the highest of all measurands. Possible reasons for this are the low concentration of cells in the samples and their small size.

Chaetoceros diadema		
species	**Number**	**%**
Chaetoceros diadema	44	68.8
Chaetoceros debilis	4	6.3
Chaetoceros constrictus	3	4.7
Chaetoceros decipiens	3	4.7
Chaetoceros costatus	2	3.1
Chaetoceros sp.	2	3.1
Chaetoceros lauderi	1	1.6
Chaetoceros lorenzianus	1	1.6
Chaetoceros fallax	1	1.6
Chaetoceros cerastosporus	1	1.6
Chaetoceros brevis	1	1.6
not id	1	1.6
Total	63	100

Heterocapsa triquetra		
species	**Number**	**%**
Heterocapsa triquetra	35	54.7
Heterocapsa sp.	20	31.3
Heterocapsa minima	2	3.1
ensiculifera sp.	1	1.6
pentapharsodinium sp.	2	3.1
not id.	4	6.3
Total	60	100

Paralia sulcata		
species	**Number**	**%**
Paralia sulcata	46	71.9
Paralia sp.	16	25.0
Paralia fenestrata	2	3.1
Total	64	100

Rhizosolenia setigera		
species	Number	%
Rhizosolenia setigera	35	54.7
Rhizosolenia hebetata	11	17.2
Rhizosolenia sp.	11	17.2
Rhizosolenia styliformis	7	10.9
Total	64	100

Thalassiosira punctigera		
species	Number	%
Thalassiosira punctigera	10	15.6
Thalassiosira sp.	23	35.9
Thalassiosira eccentrica	2	3.1
Thalassiosira rotula	1	1.6
Coscinodiscus granii	4	6.3
Actynocyclus sp.	17	26.6
Actynocyclus octonarius	7	10.9
genus	64	100
Thalassiosira	36	56.3
Actynocyclus	24	37.5
Coscinodiscus	4	6.3
Total	64	100

Pseudo-nitzschia australis		
Pseudo-nitzschia seriata cplex	45	70.3
Pseudo-nitzschia seriata	7	10.9
Pseudo-nitzschia fraudulenta	5	7.8
Pseudo-nitzschia sp.	3	4.7
Pseudo-nitzschia delicatissima Cplex	2	3.1
Pseudo-nitzschia pungens	1	1.6
Pseudo-nitzschia australis	1	1.6
Total	64	100

Not id= not identified

Table 5: Qualitative data by measurand

C.diadema gave the widest variability of answers at species level of all the measurands. All participants, identified correctly to genus level except for one 'not id', at species level there was consensus for *C.diadema* 68.8%, the other 20% were distributed across nine other species names.

97% of the analysts identified *Pseudo-nitzschia* seriata complex correctly to genus level only. 70.3% as 'seriata complex'. The rest (aprox. 30%) identified to species. *P.seriata* (10.9%) and *P.fraudulenta* (7.8%) were the most popular choices at species level, but the right answer was *P.australis* (1.6%) given by just one analyst.

Thalassiosira appeared to be the most difficult species to identify in the samples even at genus level. The reason is probably that *T.punctigera* only forms small chains of two cells, which is unusual among the *Thalassiosira* group as they tend to form larger cell chains. These chains appear to be broken down into single cells in some of the samples creating problems for analysts to identify fully and correctly, which shows in the actual statistics. So, at genus level only 56.3% of the analysts identified correctly this organism with 37.5% opting for *Actynocyclus* and 6.3% for *Coscinodiscus*, neither of these two species form cell chains. At species level, from the 56.3%, 35.9% did not go to species level and only 15.6% correctly identified *T.punctigera*. So, the consensus is weaker here among analysts.

4.9 Ocean Teacher online HAB quiz

The online HAB quiz consisted of 13 questions; annex XVI shows the questions and right answers for the online HAB quiz and annex XVII show the final grades per analyst. Question 1 (Table 6) shows the answers given to question one in the quiz. This question presented the analysts with a number of images of phytoplankton species and the analysts had to match the image with the species using a drop-down menu containing the names of the species. This question was nearly perfectly answered by most analysts. There are a small number of erroneous answers but this did not had to do with the ability to identify the species but with a problem related to the way the software in Ocean teacher shuffles questions and answers on the website.

Part of question	Model response	Actual response	Partial credit	Count	Frequency
102	1: Chaetoceros diadema	Chaetoceros diadema	12.50%	60	95.24%
102	1: Chaetoceros didymus	Chaetoceros didymus	0.00%	1	1.59%
102	1: Pseudo-nitzschia sp.	Pseudo-nitzschia sp.	0.00%	1	1.59%
102	1: Chaetoceros concavicornis	Chaetoceros concavicornis	0.00%	1	1.59%
103	2: Thalassiosira sp.	Thalassiosira sp.	12.50%	62	98.41%
103	2: Licmophora sp.	Licmophora sp.	0.00%	1	1.59%
104	3: Licmophora sp.	Licmophora sp.	12.50%	62	98.41%
104	3: Thalassiosira sp.	Thalassiosira sp.	0.00%	1	1.59%
105	4: Odontella sp.	Odontella sp.	12.50%	62	98.41%
105	4: Chaetoceros diadema	Chaetoceros diadema	0.00%	1	1.59%
106	5: Rhizosolenia sp.	Rhizosolenia sp.	12.50%	62	98.41%
106	5: Odontella sp.	Odontella sp.	0.00%	1	1.59%
107	6: Chaetoceros didymus	Chaetoceros didymus	12.50%	61	96.83%
107	6: Chaetoceros diadema	Chaetoceros diadema	0.00%	1	1.59%
107	6: Rhizosolenia sp.	Rhizosolenia sp.	0.00%	1	1.59%
108	7: Phaeocystis sp.	Phaeocystis sp.	12.50%	62	98.41%
108	7: Chaetoceros didymus	Chaetoceros didymus	0.00%	1	1.59%
109	8: Pseudo-nitzschia sp.	Pseudo-nitzschia sp.	12.50%	61	96.83%
109	8: Pseudo-nitzschia delicatissima	Pseudo-nitzschia delicatissima	0.00%	1	1.59%
109	8: Phaeocystis sp.	Phaeocystis sp.	0.00%	1	1.59%

Table 6: Question 1 model response table.

Questions 2 to 4 (Table 7) were all numerical questions. Analysts were presented with images of chain forming diatoms and they were asked to count the number of cells depicted in the images. A model response was built into the answer by the organizers and hoped the consensus answer would be similar. A tolerance of + or – 1 cell was also built in around the model response. Only 9 answers in total on the 3 questions were answered outside the specification parameters.

Q2 Model response	Actual response	Partial credit	Count	Frequency
13 (12..14)	14	100.00%	2	3.17%
13 (12..14)	12	100.00%	17	26.98%
13 (12..14)	13	100.00%	39	61.90%
[Did not match any answer]	11	0.00%	1	1.59%
[Did not match any answer]	8	0.00%	1	1.59%
[Did not match any answer]	9	0.00%	2	3.17%
[Did not match any answer]	10	0.00%	1	1.59%
[No response]		0.00%	0	0.00%
Q3 Model response	**Actual response**	**Partial credit**	**Count**	**Frequency**
16 (15..17)	15	100.00%	4	6.35%
16 (15..17)	17	100.00%	2	3.17%
16 (15..17)	16	100.00%	56	88.89%
[Did not match any answer]	13	0.00%	1	1.59%
[No response]		0.00%	0	0.00%
Q4 Model response	**Actual response**	**Partial credit**	**Count**	**Frequency**
8 (7..9)	7	100.00%	23	36.51%
8 (7..9)	8	100.00%	35	55.56%
[Did not match any answer]	6	0.00%	1	1.59%
[Did not match any answer]	3	0.00%	1	1.59%
[Did not match any answer]	2	0.00%	1	1.59%
[Did not match any answer]	6	0.00%	2	3.17%
[No response]		0.00%	0	0.00%

Table 7. Model responses to numerical questions 2,3 and 4.

Q5	Model response	Actual response	Partial credit	Count	Frequency
4	Arrow 1 points to: Interstria	Interstria	16.67%	63	100.00%
5	Arrow head 2 points to: Fibula	Fibula	16.67%	59	93.65%
5	Arrow head 2 points to: Raphe slit	Raphe slit	0.00%	3	4.76%
5	Arrow head 2 points to: Stria	Stria	0.00%	1	1.59%
6	Arrow head 3 points to: Raphe slit	Raphe slit	16.67%	58	92.06%
6	Arrow head 3 points to: Fibula	Fibula	0.00%	3	4.76%
6	Arrow head 3 points to: Poroid	Poroid	0.00%	1	1.59%
6	Arrow head 3 points to: Central interspace	Central interspace	0.00%	1	1.59%
7	Arrow 4 points to: Stria	Stria	16.67%	60	95.24%
7	Arrow 4 points to: Poroid	Poroid	0.00%	2	3.17%
7	Arrow 4 points to: Central interspace	Central interspace	0.00%	1	1.59%
8	Arrow 5 points to: Poroid	Poroid	16.67%	60	95.24%
8	Arrow 5 points to: Stria	Stria	0.00%	2	3.17%
8	Arrow 5 points to: Fibula	Fibula	0.00%	1	1.59%
9	Arrow 6 points to: Central interspace	Central interspace	16.67%	61	96.83%
9	Arrow 6 points to: Raphe slit	Raphe slit	0.00%	2	3.17%

Table 8. Model answers for question 5 on the genus *Pseudo-nitzschia*.

Q6 Model response	Actual response	Partial credit	Count	Frequency
P. seriata	P. seriata	100.00%	36	57.14%
P.seriata	P.seriata	100.00%	9	14.29%
[Did not match any answer	Pseudo-nitzschia seriata	100.00%	1	1.59%
[Did not match any answer	P. Seriata	100.00%	1	1.59%
[Did not match any answer	Pseudonitzschia seriata	100.00%	1	1.59%
[Did not match any answer]	P. multiseries	0.00%	5	7.94%
[Did not match any answer]	P.multiseries	0.00%	1	1.59%
[Did not match any answer]	Pseudo-nitzschia pungens	0.00%	1	1.59%
[Did not match any answer]	P.pungens	0.00%	1	1.59%
[Did not match any answer]	P. pungens	0.00%	1	1.59%
[Did not match any answer]	P. fraudulenta	0.00%	3	4.76%
[Did not match any answer]	Pseudo-nitzschia fraudulenta	0.00%	2	3.17%
[Did not match any answer]	speudo-nitzschia fraudulenta	0.00%	1	1.59%
Q7 Model response	**Actual response**	**Partial credit**	**Count**	**Frequency**
P. calliantha	P. calliantha	100.00%	40	63.49%
P.calliantha	P.calliantha	100.00%	10	15.87%
[Did not match any answer	Pseudo-nitzschia calliantha	100.00%	3	4.76%
[Did not match any answer	P.Caliantha	100.00%	1	1.59%
[Did not match any answer]	Pseudonitzschia mannii	0.00%	1	1.59%
[Did not match any answer]	P. mannii	0.00%	1	1.59%
[Did not match any answer]	P.pseudodelicatissima	0.00%	1	1.59%
[Did not match any answer]	pseudo-nitzschia delicatissima	0.00%	1	1.59%
[Did not match any answer]	P. turgidula	0.00%	1	1.59%
[Did not match any answer]	pseudo-nitzschia calliantha	0.00%	1	1.59%
[Did not match any answer]	P. callianta	0.00%	1	1.59%
[Did not match any answer]	P. caciantha	0.00%	1	1.59%
[Did not match any answer]	P. caliantha	0.00%	1	1.59%

Q8 Model response	Actual response	Partial credit	Count	Frequency
P. delicatissima	P. delicatissima	100.00%	42	66.67%
P.delicatissima	P.delicatissima	100.00%	11	17.46%
[Did not match any answer]	Pseudo-nitzschia delicatissima	100.00%	3	4.76%
[Did not match any answer]	pseudo-nitzschia delicatissima	100.00%	2	3.17%
[Did not match any answer]	P.Delicatissima	100.00%	1	1.59%
[Did not match any answer]	P. pseudodelicatissima	0.00%	2	3.17%
[Did not match any answer]	P. pungens	0.00%	1	1.59%
[Did not match any answer]	Pseudonitzschia decipiens	0.00%	1	1.59%
Q9 Model response	**Actual response**	**Partial credit**	**Count**	**Frequency**
P. australis	P. australis	100.00%	40	63.49%
P.australis	P.australis	100.00%	8	12.70%
[Did not match any answer]	Pseudo-nitzschia australis	100.00%	1	1.59%
[Did not match any answer]	p.australis	100.00%	1	1.59%
[Did not match any answer]	Pseudo - nitzschia australis	100.00%	1	1.59%
[Did not match any answer]	Pseudonitzschia australis	100.00%	1	1.59%
[Did not match any answer]	P. pungens	0.00%	2	3.17%
[Did not match any answer]	P. Pungens	0.00%	1	1.59%
[Did not match any answer]	pseudo-nitzschia pungens	0.00%	1	1.59%
[Did not match any answer]	Pseudo-nitzschia multiseries	0.00%	1	1.59%
[Did not match any answer]	P.multiseries	0.00%	1	1.59%
[Did not match any answer]	P. fraudulenta	0.00%	1	1.59%
[Did not match any answer]	pseudo-nitzschia fraudulenta	0.00%	1	1.59%
[Did not match any answer]	P. seriata	0.00%	1	1.59%
[Did not match any answer]	P.austrialis	0.00%	1	1.59%
[No response]	[No response]	0.00%	1	1.59%

Table 9. Model responses for question 6 to 9 on the genus *Pseudo-nitzschia*

Table 8 shows the model response and actual answers by the participants on a question on the taxonomy of the genus *Pseudo-nitzschia*. As the table indicates, most analysts answered perfectly this question with small mistakes for a handful of analysts between fibula and raphe slit.

Table 9 shows the answers to questions 6 to 9. These four questions showed images of *Pseudo-nitzschia* species and participants were asked to identify the organism to species level. These questions were 'short answer' types where the participant had to actually write the species name in the space provided.

There were a number of errors due to spelling mistakes but generally all questions were answered correctly. However, when comparing percentage of correct answers of Q5 (theoretical knowledge of the genus) over 95% and Q6,7,8,9 (Practical identification) just over 80%, there appears to be a significant difference.

Q10 Model response	Actual response	Partial credit	Count	Frequency
Eutreptiella	Eutreptiella	100.00%	39	61.90%
[Did not match any answer]	Euglena	0.00%	13	20.63%
[Did not match any answer]	Eutreptia	0.00%	4	6.35%
[Did not match any answer]	Phacus	0.00%	1	1.59%
[Did not match any answer]	Chatonella sp	0.00%	1	1.59%
[Did not match any answer]	Chattonella	0.00%	1	1.59%
[Did not match any answer]	Astasia	0.00%	1	1.59%
[Did not match any answer]	i.e. Euglena	0.00%	1	1.59%
[Did not match any answer]	eutreptia viridis	0.00%	1	1.59%
[No response]	[No response]	0.00%	1	1.59%

Table 10. Model answers for question 10

Question 10 (Table 10) of the quiz showed a video of a live cell of the genus *Eutreptiella* swimming. The video showed the typical euglenoid movement of the cell and that the two flagella were unequal, enough information to discriminate between the genus *Euglena* and *Eutreptiella*. This question caused most problems to participants with only 61.90% of correct answers.

Table 11 shows the model response plate pattern tabulation of thecate dinoflagellates of the *Protoperidinium* genus and questions 12 and 13 (Table 12) on the identification of *Protoperidinium*. The results indicate nearly perfect scores for Question 12 (~95%) and down for questions 12-13 (~85%) but slightly better than the *Pseudo-nitzschia* questions. In question 13 *P.claudicans* was identified incorrectly mainly as *P.oblongum* (12.70%) and *P.curtipes* as *P.divergens* (9.52%). In question 12, *P.leonis* was identified incorrectly mainly as *P.conicum* (9.52%) and *P.pellucidum* as *P.stenii* (11.11%).

Part of question	Q11 Model response	Actual response	Partial credit	Count	Frequency
113	Fig.1 shows: 1' ortho configuration	1' ortho configuration	16.67%	63	100.00%
114	Fig..2 shows: 1' meta configuration	1' meta configuration	16.67%	62	98.41%
114	Fig..2 shows: 1' para configuration	1' para configuration	0.00%	1	1.59%
115	Fig.3 shows: 1' para configuration	1' para configuration	16.67%	61	96.83%
115	Fig.3 shows: 1' meta configuration	1' meta configuration	0.00%	2	3.17%
116	Fig.4 shows: 2a quadra configuration	2a quadra configuration	16.67%	57	90.48%
116	Fig.4 shows: 2a hexa configuration	2a hexa configuration	0.00%	5	7.94%
116	Fig.4 shows: 2a penta configuration	2a penta configuration	0.00%	1	1.59%
117	Fig.5 shows: 2a hexa configuration	2a hexa configuration	16.67%	58	92.06%
117	Fig.5 shows: 2a quadra configuration	2a quadra configuration	0.00%	4	6.35%
117	Fig.5 shows: 2a penta configuration	2a penta configuration	0.00%	1	1.59%
118	Fig.6 shows: 2a penta configuration	2a penta configuration	16.67%	61	96.83%
118	Fig.6 shows: 2a quadra configuration	2a quadra configuration	0.00%	2	3.17%

Table 11. Model answers for question 11 on Protoperidinium

Part of question	Q12 Model response	Actual response	Partial credit	Count	Frequency
119	Species 1 is: P. claudicans	P. claudicans	50.00%	54	85.71%
119	Species 1 is: P. oblongum	P. oblongum	0.00%	8	12.70%
119	Species 1 is: P. divergens	P. divergens	0.00%	1	1.59%
120	Species 2 is: P. curtipes	P. curtipes	50.00%	55	87.30%
120	Species 2 is: P. divergens	P. divergens	0.00%	6	9.52%
120	Species 2 is: P. oblongum	P. oblongum	0.00%	1	1.59%
120	Species 2 is: P. depressum	P. depressum	0.00%	1	1.59%
Part of question	**Q13 Model response**	**Actual response**	**Partial credit**	**Count**	**Frequency**
131	Species 1 is: P. leonis	P. leonis	50.00%	53	84.13%
131	Species 1 is: P. conicum	P. conicum	0.00%	6	9.52%
131	Species 1 is: P. claudicans	P. claudicans	0.00%	2	3.17%
131	Species 1 is: P. pellucidum	P. pellucidum	0.00%	1	1.59%
131	Species 1 is: P. divergens	P. divergens	0.00%	1	1.59%
132	Species 2 is: P. pellucidum	P. pellucidum	50.00%	50	79.37%
132	Species 2 is: P. steinii	P. steinii	0.00%	7	11.11%
132	Species 2 is: P. curvipes	P. curvipes	0.00%	3	4.76%
132	Species 2 is: P. pallidum	P. pallidum	0.00%	2	3.17%
132	Species 2 is: P. leonis	P. leonis	0.00%	1	1.59%

Table 12. Model answers for question 12 and 13 on Protoperidinium

Q#	Question type	Question name	Attempts	Facility index
1	Matching	Identification of species 2014	63	97.62%
2	Numerical	Diatom cell chain counting 2 2014	63	92.06%
3	Numerical	Diatom cell chain counting 2014	63	98.41%
4	Numerical	Diatom chain cell counting 3 2014	63	92.06%
5	Matching	Pseudo-nitzschia terminology	63	95.50%
6	Short answer	Pseudo-nitzschia identification 1	63	76.19%
7	Short answer	Pseudo-nitzschia identification 2	63	85.71%
8	Short answer	Pseudo-nitzschia identification 3	63	93.65%
9	Short answer	Pseudo-nitzschia identification 4	63	82.54%
10	Short answer	Euglenoid video	63	61.90%
11	Matching	Protoperidinium identification 1, 2014	63	95.77%
12	Matching	Protoperidinium identification 2, 2014	63	86.51%
13	Matching	Protoperidinium identification 3, 2014	63	81.75%

Table 13: Statistics by question type

Table 13 shows the statistics of percentage of correct answers by question and question type. Generally, scores are high for most questions. Questions 10 (61.90%) of correct answers appear to have been the most difficult one for analysts, followed by question 6 on the genus *Pseudo-nitzschia* identification (76.19%), but most questions are above 80-90% mark with close to perfect scores for question 1 and 3. Figure 1 below is the graphical representation of table 13.

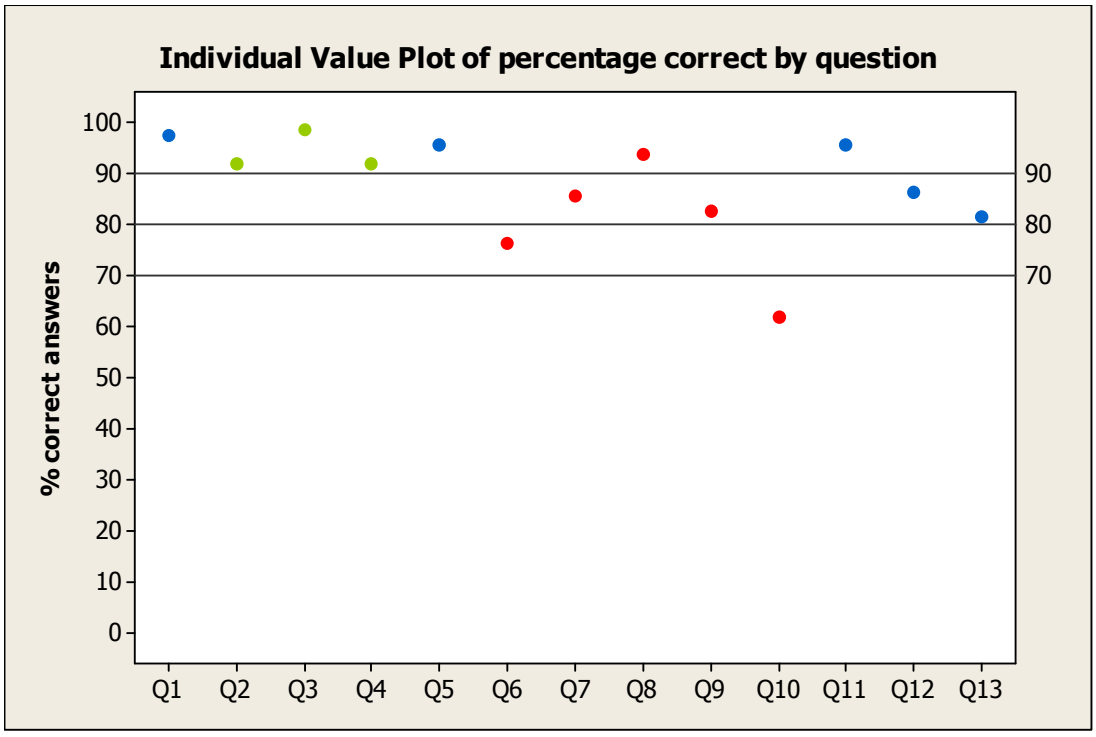


Figure 1: Individual value plot of % correct answers by question type.

Descriptive Statistics: ANALYST CODE

Variable	Grade	Total Count	N	N*	CumN	Percent	CumPct
ANALYST CODE	32.7	1	1	0	1	1.5873	1.587
	49.4	1	1	0	2	1.5873	3.175
	53.8	1	1	0	3	1.5873	4.762
	64.1	1	1	0	4	1.5873	6.349
	65.4	1	1	0	5	1.5873	7.937
	66.7	1	1	0	6	1.5873	9.524
	69.2	1	1	0	7	1.5873	11.111
	74.4	1	1	0	8	1.5873	12.698
	76.9	5	5	0	13	7.9365	20.635
	80.8	1	1	0	14	1.5873	22.222
	82.1	1	1	0	15	1.5873	23.810
	84.6	7	7	0	22	11.1111	34.921
	87.2	1	1	0	23	1.5873	36.508
	88.5	7	7	0	30	11.1111	47.619
	89.7	1	1	0	31	1.5873	49.206
	92.3	9	9	0	40	14.2857	63.492
	93.6	2	2	0	42	3.1746	66.667
	96.2	1	1	0	43	1.5873	68.254
	97.4	1	1	0	44	1.5873	69.841
	100.0	19	19	0	63	30.1587	100.000

Table 14: Cumulative frequency table of analysts scores

Table 14 shows the cumulative frequency of scores. 32 analysts (50%) scored above the 90% mark, another 18 analysts (29%)scored above the 80% mark, 6 analysts (10%) over 70% and the rest (7 analysts (11%)) below 70% needing improvement. Overall, 88% was the mean overall grade for all analysts.

5. Discussion

The present format of this intercomparison exercise is in operation since 2010 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 marine algae in lugol's preserved water samples for abundance and composition. These samples are generally spiked with algal cultures, which allows for a better control of the spiked material in terms of 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, this time, we are following the statistical methods laid out in ISO13528 to calculate the performance statistics for the test. Also, some of the forms used to write the results of the test have been re-vamped. The enumeration and identification logsheet (See Annex II), which in previous years have been set up as a Word document for analysts to enter their results and calculations, this time have been set up as an Excel spreadsheet.

The Excel spreadsheet contains an embedded reduced marine phytoplankton species list which is linked to the identification logsheet table and appears as a dropdown menu list, where analysts must choose the right entries for each sample. The advantages of using the 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 is needed as it avoids 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 to 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 is a definitive departure from previous years. ISO 13528 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 but not qualitative.

This year, for the first time 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 carried out by an expert laboratory was calculated using ProLab Plus (Annex VII) and summarized in table 1. This shows that not all items passed the homogeneity and stability test criteria. The standard ISO 13528, however, gives various ways of working around this.

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) and we have found that in all the test items used in this round the standard uncertainty is negligible. Also, 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 (table 4) but we found that the difference between the consensus values and the reference values is more than twice its uncertainty for some test items, so a source of bias is present in the methodology.

However, the comparison here is not equal as the homogeneity test is based on 10ml sub-samples and analysts analyse 25ml replicates, so the results cannot be fully compared when the volumes used between the homogeneity test and the actual test samples are different. Leaving aside the volume issue, looking at the data (Table 4), it appears that four of the counts are reasonably close together between analysts and homogeneity samples, even if they don't ultimately satisfy the comparison criteria. On the other side, there are two counts that are quite different (*P.sulcata* and *T.punctigera*) between the homogeneity samples and the analysts samples, which cannot be explained alone by the differences in volume analysed. This suggests some level of bias in the measurement method either by the participants, by the expert laboratory or both.

Another option is to include the between sample standard deviation to the assigned value standard deviation for each test item which is what we have done here. Even though not all the test items failed the homogeneity test we have decided to include the between sample standard deviation into all the calculations. It must be noted that the calculations have been done both with and without adding the in between sample standard deviation to the test items (not shown in this report) and that the differences are not really significant to the final results.

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 27 Warning signals, 7 Action signals and 5 non-identifications from 384 results but good overall agreement for all measurands and laboratories.

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 our case, 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 XIII 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. Analysts 37, 56 and 57 for example show a tendency to underestimate all their counts compare to the rest of the participants.

The k plot can be interpreted as repeatability precision measures. 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 the measurands. Analysts 7, 33, 43 and 50 stand out in this instance.

The chart of RLP against RSZ (Annex XIV) 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 XV 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.

The graphs show poor repeatability for *P.australis, R.setigera and T.punctigera* cell counts. However, there is good correlation with *C.diadema, H.triquetra* and *P.sulcata* counts for most analysts.

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. Also an incorrect identification it is given as a -3 Z-score result in the individual statement of performance certificate. The composition of species has changed from year to year and in 2014 we have used six species.

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.

The most difficult species to identify was *T.punctigera* this year. This diatom was difficult to identify because it forms small chains (unlike other species of the genus) and these have broken down in some samples as single cells and many analysts mis-identified the species. Again, the mechanical homogenisation procedure of the samples doesn't seems to favor some chain forming diatom species or even some single cell diatom species and perhaps a new strategy on homogenizing samples must be sought to avoid cell chains/single cells breaking down.

This meant that a number of analysts identified incorrectly to genus level this organism as *Coscinodiscus* (6.3%) or *Actynocyclus* (37.5%) which are not chain forming organisms. Nonetheless, we had carried out work on the SEM and light microscope at the time the culture was grown (images not shown here) and the images obtained confirm that the culture is that of *Thalassiosira* and that the occluded processes and other valve features are there, although it is possible that upon preservation some of these features may not be available for viewing on the samples. I think all these factors, together with the fact that this species only

really form two cell chains which are also sometimes found in solitary form worked against a correct identification of the cells. Only 56.3% of analysts got the identification right against 43.8% wrong (37.5% +6.3%), here the consensus is not too strong compared with the other identifications.

Homogenisation was also an issue for *R.setigera* which did tend to break down rather easily and also caused problems for *Pseudo-nitzschia* and *C.diadema,* but not for *P.sulcata* that appears to be quite robust, however analysts were able to identify these species correctly to a large extent. The use of diatom chains was a feature of this test as we wanted to test analysts against counting organisms in chains rather than single cell species and of course we wanted to see if their cell counts were comparable.

Only one analyst failed to identify *C.diadema* and four failed to identify *H.triquetra* in the samples. The reason for the higher 'non-id' rate of *H.triquetra* is presumably due not so much to the inability to recognize this species which is a rather cosmopolitan and easily recognizable armoured dinoflagellate, but rather down to the size of the organism compared to the other organisms in the samples and its low cell concentration, which have conspired to create problems for some analysts.

The case of *Pseudo-nitzschia* is somewhat exceptional in the sense that while most participants were content to go to species level with all the other species in the samples, they weren't so forthcoming with *Pseudo-nitzschia.* 70.3% of the analysts identified to genus level only as 'seriata complex'. The rest (aprox. 30%) identified to species level. *P.seriata* and *P.fraudulenta* were the most popular choices, but the right answer was *P.australis* given by just one analyst. This suggests why it is a good reason to identify *Pseudo-nitzschia* to genus level only.

Some recommendations were put forward at the workshop (Annex IV: workshop agenda) to improve the condition of the diatoms in culture like the use of orbital shakers to strengthen the valves of the diatoms through gentle movement. However, it might be that we may not be able to use some organisms for these studies or we may need to think of other strategies to homogenize the materials in the future. One proposal suggests that if an organism is a chain forming one and it is broken down in the samples due to homogenisation, then analysts should be made aware of this in advance of their analysis.

The flags for correct identifications are based on a correct genus answer rather than on species taxon as discussed in the instructions (see annex III). However, for the purpose of the intercomparison we asked analysts to identify to species level to give us a better insight on the analysts and laboratories approach to identification and while this is not used for final marks, the information is still valuable for discussion among the participants. It also gives the coordinators of the scheme invaluable data towards species selection in future exercises.

It has been observed from the data received that there is a level of conferring between colleagues working in the same laboratory which becomes obvious when analyzing the results. This sometimes means that one incorrect identification runs throughout all the analysts from the same laboratory. The advice to analysts here is always do your own work and do not confer with others for the purpose of the exercise.

Online HAB quiz

The online HAB quiz has proven very successful and original problems with the software have been ironed out as much as possible. There are still a small number of concerns, specifically with 'short answer' type questions and shuffling within questions and answers. Also, there are problems with analysts not reading or understanding what is required of them and some spelling mistakes which ultimately mean losing marks. Nevertheless, the HAB online quiz is otherwise a good addition to the exercise and this online facility helps greatly the administration and reporting of results.

This year the overall grade was 88% across all analysts with 50% of analysts scoring over 90% mark and another 29% scoring over 80% which is a good showing with a small number of analysts (11%) in need of improvement.

There was good overall consensus between participants on the numerical questions (Q2, 3, 4). Most analysts responded within the parameters of the model response and tolerance applied, but there were a small number of inconsistent answers. However, there doesn't seem to be a relationship between an erroneous answer by analysts here and their performance in the rest of the test, which suggests that perhaps they did not understand what was required of them here. Only 9 answers from a total of 192 on the 3 questions were answered outside the specification parameters which suggest that we all have a similar approach on the enumeration of cells in diatom chains with small variations due to differences in interpretation of what a viable cell is.

The numerical questions were based on counting one or two diatom chains from an image. This one cell difference between analysts increases the variability of the cell counts over a whole sample which suggests that even if we were analyzing all the same sample we would all come up with different results. This variability would depend on the number of chains to be counted.

Analysts had difficulty with question 10; a video showing a cell of *Eutreptiella* swimming. Only 61.90% of analysts answered correctly this question, although, most analysts agreed on a 'euglenoid' type answer.

The questions on *Pseudo-nitzschia* (Q5,6,7,8 & 9) and *Protoperidinium* (Q11,12 & 13) were answered well by most analysts. The marks achieved on the taxonomical questions on *Pseudo-nitzschia* (Q5) and *Protoperidinium* (Q11) was above 95% which contrast with the marks achieved on the identification of *Pseudo-nitzschia* (Q6,7,8 & 9) 80% and *Protoperidinium* species (Q12 &13) 85% which suggest a small gap between theoretical knowledge and ability to identify the species. Q5 on *Pseudo-nitzschia* taxonomy answers indicate problems for a small number of analysts to differentiate the 'fibula' from the 'raphe slit' and in Q11 answers suggest problems with differentiating between 'quadra' and 'hexa' plates in the peridinioid tabulation of *protoperidinium*.

Q6 *P.seriata* was confused with *P.multiseries* (9.53%) but the shape is different for *P.seriata* with an asymmetrical outline, Q7 *P.calliantha* was identified correctly by most analysts as the poroids have a very distinctive pattern but marks were lost due to bad spelling and grammar. Q8 *P.delicatissima* was the correct answer but a small percentage of analysts (3.17%) used *P.pseudodelicatissima* which has just one row of poroids compared to two for *P.delicatissima*. Q9 *P.australis* was mistaken for *P.pungens* (6.35%), *P.multiseries* (3.18%) and *P.fraudulenta* (3.18%) but the shape is different in these three plus the number of poroids is not the same for *P.multiseries*.

In question 12 *P.claudicans* was identified incorrectly mainly as *P.oblongum* (12.70%), both are ortho 1' plate types but the 2a plate is 'penta' in *P.claudicans* and 'quadra' in *P.oblongum* although they can be easily confused, generally *P.claudicans* is more pyriform in shape and the 1" is pentagonal in shape, compared with a more dorso-ventrally flattened *P.oblongum* with a four sided 1". *P.curtipes* was also mistaken for *P.divergens* (9.52%). Both have a meta-quadra arrangement, so it was down to the shape of the species, *P.curtipes* is equally broad than long while *P.divergens* is longer than broad. In question 13, *P.leonis* was identified incorrectly mainly as *P.conicum* (9.52%), both have an ortho-hexa configuration, but *P.conicum* can be recognized by the 'v' shape on the 1' plate. Also *P.pellucidum* was misidentified as *P.stenii* (11.11%). The first is para-hexa configuration while the latter is meta-penta.

6. **Recommendations from workshop 2014**

HAB online quiz:

- Do not use short answer type questions to avoid grammar and spelling mistakes. Use matching questions instead.
- Do not use 'shuffling' option on matching questions
- Continue using numerical questions

Workshop:

- Everyone should bring samples to the workshop from their geographical areas that may be of interest.
- Increase workshop length to 3 days.
- Participants are encouraged to present their work at these workshops

Samples:

- Send 50ml samples to correspond with the homogeneity and stability test
- Mixing technique maybe too rough for some species, consider other options for homogenisation
- Use orbital shaker to toughen up diatom cultures through movement.

Homogeneity test:

- Use bigger volume (50ml) samples, then divide the sample in two 25ml portions

Bio Volume:

- Introduce the measurement of Biovolume for 2015 samples

ANNEX I: Form 1 return slip and checklist

Bequalm Intercomparison PHY-ICN-14-MI1

FORM 1: RETURN SLIP AND CHECKLIST

Please ensure to complete the table below upon receipt of samples, then fax 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 (Please circle the relevant answer)		
Please enter Sample numbers received _____	YES	NO
Set of Instructions	YES	NO
Enumeration and identification result log sheet **(Form 2)**	YES	NO

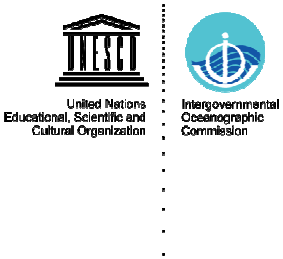
I confirm that I have received the items, as detailed above.

(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



Bequalm 2014 Phytoplankton Intercomparison Exercise

Analyst Name:	
Laboratory Code:	
Analyst Code :	

Settlement date:			
Volume Chamber (ml)			
Analysis date:			
Sample No:			

Organism	Cell count	Cell count	Cell count	Multiplication factor			Number cells/L	Number cells/L	Number cells/L	Average
										#DIV/0!
										#DIV/0!
										#DIV/0!
										#DIV/0!
										#DIV/0!
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Comments:

Form 2: Results logsheet

Marine Institute-IOC- BEQUALM-NMBAQC Phytoplankton Proficiency Test

PHY-ICN-14-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**

ANNEX III

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 continues under the Marine Institute- IOC -BEQUALM-NMBAQC banner.

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 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 Bequalm-NMBAQC schemes. Registration to the exercise is through the Marine institute. You need to contact our administrator Maeve Gilmartin at maeve.gilmartin@marine.ie .

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.

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) 30ml volume in plastic sterilin tubes.

ANNEX III

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 it and send it via e-mail to rafael.salas@marine.ie 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 (rafael.salas@marine.ie), 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 4th 2014**.

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.

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).

Inverted Microscope: 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

ANNEX III

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.

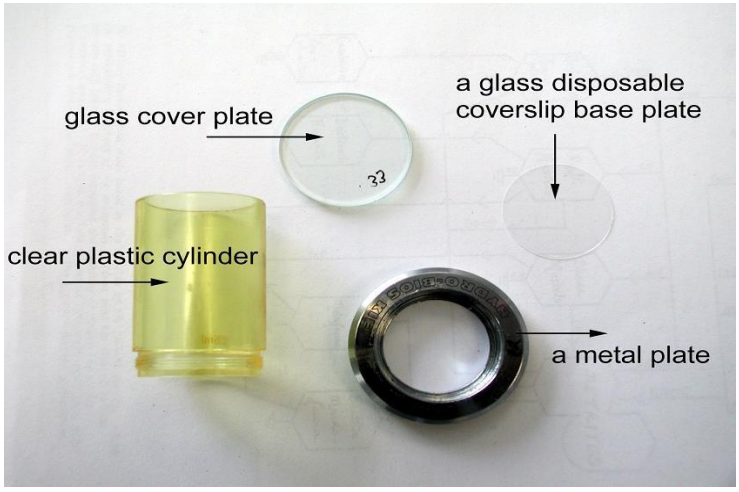

Fig 1: Sedimentation counting chamber

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. Samples

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

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The set consist of four samples. Three must be analysed and one is a spare in case of leakages or breaks. These are made up in sterile filtered Seawater and spiked with culture material of one or more organisms. Participants are asked to carry out a whole chamber 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 then homogenized following the IOC Manual on Harmful Marine Algae technique of 100 times sample inversion to extract sub-samples.

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 type. See figure 1.

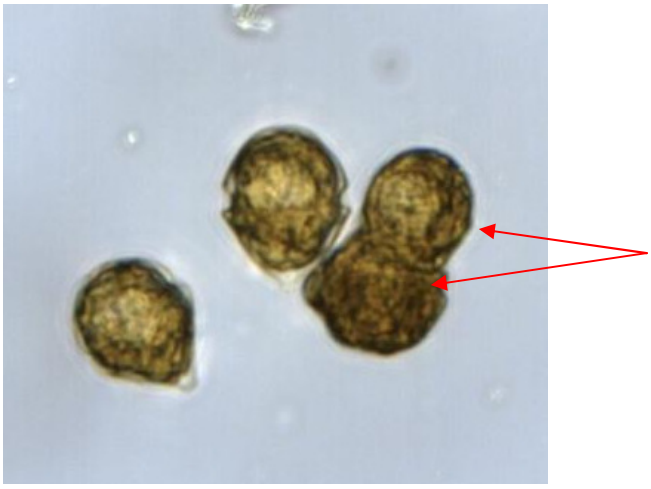


Figure 1: Two Cells fusing

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

ANNEX III

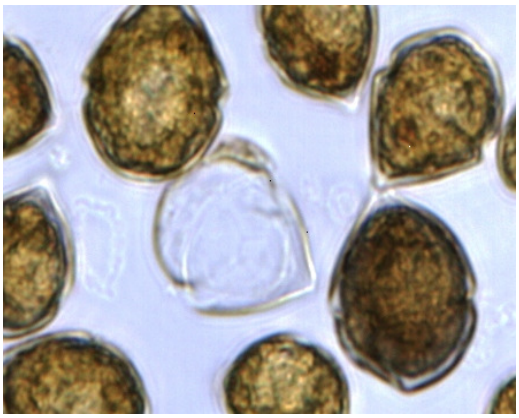


Figure 2: Empty theca

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.

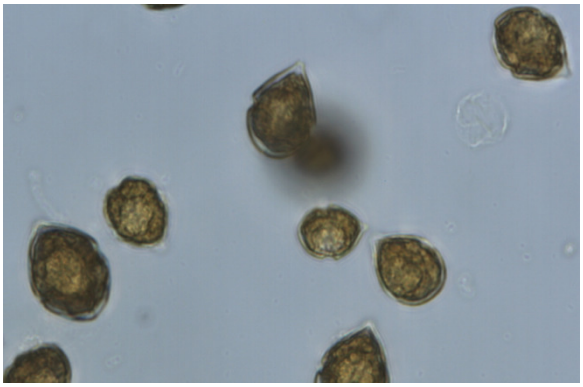


Figure 3: Big versus small cells

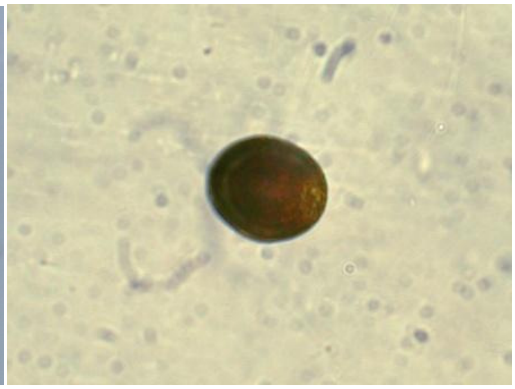


Figure 4: Plasmolised cell

When counting cell chains, only count fully intact and divided cells, counting half cells should be avoided (fig.5).

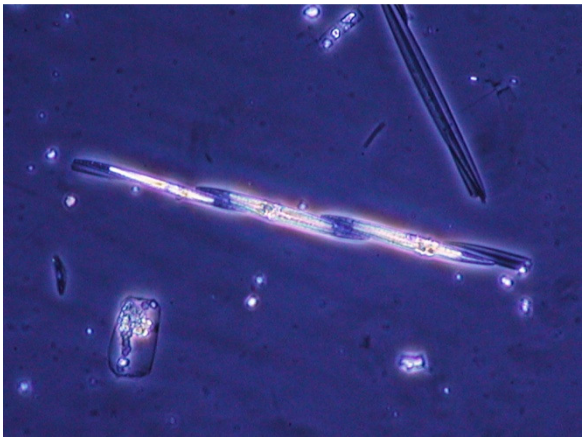


Figure 5

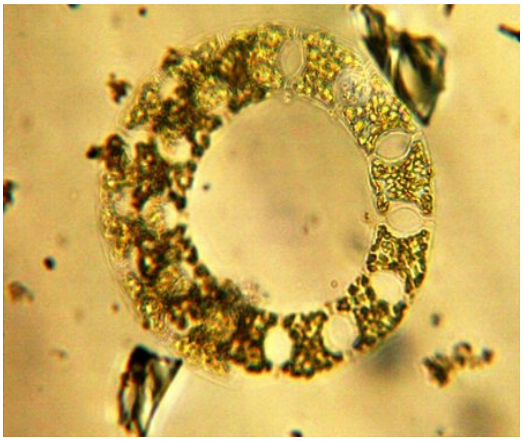


Figure 6

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

ANNEX III

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

a) Any cells that are dividing or fusing, no matter how advance the stage of division or fusion is should be counted as one cell.

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

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

d) Plasmolised cells should not be counted

e) Aberrant forms should be counted

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

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

h) 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.

These rules are only applicable to this intercomparison exercise.

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 2014. 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.

ANNEX III

In order to access the exercise you need to go to the webpage http://classroom.oceanteacher.org/ and login. Analysts which took part in the exercise in 2011 or 2012 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 http://classroom.oceanteacher.org/ 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 already have registered in the previous three years (2011-2013), 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. This year as in 2013 participants will be able to self-enrol for this exercise, so once you are registered and logged in you must supply an enrolment key to access the exercise. This key is **Beq2014**. 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 2014 link will appear, click on it, enter your key (**Beq2014**) and start your quiz. Make sure you enter the right course.

Analysts will have 4 weeks 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). 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.

ANNEX III

10. Points to remember

1. **All results must be the analysts' own work. Conferring with other analysts is not allowed.**

2. The Excel worksheet Form 2: Enumeration and identification results log sheet must be received by the Marine Institute, Phytoplankton unit by **Friday July 4th 2014**.

ANNEX IV: Workshop agenda

Agenda Bequalm Phytoplankton Intercomparison workshop

Danhostel, Hillerød, Denmark, 1-3 Dec 2014.

Monday 1 –Wednesday 3 Dec. 2014

	Morning 9.00-12.00pm	**Afternoon 13.30-17.00**
Monday, 1 Dec	Intercomparison exercise results Enumeration and identification exercise results. Ocean teacher online HABs quiz exercise results. Prolab plus database (Rafael Salas) Discussion of exercise and ideas for 2015 (All)	"Seek and you shall find: A case study of an *Alexandrium ostenfeldii* bloom in the Netherlands." (Anneke van den Oever) Harmful algae, toxins and fish kills (P.J Hansen, Univ. of Copenhagen)
Tuesday, 2 Dec	Lecture and microscope demonstration: Ichthyotoxic flagellates (J.Larsen) 'Which Lugol's is the best 'solution'?' (Oliver Williams)	Lecture and microscope demonstration: Ichthyotoxic flagellates, continued (J.Larsen)
Wednesday 3 Dec	Field samples from participants (microscopy and identification) All	Departure

Coffee/Tea times 11:00am and 15:30pm

Lunch 13:00-14:00 pm

ANNEX V: Participating Laboratories

Number of Laboratories	Company Name	Address
1	IMARES	Korringaweg 5 4401 NT Yerseke The Netherlands
2	Laboratorio de Control de Calidad de los Recursos Pesqueros	Agencia de Gestión Agraria y Pesquera de Andalucía Ctra. Punta Umbría - Cartaya km.12 C.P.21459 (Huelva)
3	CEFAS Laboratory	Pakefield Road Lowestoft Suffolk NR33 0HT
4	SAMS Research Services Ltd	Scottish Marine Institute Oban Argyll PA37 1QA Scotland
5	Koeman en Bijkerk bv	Oosterweg 127, 9751PE Haren, The Netherlands
6	Certificaciones Del Peru	Avenida Santa Rosa 601 La Perla Callao 04 Peru
7	IRTA E-43540 Sant Carles de la Ràpita (Tarragona) Spain	Ctra. de Poble Nou, Km 5,5
8	Cawthron Insitute	Phytoplankton laboratory 98 Halifax Street East Nelson 7010 New Zealand
9	Agri Food and Biosciences Institute	Fisheries and Aquatic Ecoystems Branch Newforge Lane Belfast BT9 5PX
10	Centre régional de l'INRH (Institut National de Recherche Halieutique	Aghsdis Nouveau port Morocco
11	Centre régional de l'INRH (Institut National de Recherche Halieutique	Bd Sidi Abderhmane, Casablanca 20030, Maroc
12	Centre régional de l'INRH (Institut National de Recherche Halieutique	Port de Pêche, BP75, Laayounne, Maroc
13	Centre régional de l'INRH (Institut National de Recherche Halieutique	Cap Malabata Dradeb, BP 5268, Tanger, Maroc
14	Centre régional de l'INRH (Institut National de Recherche Halieutique	13 Boulevard Zerktouni, BP 493, Nador-Maroc
15	Centre régional de l'INRH (Institut National de Recherche Halieutique	bis 73000, BP127, Dakhla, Maroc
16	Marine Scotland Science	Marine Laboratory 375 Victoria Road Aberdeen AB11 9DB UK
17	UMR 5119 ECOSYM CNRS-IRD-UM2-IFREMER-UM1 Université Montpellier 2	Place Eugène Bataillon cc093 34095 Montpellier cedex 5
18	SEPA ASB Angus Smith Building, 6 Parklands Avenue	Eurocentral, Holytown North Lanarkshire ML1 4WQ UK
19	SAHFOS The Laboratory	Citadel Hill Plymouth Devon PL1 2PB
20	IFREMER	Center de brest CS 10070 29280 Plouzane FRANCE
21	IFREMER LER-BL	IFREMER LER/BL150,Quai GambettaBP 699 62321 BOULOGNE SUR MERFRANCE
22	IFREMER LER-N	IFREMER LER/NAvenue du Gal De Gaulle B.P. 3214520 PORT-EN-BESSINFRANCE
23	IFREMER LER-BN	CRESCOIFREMER LER/BN38, rue du Port-BlancBP 8010835801 DINARD CEDEXFRANCE
24	IFREMER LER-MPL	IFREMER -LER/MPL12, rue des RésistantsB.P. 8656470 LA TRINITE-SUR-MERFRANCE
25	IFREMER DYNECO-VIGIES	IFREMER -DYNECO-VIGIESRue de l'Ile d'YeuB.P. 2110544311 NANTES CEDEX 03FRANCE
26	IFREMER LER-PC	IFREMER LER/PCSite de La RochellePlace Gaby Coll BP 717137 L'HOUMEAUFRANCE
27	IFREMER LER-AR	IFREMER LER/ARQuai du Commandant Silhouette33120 ARCACHONFRANCE
28	IFREMER LER-LR	IFREMER LER/LRAvenue Jean MONNET CS 3017134203 SETE cedexFRANCE
29	IFREMER LER-PAC	IFREMER LER/PACZ.P BrégaillonCS 2030383507 LA SEYNE SUR MER CedexFRANCE
30	ORSA	Loch Melfort Arduaine Argyll PA34 4XQ Scotland
31	SMHI	Sven Källfelts gata 15 426 71 Västra Frölunda Sweden
32	UMR BOREA Biologie des Organismes et Ecosystèmes Aquatiques MNHN	UNIVERSITE DE CAEN BASSE-NORMANDIE Esplanade de la Paix CS 14032 14032 CAEN
33	Institut National des sciences et Technologies de la Mer-	Centre de Sfax- Tunisie BP1035- 3018- Sfax Tunisie
34	Jacobs UK	Kenneth Dibben House Enterprise Road Southampton Science Park SO16 7NS
35	Laboratory Unit of Harmful Marine Microalgae School of Biology	Biology Building, 8th Floor Office 8.27 Aristotle University of Thessaloniki, Thessaloniki Greece 54124
36	CLS	Rosmuc, Carna, Co.Galway, Ireland
37	Marine Institute Bantry	Gearhies pier, Bantry, Co.Cork, Ireland
38	Marine Institute Galway	Rinville, Oranmore, Co.Galway, Ireland
39	Institute of Oceanography and Fisheries	Šetalište I. Meštrovića 63, 21000 Split; Croatia
40	APEM Limited	Riverview, A17 Embankment Business Park, Heaton Mersey, Stockport, SK4 3GN. UK

ANNEX VI: Statement of performance certificate

Biological Effects Quality Assurance in Monitoring Programmes /
National Marine Biological Analytical Quality Control Scheme /
Marine Institute
STATEMENT OF PERFORMANCE
Phytoplankton Component of Community Analysis
Year 2014

Participant details:
Name of organisation:
Country:
Participant:
Year of joining:
Years of participation:

Component Name	Subcontracted	Results Z-score (+/- 2 Sigma limits)		identification
Phytoplankton abundance and composition PHY-ICN-14-M11	Marine Institute	*Chaetoceros diadema*		
		Rhizosolenia setigera		
		Pseudo-nitzschia australis		
		Paralia sulcata		
		Heterocapsa triquetra		
		Thalassiosira punctigera		
Overall Result Taxonomic quiz (Pass Mark 70%, over 90% proficient)				
Phytoplankton Taxonomy quiz PHY-ICN-14-M11	IOC Science and communication Centre on Harmful algae			

Statement Issued: XX/XX/2014
Statement Number: MI-BQM-14-001

Summary of results:

n/a: component not applicable to the participant; n/p: Participant not participating in this component;
n/r: no data received from participant
The list shows the results for all components in which the laboratory participated. See over for details.
Notes:

Details certified by:

Joe Silke
Section manager

Rafael Gallardo Salas
Scientific Technical Officer

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

Chaetoceros diadema homogeneity test

BEQ2014

Survey of homogeneity test results

Sample: **WATER2** Date: 16/01/2015
Measurand: **Chaetoceros**

Mean:	1430
Analytical standard deviation:	259
Heterogeneity standard deviation s(samples):	78
Target standard deviation:	745 (Manual)

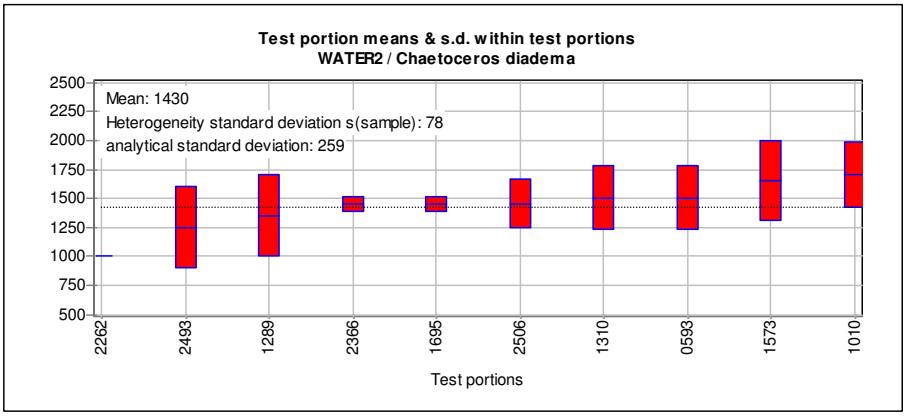
Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the test portions of sample WATER2 were randomly selected, and the measurand Chaetoceros diadema was analyzed 2 times. The mean across all 10 test portions is 1430, the standard deviation within test portions s(analytical) (=analytical precision) is 259, and the standard deviation between test portions s(sample) is 78.

F-Test: statistical test on significant heterogeneity
According to the F-test, the heterogeneity standard deviation is not significantly different from 0 (significance level 5%), therefore the sample can be considered sufficiently homogeneous according to this criterion.

ISO 13528: Check for sufficient homogeneity
According to ISO 13528, the heterogeneity standard deviation s(sample) between the test portions of the sample should not exceed 30 % of the target standard deviation.
The heterogeneity standard deviation is less than 30% of the target s.d. 745 (Manual), therefore the sample can be considered adequately homogeneous according to ISO 13528.

Harmonized Protocol: test on significant heterogeneity
The analytical precision of the method does not exceed 50% of the target s.d. 745 (Manual). Therefore the evaluation according to the Harmonized Protocol can be carried out for this sample: The heterogeneity standard deviation is less than 30% of the target s.d., therefore the sample can be considered homogeneous.



ANNEX VII: *Chaetoceros diadema* stability test

BEQ2014

Survey of stability test results

Sample: **WATER2** Date: 16/01/2015
Measurand: **Chaetoceros**

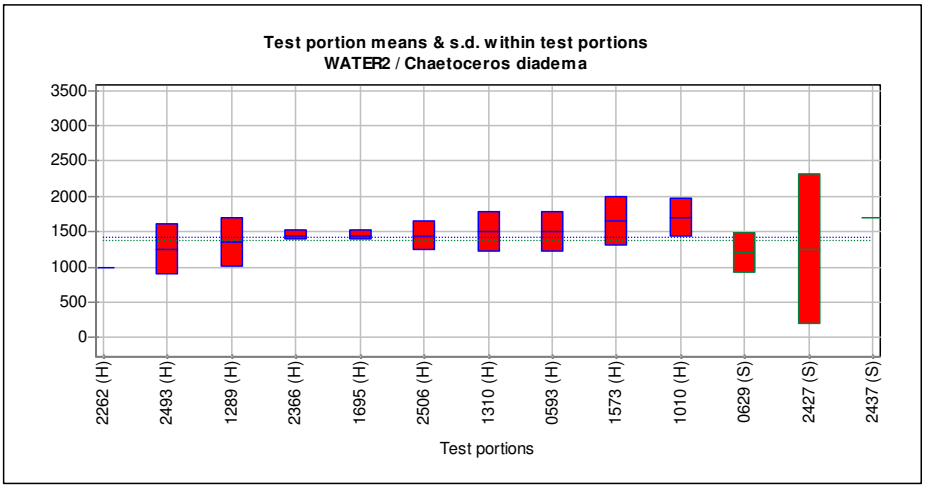
Mean of homogeneity:	1430
Mean of stability:	1383
Target standard deviation:	745 (Manual)

Results of Stability Test

For the test of stability, 3 of the test portions of sample WATER2 have been selected randomly and the measurand Chaetoceros diadema has been analysed 2 times.
The mean value across all test portions of the homogeneity analysis equals 1430, the mean value across all test portions of the stability analysis equals 1383.
Therefore, the mean value of the stability analysis lies 3.3 % below the mean value of the homogeneity analysis.

According to ISO 13528, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the target standard deviation.
Therefore, given the target standard deviation of 745, the sample may be considered as adequately stable according to ISO 13528.

According to the Harmonized Protocol it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of signficance 5%).
The difference of the mean values is not statistically significant. Therefore - according to the Harmonized Protocol - the sample can be considered adequately stable.



ANNEX VII: *Rhizosolenia setigera* homogeneity test

BEQ2014

Survey of homogeneity test results

Sample: WATER2 Date: 16/01/2015
Measurand: Rhizosolenia

Mean:	15750
Analytical standard deviation:	856
Heterogeneity standard deviation s(samples):	1373
Target standard deviation:	2767 (Manual)

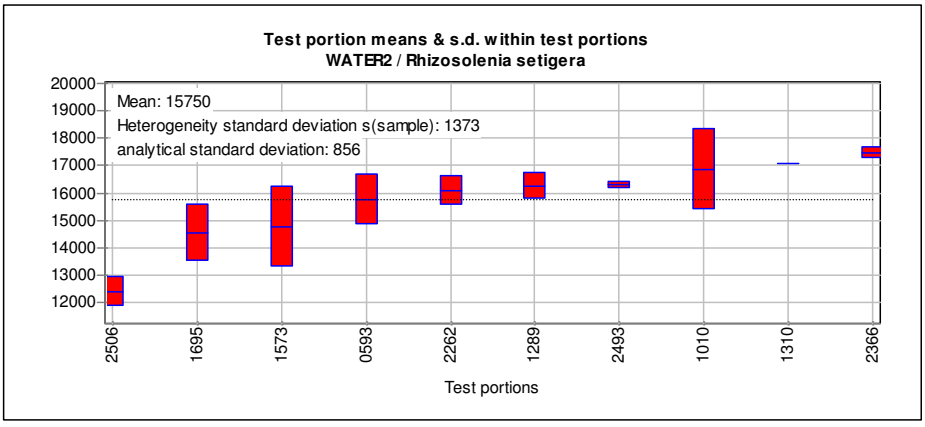
Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the test portions of sample WATER2 were randomly selected, and the measurand Rhizosolenia setigera was analyzed 2 times. The mean across all 10 test portions is 15750, the standard deviation within test portions s(analytical) (=analytical precision) is 856, and the standard deviation between test portions s(sample) is 1373.

F-Test: statistical test on significant heterogeneity
According to the F-test, the heterogeneity standard deviation is significantly different from 0 (significance level 5%), therefore the sample should be considered heterogeneous according to this criterion.

ISO 13528: Check for sufficient homogeneity
According to ISO 13528, the heterogeneity standard deviation s(sample) between the test portions of the sample should not exceed 30 % of the target standard deviation.
The heterogeneity standard deviation is greater than 30% of the target s.d. 2767 (Manual), therefore the sample should be considered heterogeneous.

Harmonized Protocol: test on significant heterogeneity
The analytical precision of the method does not exceed 50% of the target s.d. 2767 (Manual). Therefore the evaluation according to the Harmonized Protocol can be carried out for this sample: Even though the heterogeneity standard deviation is greater than 30% of the target s.d., this is not statistically significantly the case, and the sample can thus be considered homogeneous.



ANNEX VII: *Rhizosolenia setigera* stability test

BEQ2014

Survey of stability test results

Sample: WATER2 Date: 16/01/2015
Measurand: Rhizosolenia

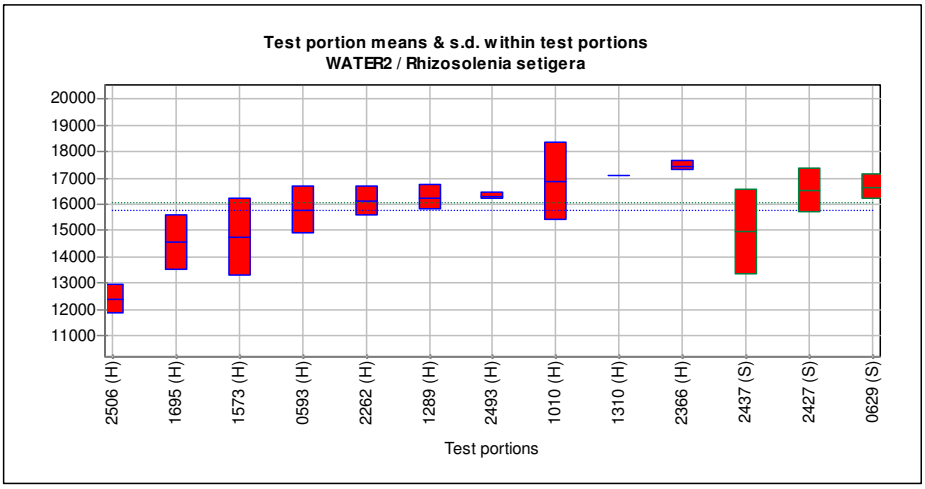
Mean of homogeneity:	15750
Mean of stability:	16033
Target standard deviation:	2767 (Manual)

Results of Stability Test

For the test of stability, 3 of the test portions of sample WATER2 have been selected randomly and the measurand Rhizosolenia setigera has been analysed 2 times.
The mean value across all test portions of the homogeneity analysis equals 15750, the mean value across all test portions of the stability analysis equals 16033.
Therefore, the mean value of the stability analysis lies 1.8 % above the mean value of the homogeneity analysis.

According to ISO 13528, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the target standard deviation.
Therefore, given the target standard deviation of 2767, the sample may be considered as adequately stable according to ISO 13528.

According to the Harmonized Protocol it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of signficance 5%).
The difference of the mean values is not statistically significant. Therefore - according to the Harmonized Protocol - the sample can be considered adequately stable.



ANNEX VII: *Pseudo-nitzschia australis* homogeneity test

BEQ2014

Survey of homogeneity test results

Sample: **WATER2** Date: 16/01/2015
Measurand: **Pseudo-**

Mean:	22565
Analytical standard deviation:	1344
Heterogeneity standard deviation s(samples):	89
Target standard deviation:	9669 (Manual)

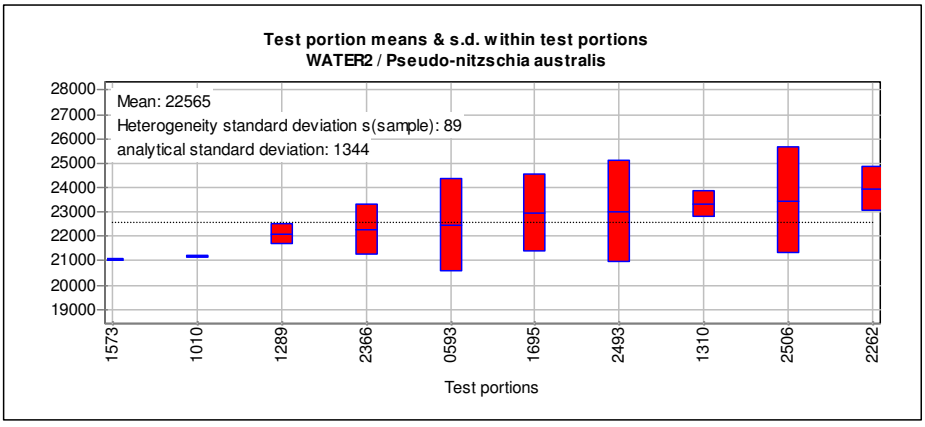
Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the test portions of sample WATER2 were randomly selected, and the measurand Pseudo-nitzschia australis was analyzed 2 times. The mean across all 10 test portions is 22565, the standard deviation within test portions s(analytical) (=analytical precision) is 1344, and the standard deviation between test portions s(sample) is 89.

F-Test: statistical test on significant heterogeneity
According to the F-test, the heterogeneity standard deviation is not significantly different from 0 (significance level 5%), therefore the sample can be considered sufficiently homogeneous according to this criterion.

ISO 13528: Check for sufficient homogeneity
According to ISO 13528, the heterogeneity standard deviation s(sample) between the test portions of the sample should not exceed 30 % of the target standard deviation.
The heterogeneity standard deviation is less than 30% of the target s.d. 9669 (Manual), therefore the sample can be considered adequately homogeneous according to ISO 13528.

Harmonized Protocol: test on significant heterogeneity
The analytical precision of the method does not exceed 50% of the target s.d. 9669 (Manual). Therefore the evaluation according to the Harmonized Protocol can be carried out for this sample: The heterogeneity standard deviation is less than 30% of the target s.d., therefore the sample can be considered homogeneous.



ANNEX VII: *Pseudo-nitzschia australis* stability test

BEQ2014

Survey of stability test results

Sample: **WATER2** Date: 16/01/2015
Measurand: **Pseudo-**

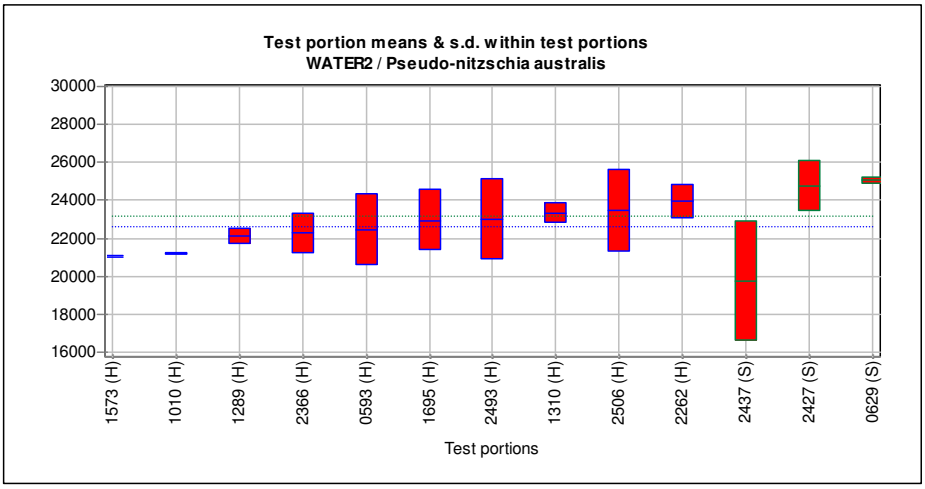
Mean of homogeneity:	22565
Mean of stability:	23183
Target standard deviation:	9669 (Manual)

Results of Stability Test

For the test of stability, 3 of the test portions of sample WATER2 have been selected randomly and the measurand Pseudo-nitzschia australis has been analysed 2 times.
The mean value across all test portions of the homogeneity analysis equals 22565, the mean value across all test portions of the stability analysis equals 23183.
Therefore, the mean value of the stability analysis lies 2.7 % above the mean value of the homogeneity analysis.

According to ISO 13528, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the target standard deviation.
Therefore, given the target standard deviation of 9669, the sample may be considered as adequately stable according to ISO 13528.

According to the Harmonized Protocol it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of signficance 5%).
The difference of the mean values is not statistically significant. Therefore - according to the Harmonized Protocol - the sample can be considered adequately stable.



ANNEX VII: *Paralia sulcata* homogeneity test

BEQ2014

Survey of homogeneity test results

Sample: **WATER2** Date: 16/01/2015
Measurand: **Paralia sulcata**

Mean:	5910
Analytical standard deviation:	1789
Heterogeneity standard deviation s(samples):	0
Target standard deviation:	2243 (Manual)

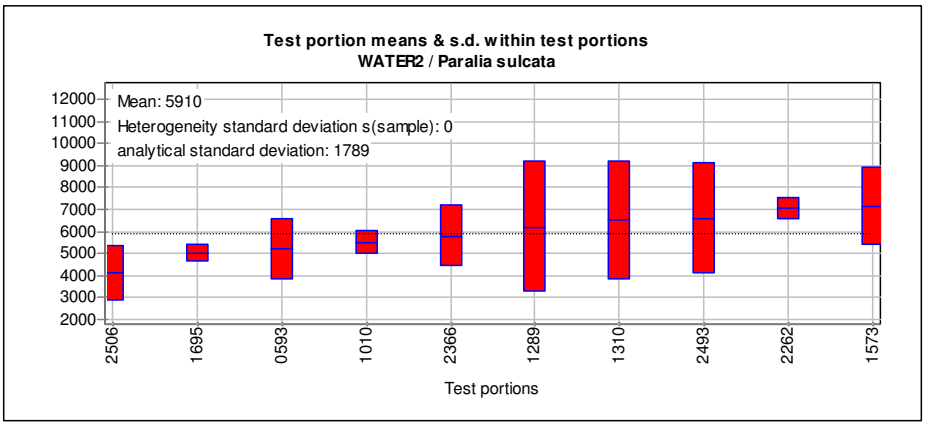
Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the test portions of sample WATER2 were randomly selected, and the measurand Paralia sulcata was analyzed 2 times. The mean across all 10 test portions is 5910, the standard deviation within test portions s (analytical) (=analytical precision) is 1789, and the standard deviation between test portions s(sample) is 0.

F-Test: statistical test on significant heterogeneity
The heterogeneity standard deviation s(sample) is 0, and hence no statistically significant difference to zero can be detected by the F-test.

ISO 13528: Check for sufficient homogeneity
According to ISO 13528, the heterogeneity standard deviation s(sample) between the test portions of the sample should not exceed 30 % of the target standard deviation.
The heterogeneity standard deviation is less than 30% of the target s.d. 2243 (Manual), therefore the sample can be considered adequately homogeneous according to ISO 13528.

Harmonized Protocol: test on significant heterogeneity
For the specified target standard deviation 2243 (Manual), the analytical precision of the method does not fulfil the requirements of the Harmonized Protocol (s(analytical) > 50% of the target standard deviation), and it may not be possible to determine the heterogeneity of the samples. Accordingly, an adequate homogeneity test is not possible.



ANNEX VII: *Paralia sulcata* stability test

BEQ2014

Survey of stability test results

Sample: **WATER2** Date: 16/01/2015
Measurand: **Paralia sulcata**

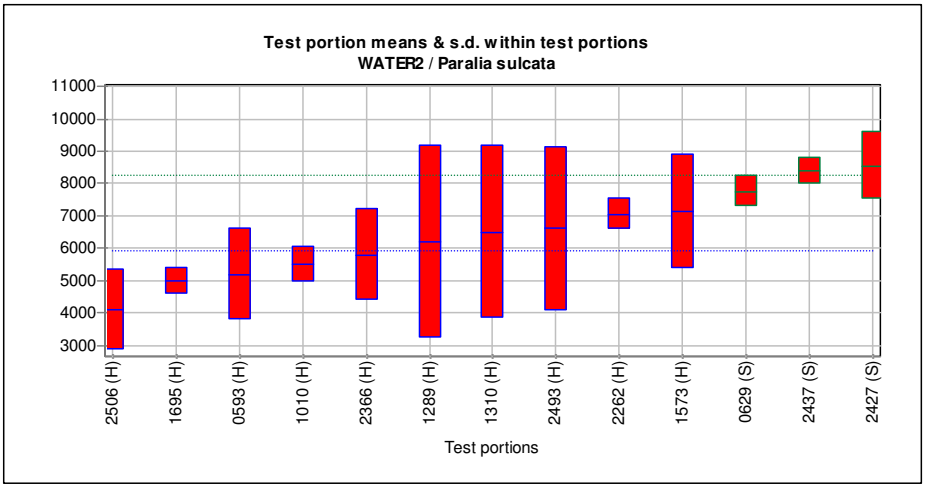
Mean of homogeneity:	5910
Mean of stability:	8233
Target standard deviation:	2243 (Manual)

Results of Stability Test

For the test of stability, 3 of the test portions of sample WATER2 have been selected randomly and the measurand Paralia sulcata has been analysed 2 times.
The mean value across all test portions of the homogeneity analysis equals 5910, the mean value across all test portions of the stability analysis equals 8233.
Therefore, the mean value of the stability analysis lies 39.3 % above the mean value of the homogeneity analysis.

According to ISO 13528, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the target standard deviation.
Therefore, given the target standard deviation of 2243, the sample may not be considered as adequately stable according to ISO 13528.

According to the Harmonized Protocol it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of signficance 5%).
There is a statistically significant difference between the mean values. Therefore - according to the Harmonized Protocol - the sample cannot be considered to be stable.



ANNEX VII: *Heterocapsa triquetra* homogeneity test

BEQ2014

Survey of homogeneity test results

Sample: **WATER2** Date: 16/01/2015
Measurand: **Heterocapsa**

Mean:	715
Analytical standard deviation:	328
Heterogeneity standard deviation s(samples):	177
Target standard deviation:	461 (Manual)

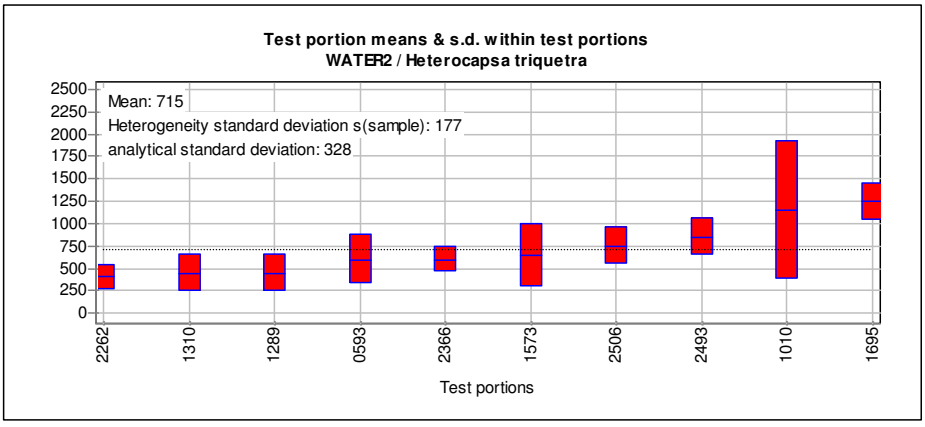
Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the test portions of sample WATER2 were randomly selected, and the measurand Heterocapsa triquetra was analyzed 2 times. The mean across all 10 test portions is 715, the standard deviation within test portions s(analytical) (=analytical precision) is 328, and the standard deviation between test portions s(sample) is 177.

F-Test: statistical test on significant heterogeneity
According to the F-test, the heterogeneity standard deviation is not significantly different from 0 (significance level 5%), therefore the sample can be considered sufficiently homogeneous according to this criterion.

ISO 13528: Check for sufficient homogeneity
According to ISO 13528, the heterogeneity standard deviation s(sample) between the test portions of the sample should not exceed 30 % of the target standard deviation.
The heterogeneity standard deviation is greater than 30% of the target s.d. 461 (Manual), therefore the sample should be considered heterogeneous.

Harmonized Protocol: test on significant heterogeneity
For the specified target standard deviation 461 (Manual), the analytical precision of the method does not fulfil the requirements of the Harmonized Protocol (s(analytical) > 50% of the target standard deviation), and it may not be possible to determine the heterogeneity of the samples. Accordingly, an adequate homogeneity test is not possible.



ANNEX VII: *Heterocapsa triquetra* stability test

BEQ2014

Survey of stability test results

Sample: **WATER2** Date: 16/01/2015
Measurand: **Heterocapsa**

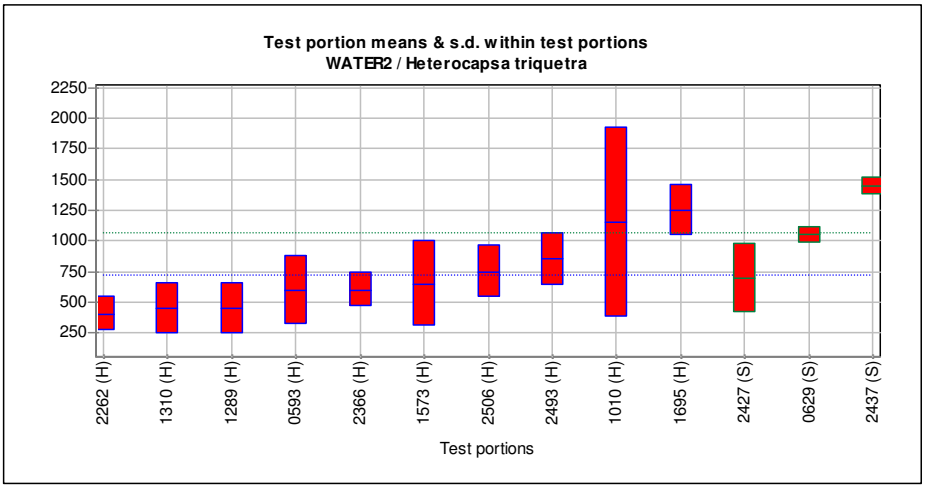
Mean of homogeneity:	715
Mean of stability:	1067
Target standard deviation:	461 (Manual)

Results of Stability Test

For the test of stability, 3 of the test portions of sample WATER2 have been selected randomly and the measurand Heterocapsa triquetra has been analysed 2 times.
The mean value across all test portions of the homogeneity analysis equals 715, the mean value across all test portions of the stability analysis equals 1067.
Therefore, the mean value of the stability analysis lies 49.2 % above the mean value of the homogeneity analysis.

According to ISO 13528, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the target standard deviation.
Therefore, given the target standard deviation of 461, the sample may not be considered as adequately stable according to ISO 13528.

According to the Harmonized Protocol it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of signficance 5%).
There is a statistically significant difference between the mean values. Therefore - according to the Harmonized Protocol - the sample cannot be considered to be stable.



ANNEX VII: *Thalassiosira punctigera* homogeneity test

BEQ2014

Survey of homogeneity test results

Sample: **WATER2** Date: 16/01/2015
Measurand: **Thalassiosira**

Mean:	10190
Analytical standard deviation:	1525
Heterogeneity standard deviation s(samples):	0
Target standard deviation:	1390 (Manual)

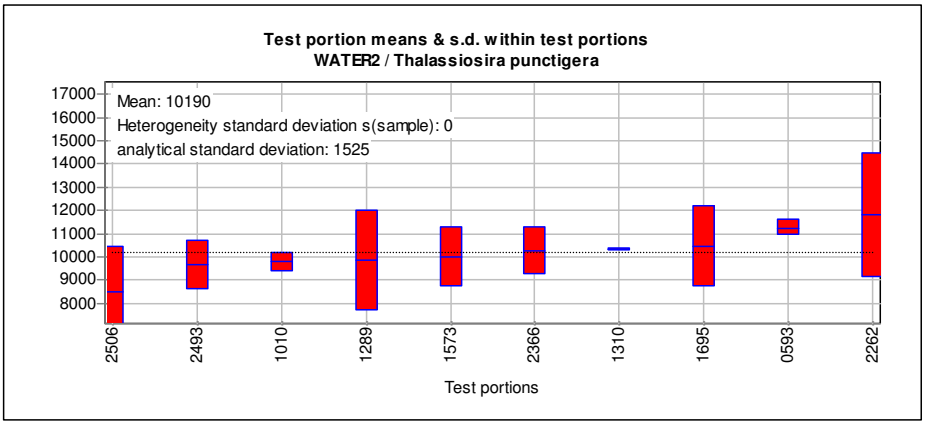
Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the test portions of sample WATER2 were randomly selected, and the measurand Thalassiosira punctigera was analyzed 2 times. The mean across all 10 test portions is 10190, the standard deviation within test portions s(analytical) (=analytical precision) is 1525, and the standard deviation between test portions s(sample) is 0.

F-Test: statistical test on significant heterogeneity
The heterogeneity standard deviation s(sample) is 0, and hence no statistically significant difference to zero can be detected by the F-test.

ISO 13528: Check for sufficient homogeneity
According to ISO 13528, the heterogeneity standard deviation s(sample) between the test portions of the sample should not exceed 30 % of the target standard deviation.
The heterogeneity standard deviation is less than 30% of the target s.d. 1390 (Manual), therefore the sample can be considered adequately homogeneous according to ISO 13528.

Harmonized Protocol: test on significant heterogeneity
For the specified target standard deviation 1390 (Manual), the analytical precision of the method does not fulfil the requirements of the Harmonized Protocol (s(analytical) > 50% of the target standard deviation), and it may not be possible to determine the heterogeneity of the samples. Accordingly, an adequate homogeneity test is not possible.



ANNEX VII: *Thalassiosira punctigera* stability test

BEQ2014

Survey of stability test results

Sample: **WATER2** Date: 16/01/2015
Measurand: **Thalassiosira**

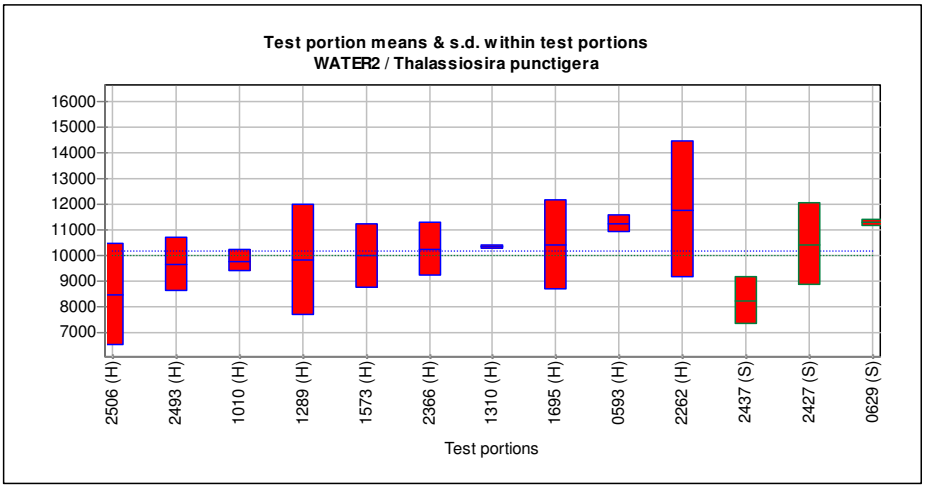
Mean of homogeneity:	10190
Mean of stability:	10000
Target standard deviation:	1390 (Manual)

Results of Stability Test

For the test of stability, 3 of the test portions of sample WATER2 have been selected randomly and the measurand Thalassiosira punctigera has been analysed 2 times.
The mean value across all test portions of the homogeneity analysis equals 10190, the mean value across all test portions of the stability analysis equals 10000.
Therefore, the mean value of the stability analysis lies 1.9 % below the mean value of the homogeneity analysis.

According to ISO 13528, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the target standard deviation.
Therefore, given the target standard deviation of 1390, the sample may be considered as adequately stable according to ISO 13528.

According to the Harmonized Protocol it is checked whether the mean values of the homogeneity analysis and the stability analysis differ significantly (level of signficance 5%).
The difference of the mean values is not statistically significant. Therefore - according to the Harmonized Protocol - the sample can be considered adequately stable.



ANNEX VIII: Analysts results

ANALYST CODE	SAMPLE CODES			Chaetoceros diadema (cells/L)			Rhizosolenia setigera (cells/L)			Pseudo-nitzschia australis (cells/L)		
				sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3
16	163	209	254	3500	3500	3000	15000	12000	12000	29500	39500	29500
20	253	240	208	1280	3240	1720	13880	14880	14160	18520	14360	29000
9	38	148	190	3680	920	2760	14640	13800	15360	11520	14000	12600
13	166	72	260	1240	1880	2280	13520	13760	14040	13560	19120	10520
7	210	151	20	2720	1960	1480	7880	12040	16560	24280	25320	33880
1	206	211	252	2480	2120	1640	13760	13120	13960	16760	13720	10240
35	54	10	36	3000	1200	2000	14480	14760	13440	14680	11040	11920
34	56	182	8	1320	1480	1120	7960	7520	7920	19800	19760	20400
8	87	133	183	3280	1840	1720	15640	16960	15200	8680	7160	9920
61	128	58	82	1240	1200	1080	13680	12560	13560	8320	8200	9240
22	60	193	262	1933	2567	2667	14250	17367	15033	11667	14367	11667
10	81	110	281	1680	960	1520	12920	14080	14040	18080	21720	23880
41	28	272	297	760	1000	1200	13400	12840	11960	21120	20400	25160
30	30	35	169	2080	1040	1360	11160	10000	13560	23760	26720	21360
40	64	143	229	1920	2600	1240	13840	12360	13360	24961	25190	27709
23	68	175	203	800	1160	1080	16728	23052	20400	33660	41616	38760
54	17	201	277	1400	1080	1400	19236	22671	21755	47174	55876	49006
42	15	284	299	1300	2900	3000	18000	17000	16000	29200	24000	35000
6	5	98	186	3737	2195	2612	15984	15178	14155	15318	15847	16379
19	19	101	165	3680	1520	1880	n/a	n/a	n/a	11000	8840	7920
37	25	32	47	960	1320	1120	7200	6800	4800	7120	10800	11200
59	195	22	4	320	520	960	14360	9360	9960	6000	14320	14080
57	103	71	268	320	200	360	12400	13120	12720	23840	13880	18680
58	79	286	179	2040	2360	2320	14520	12840	14400	31120	35320	35400
60	102	120	214	not id	not id	not id	15000	15320	14440	13280	15600	23360
56	237	244	250	200	760	1680	1760	2080	5400	2080	2720	4880
36	149	152	293	3720	2400	2160	n/a	n/a	n/a	12360	11480	14360
14	12	177	180	48	840	2040	13480	12560	15240	17320	19800	24560
17	126	251	263	720	2120	1600	15200	14880	14960	8680	16800	23960
3	37	67	181	1400	880	1440	16800	16240	14160	20920	33680	30000
26	26	43	94	1760	680	2840	9000	8440	7640	21200	24080	28000
43	213	259	279	nr	2960	2160	520	6840	8480	4120	17440	23960
21	230	238	246	760	1080	1880	14040	12960	18640	33440	40920	47200
12	45	94	296	1280	760	1200	13360	13760	14120	18720	24080	17600
47	16	34	112	1640	3280	2880	9360	8640	10400	8480	9840	17000
2	88	99	106	1000	600	2000	16400	16000	15200	32800	28300	28600
55	70	217	200	1400	2600	1500	15000	15400	18700	21700	27400	25200
51	40	216	256	1850	3050	2450	16400	14250	13750	4750	9950	10050
49	194	140	108	2800	2360	3080	9120	9560	9680	9720	13640	9280
25	235	275	295	1080	2040	1960	14480	11360	16480	22120	22200	9080
4	125	287	226	3100	1900	4400	15700	14300	15300	10800	10700	7600
48	111	147	73	1280	2120	2680	15040	16280	15920	13520	7040	9360
45	3	53	61	1900	1600	1500	8000	8800	6300	47800	56400	36200
39	66	85	167	2000	3080	2040	9000	8000	8560	30000	18320	16200
5	114	225	298	3200	900	1500	14950	14650	13750	14850	14400	15650
18	62	117	77	2548	2058	3822	14504	18032	15337	19306	32487	34349
33	86	42	270	1750	1150	1050	17700	16750	2900	35500	45150	41050
44	9	11	170	2100	600	2200	9800	8650	8600	6100	7100	9100
28	267	89	285	3500	2900	3500	10200	8300	9600	8300	10200	20500
24	119	156	160	800	500	800	14100	9700	13533	11200	11900	8000
11	292	232	127	867	1000	300	14467	10400	14000	21000	19133	9767
50	290	227	243	633	900	600	17067	21100	9867	11067	9400	12667
53	142	159	188	2080	1800	2760	15200	15880	14800	21720	21040	24680
52	46	104	218	1565	2174	2044	15827	14653	18044	7609	12479	13174
46	196	239	278	2174	3261	2131	17088	14305	15435	8435	11392	14305
27	63	199	124	2720	1920	2400	9680	13520	9520	38400	46160	36240
38	261	234	23	1000	880	960	12720	12320	12480	52520	54880	53840
15	59	173	242	2240	1800	1960	15120	15680	15040	28040	33320	33280
31	136	139	207	1560	2360	2160	7400	7640	7520	28520	50080	41600
32	7	57	137	1960	1320	1520	15040	14960	14200	37520	44000	47560
29	24	154	197	1720	2520	1160	14520	16520	15760	18240	16120	22600
64	75	90	185	1385	577	1731	15961	12230	13230	12653	12999	7615
62	18	84	283	1538	2577	962	15769	17038	16000	12653	12346	15460
63	166	145	255	1423	2808	2115	13846	14269	14653	13653	14922	16884

ANNEX VIII

ANALYST CODE	SAMPLE CODES			Heterocapsa triquetra (cells/L)			paralia sulcata (cells/L)			Thalassiosira punctigera (cells/L)		
				sample 1	sample 2	sample 3	sample 1	sample 2	sample 3	sample 1	sample 2	sample 3
16	163	209	254	not id	not id	not id	7500	8000	12500	10500	7500	8500
20	253	240	208	1480	1280	880	7640	11200	7040	3520	3800	3560
9	38	148	190	320	480	240	10120	8280	9720	3200	3480	3840
13	166	72	260	520	560	320	9640	9440	9440	5680	3840	3240
7	210	151	20	80	0	40	5720	12520	10680	5040	3280	2080
1	206	211	252	760	360	440	9040	12720	10680	4120	4440	4000
35	54	10	36	600	280	320	10160	10080	8920	3200	3000	4760
34	56	182	8	not id	not id	not id	5400	5000	6280	4360	3160	3520
8	87	133	183	920	800	720	9600	8440	7640	2080	3720	3240
61	128	58	82	1000	720	680	9440	13400	13320	2080	2120	1880
22	60	193	262	1400	1733	1100	10000	8567	9867	4850	2833	3200
10	81	110	281	1160	1000	800	8800	9360	6200	1840	1400	1880
41	28	272	297	720	640	880	7760	8320	8400	1120	1440	880
30	30	35	169	1360	960	1120	6520	6080	5800	1120	880	1040
40	64	143	229	280	480	320	11160	9460	13760	2000	2240	2720
23	68	175	203	240	160	200	10640	6160	10680	2600	2640	3240
54	17	201	277	960	880	1040	8920	8840	11360	2160	1800	2760
42	15	284	299	1000	700	1300	8200	10900	7600	3600	3200	3700
6	5	98	186	1147	893	812	13727	9523	11437	5365	5766	4060
19	19	101	165	1320	1120	960	10720	13120	10360	5680	6720	5840
37	25	32	47	40	80	80	1800	1880	2200	2320	3200	2400
59	195	22	4	280	280	200	5120	5800	4160	2760	3560	2680
57	103	71	268	not id	not id	not id	4160	4560	4080	1480	840	1280
58	79	286	179	1000	1160	1320	10680	12440	10520	1080	1600	1160
60	102	120	214	920	1040	1200	6440	5760	7840	880	880	920
56	237	244	250	not id	not id	not id	560	240	1680	240	440	1040
36	149	152	293	760	840	600	8880	8920	10320	2080	2440	3080
14	12	177	180	680	1040	1040	9840	10880	10880	2200	2240	1760
17	126	251	263	600	400	680	6800	8880	8280	2440	3880	2800
3	37	67	181	520	600	720	10760	8320	3960	2880	3880	2000
26	26	43	94	1080	760	1040	8600	10560	9760	2280	2200	2800
43	213	259	279	4200	880	1160	1280	7280	10240	200	3000	2760
21	230	238	246	nr	280	200	4400	8960	9360	3200	3600	3520
12	45	94	296	840	1000	760	13080	14120	11200	2840	3400	3400
47	16	34	112	240	40	600	5920	5720	8800	2320	1400	1920
2	88	99	106	1100	700	1000	10400	7700	11500	1600	1700	1600
55	70	217	200	1400	900	1500	15400	16500	11600	5100	3500	2900
51	40	216	256	100	50	400	14350	5650	10800	4350	4050	4900
49	194	140	108	360	520	320	6640	7600	7480	2720	1880	2560
25	235	275	295	400	200	80	6720	9000	9040	2720	1720	1880
4	125	287	226	500	500	300	11900	7700	11000	4400	4000	4100
48	111	147	73	640	440	480	11720	10400	8680	2320	1680	2320
45	3	53	61	500	800	300	3800	5100	8400	500	2000	1300
39	66	85	167	1160	1120	760	12120	11120	9920	1840	2760	2240
5	114	225	298	1000	800	650	8600	9000	7150	2150	2500	2900
18	62	117	77	980	1519	1029	9898	9163	7350	2254	1960	2597
33	86	42	270	1050	1000	1950	10250	9600	7300	3550	2800	2250
44	9	11	170	900	500	1100	5600	12400	10700	3400	3300	3500
28	267	89	285	1300	1200	900	14600	8400	16600	3500	2600	3400
24	119	156	160	500	500	900	9700	8300	5900	1800	1800	1800
11	292	232	127	233	600	567	12900	7133	10067	3467	2733	2800
50	290	227	243	433	367	267	8300	9133	6367	10833	8933	8600
53	142	159	188	80	40	80	8280	8160	8200	3000	3520	3080
52	46	104	218	957	652	1087	14261	10566	8566	2609	3870	2087
46	196	239	278	957	739	1000	11653	7652	7609	2870	4565	4000
27	63	199	124	0	0	80	12240	18720	8480	6000	6800	4960
38	261	234	23	1000	1080	1040	11800	11800	11320	3800	4280	4400
15	59	173	242	800	880	760	9120	10560	9280	6720	7200	6960
31	136	139	207	440	800	640	8400	11880	10720	4480	7000	6840
32	7	57	137	1320	1160	960	6960	4880	5040	5600	6160	6640
29	24	154	197	440	720	600	11400	13360	13200	3320	2800	2560
64	75	90	185	385	192	269	5846	6192	8923	1461	3615	1154
62	18	84	283	1077	1269	1500	10500	4538	8538	3769	4077	4000
63	166	145	255	1154	1077	885	8654	10500	11000	2654	3500	2923

Annex IX:

Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528

C.diadema iteration

ANALYST CODE	Average	X-X*	X*i	it2	it3	it4	it5
57	293	1540	632	672	687	691	692
59	600	1233	632	672	687	691	692
24	700	1133	700	700	700	700	700
50	711	1122	711	711	711	711	711
11	722	1111	722	722	722	722	722
56	880	953	880	880	880	880	880
38	947	887	947	947	947	947	947
14	976	857	976	976	976	976	976
41	987	847	987	987	987	987	987
23	1013	820	1013	1013	1013	1013	1013
12	1080	753	1080	1080	1080	1080	1080
37	1133	700	1133	1133	1133	1133	1133
61	1173	660	1173	1173	1173	1173	1173
2	1200	633	1200	1200	1200	1200	1200
64	1231	603	1231	1231	1231	1231	1231
3	1240	593	1240	1240	1240	1240	1240
21	1240	593	1240	1240	1240	1240	1240
54	1293	540	1293	1293	1293	1293	1293
34	1307	527	1307	1307	1307	1307	1307
33	1317	517	1317	1317	1317	1317	1317
10	1387	447	1387	1387	1387	1387	1387
17	1480	353	1480	1480	1480	1480	1480
30	1493	340	1493	1493	1493	1493	1493
32	1600	233	1600	1600	1600	1600	1600
44	1633	200	1633	1633	1633	1633	1633
45	1667	167	1667	1667	1667	1667	1667
62	1692	141	1692	1692	1692	1692	1692
25	1693	140	1693	1693	1693	1693	1693
26	1760	73	1760	1760	1760	1760	1760
13	1800	33	1800	1800	1800	1800	1800
29	1800	33	1800	1800	1800	1800	1800
55	1833	0	1833	1833	1833	1833	1833
5	1867	33	1867	1867	1867	1867	1867
40	1920	87	1920	1920	1920	1920	1920
52	1928	94	1928	1928	1928	1928	1928
15	2000	167	2000	2000	2000	2000	2000
48	2027	193	2027	2027	2027	2027	2027
31	2027	193	2027	2027	2027	2027	2027
7	2053	220	2053	2053	2053	2053	2053
35	2067	233	2067	2067	2067	2067	2067
20	2080	247	2080	2080	2080	2080	2080
1	2080	247	2080	2080	2080	2080	2080
63	2115	282	2115	2115	2115	2115	2115
53	2213	380	2213	2213	2213	2213	2213
58	2240	407	2240	2240	2240	2240	2240
8	2280	447	2280	2280	2280	2280	2280
27	2347	513	2347	2347	2347	2347	2347
19	2360	527	2360	2360	2360	2360	2360
39	2373	540	2373	2373	2373	2373	2373
22	2389	556	2389	2389	2389	2389	2389
42	2400	567	2400	2400	2400	2400	2400
51	2450	617	2450	2450	2450	2450	2450
9	2453	620	2453	2453	2453	2453	2453
46	2522	689	2522	2522	2522	2522	2522
43	2560	727	2560	2560	2560	2560	2560
47	2600	767	2600	2600	2600	2600	2600
49	2747	913	2747	2747	2747	2747	2747
36	2760	927	2760	2760	2760	2760	2760
18	2809	976	2809	2809	2809	2809	2809
6	2848	1015	2848	2848	2848	2848	2848
4	3133	1300	3035	2943	2922	2917	2916
28	3300	1467	3035	2943	2922	2917	2916
16	3333	1500	3035	2943	2922	2917	2916
60	not id	not id	not id	not id	not id	not id	not id
Average *X*	1812		1807	1804	1804	1804	1804
SD *S*	700		667	657	655	654	654
robust average *X**	1833	new *X**	1807	1804	1804	1804	1804
robust stdev *S**	801	new *S**	757	745	742	741	741
$\delta = 1.5S^*$	1201		1135	1118	1113	1112	1112
X*- δ	632		672	687	691	692	692
X*+ δ	3035		2943	2922	2917	2916	2916
no of analysts *P*	63		63	63	63	63	63
Between Samples SD	78	From homogeneity test					
new stdev for CDIA	745						

Annex IX: *R.setigera* iteration

ANALYST CODE	Average	X-X*	X*i	it2	it3	it4
56	3080	10784	10398	10398	10398	10398
43	5280	8584	10398	10398	10398	10398
37	6267	7597	10398	10398	10398	10398
31	7520	6344	10398	10398	10398	10398
45	7700	6164	10398	10398	10398	10398
34	7800	6064	10398	10398	10398	10398
26	8360	5504	10398	10398	10398	10398
39	8520	5344	10398	10398	10398	10398
44	9017	4847	10398	10398	10398	10398
28	9367	4497	10398	10398	10398	10398
49	9453	4410	10398	10398	10398	10398
47	9467	4397	10398	10398	10398	10398
27	10907	2957	10907	10907	10907	10907
59	11227	2637	11227	11227	11227	11227
30	11573	2290	11573	11573	11573	11573
7	12160	1704	12160	12160	12160	12160
24	12444	1419	12444	12444	12444	12444
33	12450	1414	12450	12450	12450	12450
38	12507	1357	12507	12507	12507	12507
41	12733	1130	12733	12733	12733	12733
57	12747	1117	12747	12747	12747	12747
11	12956	908	12956	12956	12956	12956
16	13000	864	13000	13000	13000	13000
40	13187	677	13187	13187	13187	13187
61	13267	597	13267	13267	13267	13267
1	13613	250	13613	13613	13613	13613
10	13680	184	13680	13680	13680	13680
12	13747	117	13747	13747	13747	13747
14	13760	104	13760	13760	13760	13760
13	13773	90	13773	13773	13773	13773
64	13807	56	13807	13807	13807	13807
58	13920	56	13920	13920	13920	13920
25	14107	243	14107	14107	14107	14107
35	14227	363	14227	14227	14227	14227
63	14256	392	14256	14256	14256	14256
20	14307	443	14307	14307	14307	14307
5	14450	586	14450	14450	14450	14450
9	14600	736	14600	14600	14600	14600
32	14733	870	14733	14733	14733	14733
51	14800	936	14800	14800	14800	14800
60	14920	1056	14920	14920	14920	14920
17	15013	1150	15013	15013	15013	15013
4	15100	1236	15100	15100	15100	15100
6	15106	1242	15106	15106	15106	15106
21	15213	1350	15213	15213	15213	15213
15	15280	1416	15280	15280	15280	15280
53	15293	1430	15293	15293	15293	15293
22	15550	1686	15550	15550	15550	15550
29	15600	1736	15600	15600	15600	15600
46	15609	1746	15609	15609	15609	15609
3	15733	1870	15733	15733	15733	15733
48	15747	1883	15747	15747	15747	15747
2	15867	2003	15867	15867	15867	15867
8	15933	2070	15933	15933	15933	15933
18	15958	2094	15958	15958	15958	15958
50	16011	2147	16011	16011	16011	16011
52	16175	2311	16175	16175	16175	16175
62	16269	2405	16269	16269	16269	16269
55	16367	2503	16367	16367	16367	16367
42	17000	3136	17000	17000	17000	17000
23	20060	6196	17330	17258	17249	17248
54	21221	7357	17330	17258	17249	17248
Average *X*	13222		13647	13645	13644	13644
SD *S*	3341		2123	2119	2118	2118
robust average *X**	13864	new *X**	13647	13645	13644	13644
robust stdev *S**	2311	new *S**	2407	2403	2402	2402
$\delta = 1.5S^*$	3466		3611	3604	3603	3603
$X^* - \delta$	10398		10036	10041	10041	10041
$X^* + \delta$	17330		17258	17249	17248	17248
no of analysts *P*	62		62	62	62	62
Between Samples SD	1373	From homogeneity test				
new stdev for RSET	2767					

Annex IX: *P. australis* iteration

ANALYST CODE	Averag	X-X*	X*i	it2	it3	it4
56	3227	14380	3227	4687	4781	4787
44	7433	10173	7433	7433	7433	7433
51	8250	9357	8250	8250	8250	8250
8	8587	9020	8587	8587	8587	8587
61	8587	9020	8587	8587	8587	8587
19	9253	8353	9253	9253	9253	9253
4	9700	7907	9700	9700	9700	9700
37	9707	7900	9707	9707	9707	9707
48	9973	7633	9973	9973	9973	9973
24	10367	7240	10367	10367	10367	10367
49	10880	6727	10880	10880	10880	10880
50	11045	6562	11067	11067	11067	11067
52	11087	6519	11087	11087	11087	11087
64	11089	6517	11089	11089	11089	11089
46	11377	6229	11377	11377	11377	11377
59	11467	6140	11467	11467	11467	11467
47	11773	5833	11773	11773	11773	11773
35	12547	5060	12547	12547	12547	12547
22	12567	5040	12567	12567	12567	12567
9	12707	4900	12707	12707	12707	12707
36	12733	4873	12733	12733	12733	12733
28	13000	4607	13000	13000	13000	13000
62	13486	4120	13486	13486	13486	13486
1	13573	4033	13573	13573	13573	13573
13	14400	3207	14400	14400	14400	14400
5	14967	2640	14967	14967	14967	14967
63	15153	2453	15153	15153	15153	15153
43	15173	2433	15173	15173	15173	15173
6	15848	1759	15848	15848	15848	15848
17	16480	1127	16480	16480	16480	16480
11	16633	973	16633	16633	16633	16633
60	17413	193	17413	17413	17413	17413
25	17800	193	17800	17800	17800	17800
57	18800	1193	18787	18787	18787	18787
29	18987	1380	18800	18800	18800	18800
34	19987	2380	19987	19987	19987	19987
12	20133	2527	20133	20133	20133	20133
14	20560	2953	20560	20560	20560	20560
20	20627	3020	20627	20627	20627	20627
10	21227	3620	21227	21227	21227	21227
39	21507	3900	21507	21507	21507	21507
41	22227	4620	22227	22227	22227	22227
53	22480	4873	22480	22480	22480	22480
30	23947	6340	23947	23947	23947	23947
26	24427	6820	24427	24427	24427	24427
55	24767	7160	24767	24767	24767	24767
40	25953	8347	25953	25953	25953	25953
7	27827	10220	27827	27827	27827	27827
3	28200	10593	28200	28200	28200	28200
18	28714	11107	28714	28714	28714	28714
42	29400	11793	29400	29400	29400	29400
2	29900	12293	29900	29900	29900	29900
15	31547	13940	31547	31547	31547	31547
16	32833	15227	32156	32156	32156	32156
58	33947	16340	32156	32156	32156	32156
23	38012	20405	32156	32156	32156	32156
31	40067	22460	32156	32156	32156	32156
27	40267	22660	32156	32156	32156	32156
21	40520	22913	32156	32156	32156	32156
33	40567	22960	32156	32156	32156	32156
32	43027	25420	32156	32156	32156	32156
45	46800	29193	32156	32156	32156	32156
54	50685	33079	32156	32156	32156	32156
38	53747	36140	32156	32156	32156	32156
Average *X*	20937		19266	19289	19290	19291
SD *S*	11718		8571	8529	8527	8527
robust average *X**	17607	new *X**	19266	19289	19290	19291
robust stdev *S**	9700	new *S**	9719	9672	9669	9669
δ= 1.5*S**	14549		14579	14508	14504	14504
X*- δ	3057		4687	4781	4787	4787
X*+ δ	32156		33845	33797	33794	33794
no of analysts *P*	64		64	64	64	64
Between Samples SD	89	From homogeneity test				
new stdev for PAUS	9669					

Annex IX: *P.sulcata* iteration

ANALYST CODE	Average	X-X*	X*i	it2	it3	it4	it5	it6
56	827	8527	6291	6291	6291	6291	6291	6291
37	1960	7393	6291	6291	6291	6291	6291	6291
57	4267	5087	6291	6291	6291	6291	6291	6291
59	5027	4327	6291	6291	6291	6291	6291	6291
34	5560	3793	6291	6291	6291	6291	6291	6291
32	5627	3727	6291	6291	6291	6291	6291	6291
45	5767	3587	6291	6291	6291	6291	6291	6291
30	6133	3220	6291	6291	6291	6291	6291	6291
43	6267	3087	6291	6291	6291	6291	6291	6291
60	6680	2673	6680	6680	6680	6680	6680	6680
47	6813	2540	6813	6813	6813	6813	6813	6813
64	6987	2366	6987	6987	6987	6987	6987	6987
49	7240	2113	7240	7240	7240	7240	7240	7240
21	7573	1780	7573	7573	7573	7573	7573	7573
3	7680	1673	7680	7680	7680	7680	7680	7680
62	7859	1495	7859	7859	7859	7859	7859	7859
50	7933	1420	7933	7933	7933	7933	7933	7933
24	7967	1387	7967	7967	7967	7967	7967	7967
17	7987	1367	7987	7987	7987	7987	7987	7987
10	8120	1233	8120	8120	8120	8120	8120	8120
41	8160	1193	8160	8160	8160	8160	8160	8160
53	8213	1140	8213	8213	8213	8213	8213	8213
5	8250	1103	8250	8250	8250	8250	8250	8250
25	8253	1100	8253	8253	8253	8253	8253	8253
8	8560	793	8560	8560	8560	8560	8560	8560
20	8627	727	8627	8627	8627	8627	8627	8627
18	8804	550	8804	8804	8804	8804	8804	8804
42	8900	453	8900	8900	8900	8900	8900	8900
46	8971	382	8971	8971	8971	8971	8971	8971
33	9050	303	9050	9050	9050	9050	9050	9050
23	9160	193	9160	9160	9160	9160	9160	9160
16	9333	20	9333	9333	9333	9333	9333	9333
9	9373	20	9373	9373	9373	9373	9373	9373
36	9373	20	9373	9373	9373	9373	9373	9373
22	9478	125	9478	9478	9478	9478	9478	9478
13	9507	153	9507	9507	9507	9507	9507	9507
44	9567	213	9567	9567	9567	9567	9567	9567
7	9640	287	9640	9640	9640	9640	9640	9640
26	9640	287	9640	9640	9640	9640	9640	9640
15	9653	300	9653	9653	9653	9653	9653	9653
54	9707	353	9707	9707	9707	9707	9707	9707
35	9720	367	9720	9720	9720	9720	9720	9720
2	9867	513	9867	9867	9867	9867	9867	9867
11	10033	680	10033	10033	10033	10033	10033	10033
63	10051	698	10051	10051	10051	10051	10051	10051
4	10200	847	10200	10200	10200	10200	10200	10200
51	10267	913	10267	10267	10267	10267	10267	10267
48	10267	913	10267	10267	10267	10267	10267	10267
31	10333	980	10333	10333	10333	10333	10333	10333
14	10533	1180	10533	10533	10533	10533	10533	10533
1	10813	1460	10813	10813	10813	10813	10813	10813
39	11053	1700	11053	11053	11053	11053	11053	11053
52	11131	1778	11131	11131	11131	11131	11131	11131
58	11213	1860	11213	11213	11213	11213	11213	11213
19	11400	2047	11400	11400	11400	11400	11400	11400
40	11460	2107	11460	11460	11460	11460	11460	11460
6	11562	2209	11562	11562	11562	11562	11562	11562
38	11640	2287	11640	11640	11640	11640	11640	11640
61	12053	2700	12053	12053	12053	12053	12053	12053
29	12653	3300	12416	12318	12287	12278	12275	12274
12	12800	3447	12416	12318	12287	12278	12275	12274
27	13147	3793	12416	12318	12287	12278	12275	12274
28	13200	3847	12416	12318	12287	12278	12275	12274
55	14500	5147	12416	12318	12287	12278	12275	12274
Average *X*	8975		9146	9139	9136	9135	9135	9135
SD *S*	2497		1865	1851	1847	1846	1845	1845
robust average *X**	9353	new *X**	9146	9139	9136	9135	9135	9135
robust stdev *S**	2042	new *S**	2115	2099	2095	2093	2093	2093
δ= 1.5*S**	3062		3172	3149	3142	3140	3139	3139
X*- δ	6291		5974	5990	5994	5996	5996	5996
X*+ δ	12416		12318	12287	12278	12275	12274	12274
no of analysts *P*	64		64	64	64	64	64	64
Between Samples SD	808	From homogeneity test						
new stdev for PSUL	2243							

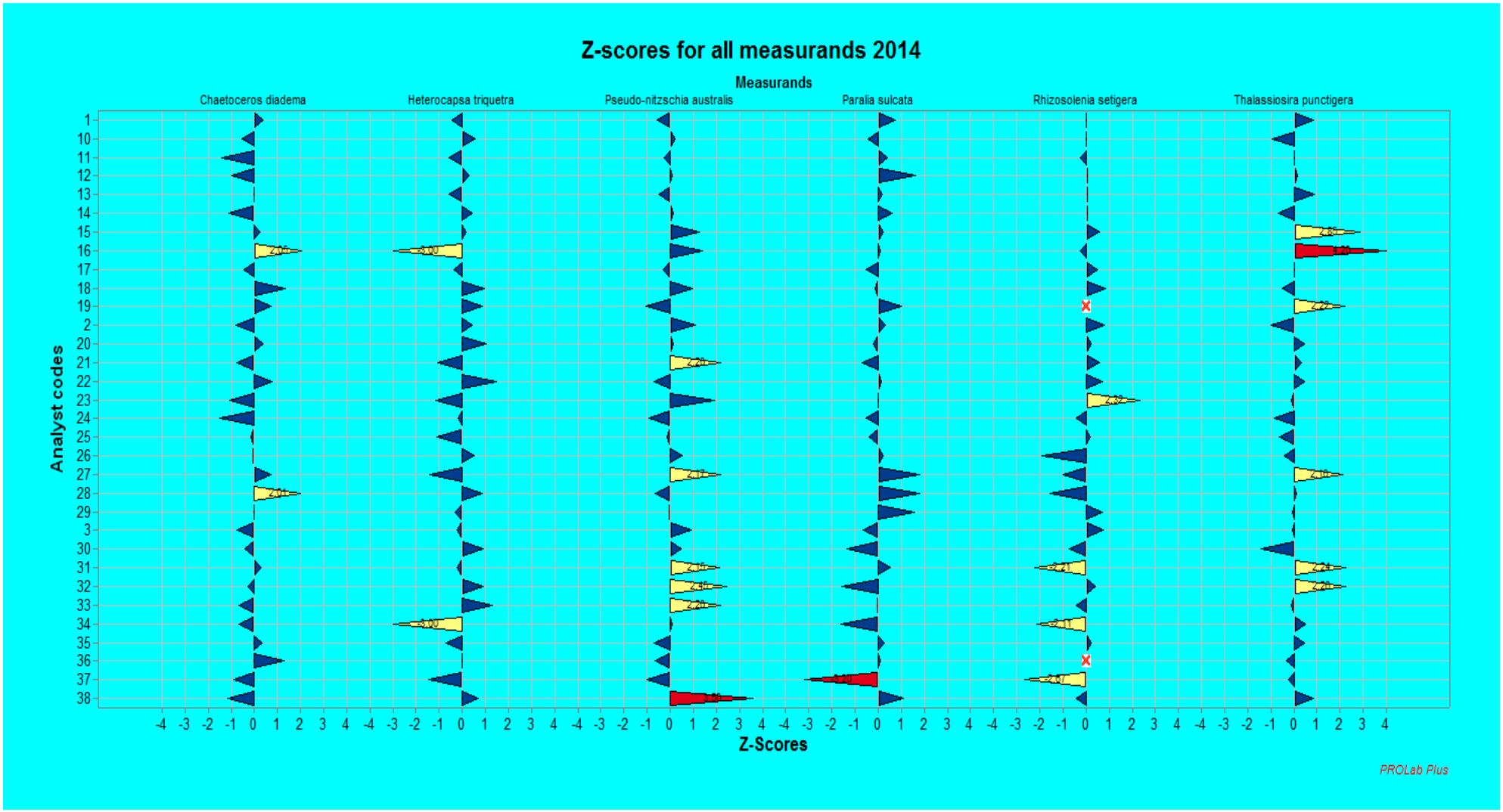
Annex IX: *H.triquetra* iteration

ANALYST CODE	Averag	X-X*	X*i	it2	it3	it4	it5
7	40	767	80	80	85	87	88
37	67	740	80	80	85	87	88
53	67	740	80	80	85	87	88
27	80	727	80	80	85	87	88
51	183	623	183	183	183	183	183
23	200	607	200	200	200	200	200
25	227	580	227	227	227	227	227
21	240	567	240	240	240	240	240
59	253	553	253	253	253	253	253
64	282	525	282	282	282	282	282
47	293	513	293	293	293	293	293
9	347	460	347	347	347	347	347
50	356	451	356	356	356	356	356
40	360	447	360	360	360	360	360
35	400	407	400	400	400	400	400
49	400	407	400	400	400	400	400
4	433	373	433	433	433	433	433
13	467	340	467	467	467	467	467
11	467	340	467	467	467	467	467
1	520	287	520	520	520	520	520
48	520	287	520	520	520	520	520
45	533	273	533	533	533	533	533
17	560	247	560	560	560	560	560
29	587	220	587	587	587	587	587
3	613	193	613	613	613	613	613
31	627	180	627	627	627	627	627
24	633	173	633	633	633	633	633
36	733	73	733	733	733	733	733
41	747	60	747	747	747	747	747
61	800	7	800	800	800	800	800
8	813	7	813	813	813	813	813
15	813	7	813	813	813	813	813
5	817	10	817	817	817	817	817
44	833	27	833	833	833	833	833
12	867	60	867	867	867	867	867
52	899	92	899	899	899	899	899
46	899	92	899	899	899	899	899
14	920	113	920	920	920	920	920
2	933	127	933	933	933	933	933
6	951	144	951	951	951	951	951
54	960	153	960	960	960	960	960
26	960	153	960	960	960	960	960
10	987	180	987	987	987	987	987
42	1000	193	1000	1000	1000	1000	1000
39	1013	207	1013	1013	1013	1013	1013
63	1038	232	1038	1038	1038	1038	1038
38	1040	233	1040	1040	1040	1040	1040
60	1053	247	1053	1053	1053	1053	1053
19	1133	327	1133	1133	1133	1133	1133
28	1133	327	1133	1133	1133	1133	1133
30	1147	340	1147	1147	1147	1147	1147
32	1147	340	1147	1147	1147	1147	1147
58	1160	353	1160	1160	1160	1160	1160
18	1176	369	1176	1176	1176	1176	1176
20	1213	407	1213	1213	1213	1213	1213
55	1267	460	1267	1267	1267	1267	1267
62	1282	475	1282	1282	1282	1282	1282
33	1333	527	1333	1333	1333	1333	1333
22	1411	604	1411	1383	1370	1368	1367
43	2080	1273	1533	1383	1370	1368	1367
16	not id	not id	not id	not id	not id	not id	not id
34	not id	not id	not id	not id	not id	not id	not id
57	not id	not id	not id	not id	not id	not id	not id
56	not id	not id	not id	not id	not id	not id	not id
Average *X*	739		731	728	727	728	728
SD *S*	410		383	378	376	376	376
robust average *X**	807	new *X**	731	728	727	728	728
robust stdev *S**	484	new *S**	435	428	427	426	426
δ= 1.5*S**	727		652	643	640	640	639
X*- δ	80		78	85	87	88	88
X*+ δ	1533		1383	1370	1368	1367	1367
no of analysts *P*	60		60	60	60	60	60
Between Samples SD		177	From homogeneity test				
new stdev for HTRIQ		461					

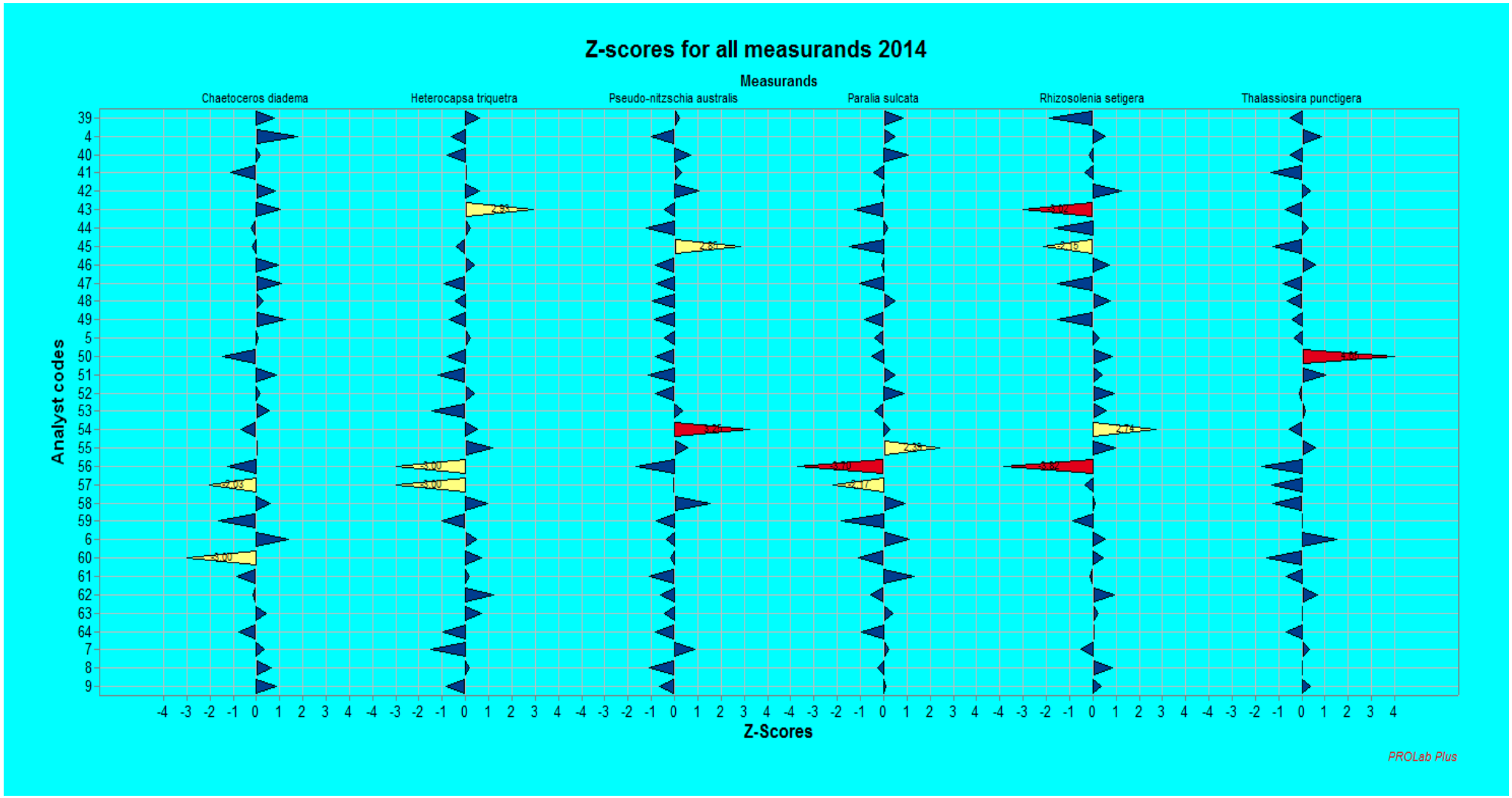
Annex IX: *T.punctigera* iteration

ANALYST CODE	Averag	X-X*	X*i	it2
56	573	2427	1170	1170
60	893	2107	1170	1170
30	1013	1987	1170	1170
41	1147	1853	1170	1170
57	1200	1800	1200	1200
45	1267	1733	1267	1267
58	1280	1720	1280	1280
2	1633	1367	1633	1633
10	1707	1293	1707	1707
24	1800	1200	1800	1800
47	1880	1120	1880	1880
43	1987	1013	1987	1987
61	2027	973	2027	2027
14	2067	933	2067	2067
64	2077	923	2077	2077
25	2107	893	2107	2107
48	2107	893	2107	2107
54	2240	760	2240	2240
18	2270	730	2270	2270
39	2280	720	2280	2280
40	2320	680	2320	2320
49	2387	613	2387	2387
26	2427	573	2427	2427
5	2517	483	2517	2517
36	2533	467	2533	2533
37	2640	360	2640	2640
23	2827	173	2827	2827
52	2855	145	2855	2855
33	2867	133	2867	2867
29	2893	107	2893	2893
3	2920	80	2920	2920
59	3000	0	3000	3000
11	3000	0	3000	3000
8	3013	13	3013	3013
63	3026	26	3026	3026
17	3040	40	3040	3040
28	3167	167	3167	3167
53	3200	200	3200	3200
12	3213	213	3213	3213
44	3400	400	3400	3400
21	3440	440	3440	3440
7	3467	467	3467	3467
42	3500	500	3500	3500
9	3507	507	3507	3507
20	3627	627	3627	3627
22	3628	628	3628	3628
35	3653	653	3653	3653
34	3680	680	3680	3680
46	3812	812	3812	3812
55	3833	833	3833	3833
62	3949	949	3949	3949
38	4160	1160	4160	4160
4	4167	1167	4167	4167
1	4187	1187	4187	4187
13	4253	1253	4253	4253
51	4433	1433	4433	4433
6	5064	2064	4830	4830
27	5920	2920	4830	4830
19	6080	3080	4830	4830
31	6107	3107	4830	4830
32	6133	3133	4830	4830
15	6960	3960	4830	4830
16	8833	5833	4830	4830
50	9455	6455	4830	4830
Average *X*	3229		2997	2997
SD *S*	1711		1107	1107
robust average *X**	3000	new *X**	2997	2997
robust stdev *S**	1220	new *S**	1256	1256
δ= 1.5*S**	1830		1883	1883
X*- δ	1170		1113	1113
X*+ δ	4830		4880	4880
no of analysts *P*	64		64	64
Between Samples SD	596	From homogeneity test		
new stdev for TPUNCT	1390			

ANNEX X: Summary of Z-scores for all measurands



ANNEX X


Z-scores for all measurands 2014
Measurands
Chaetoceros diadema
Heterocapsa triquetra
Pseudo-nitzschia australis
Paralia sulcata
Rhizosolenia setigera
Thalassiosira punctigera
Analyst codes
Z-Scores
PROLab Plus

ANNEX XI: Summary of laboratory means

Summary of laboratory means

Sample 002

	Chaetoceros diadema	Z score	Heterocapsa triquetra	Z score	Pseudo-nitzschia australis	Z score	Paralia sulcata	Z score	Rhizosolenia setigera	Z score	Thalassiosira punctigera	Z score
Unit	cells/Litre		cells/Litre		cells/Litre		cells/Litre		cells/Litre		cells/Litre	
1	2080	0.37	520	-0.45	13573	-0.59	10813	0.75	13613	0.05	4187	0.86
2	1200	-0.81	933	0.45	29900	1.10	9867	0.33	15867	0.87	1633	-0.98
3	1240	-0.76	613	-0.25	28200	0.92	7680	-0.65	15733	0.82	2920	-0.06
4	3133	1.78	433	-0.64	9700	-0.99	10200	0.47	15100	0.59	4167	0.84
5	1867	0.08	817	0.19	14967	-0.45	8250	-0.39	14450	0.36	2517	-0.35
6	2848	1.40	951	0.48	15848	-0.36	11562	1.08	15106	0.59	5064	1.49
7	2053	0.33	40	-1.49	27827	0.88	9640	0.23	12160	-0.47	3467	0.34
8	2280	0.64	813	0.19	8587	-1.11	8560	-0.26	15933	0.89	3013	0.01
9	2453	0.87	347	-0.83	12707	-0.68	9373	0.11	14600	0.41	3507	0.37
10	1387	-0.56	987	0.56	21227	0.20	8120	-0.45	13680	0.08	1707	-0.93
11	733	-1.44	467	-0.57	16633	-0.27	10033	0.40	12956	-0.18	3000	0.00
12	1080	-0.97	867	0.30	20133	0.09	12800	1.63	13747	0.10	3213	0.16
13	1800	-0.01	467	-0.57	14400	-0.51	9507	0.17	13773	0.11	4253	0.90
14	976	-1.11	920	0.42	20560	0.13	10533	0.62	13760	0.11	2067	-0.67
15	2000	0.26	813	0.19	31547	1.27	9653	0.23	15280	0.66	6960	2.85 E
16	3333	2.05 E	< 0		32833	1.40	9333	0.09	13000	-0.17	8833	4.20 E
17	1480	-0.43	560	-0.36	16480	-0.29	7987	-0.51	15013	0.56	3040	0.03
18	2809	1.35	1176	0.97	28714	0.97	8804	-0.15	15958	0.90	2270	-0.52
19	2360	0.75	1133	0.88	9253	-1.04	11400	1.01	n/a		6080	2.22 E
20	2080	0.37	1213	1.05	20627	0.14	8627	-0.23	14307	0.30	3627	0.45
21	1240	-0.76	240	-1.06	40520	2.20 E	7573	-0.70	15213	0.63	3440	0.32
22	2389	0.79	1411	1.48	12567	-0.70	9478	0.15	15550	0.75	3628	0.45
23	1013	-1.06	200	-1.15	38012	1.94	9160	0.01	20060	2.38 E	2827	-0.12
24	700	-1.48	633	-0.21	10367	-0.92	7967	-0.52	12444	-0.37	1800	-0.86

ANNEX XI

	Chaetoceros diadema	Z score		Heterocapsa triquetra	Z score		Pseudo-nitzschia australis	Z score		Paralia sulcata	Z score		Rhizosolenia setigera	Z score		Thalassiosira punctigera	Z score	
25	1693	-0.15		227	-1.09		17800	-0.15		8253	-0.39		14107	0.23		2107	-0.64	
26	1760	-0.06		960	0.50		24427	0.53		9640	0.23		8360	-1.84		2427	-0.41	
27	2347	0.73		80	-1.41		40267	2.17	E	13147	1.79		10907	-0.92		5920	2.10	E
28	3300	2.01	E	1133	0.88		13000	-0.65		13200	1.81		9367	-1.48		3167	0.12	
29	1800	-0.01		587	-0.31		18987	-0.03		12653	1.57		15600	0.77		2893	-0.07	
30	1493	-0.42		1147	0.91		23947	0.48		6133	-1.34		11573	-0.68		1013	-1.43	
31	2027	0.30		627	-0.22		40067	2.15	E	10333	0.53		7520	-2.15	E	6107	2.24	E
32	1600	-0.27		1147	0.91		43027	2.45	E	5627	-1.56		14733	0.46		6133	2.26	E
33	1317	-0.65		1333	1.31		40567	2.20	E	9050	-0.04		12450	-0.37		2867	-0.09	
34	1307	-0.67		< 0			19987	0.07		5560	-1.59		7800	-2.05	E	3680	0.49	
35	2067	0.35		400	-0.71		12547	-0.70		9720	0.26		14227	0.28		3653	0.47	
36	2760	1.28		733	0.01		12733	-0.68		9373	0.11		n/a			2533	-0.33	
37	1133	-0.90		67	-1.43		9707	-0.99		1960	-3.20	E	6267	-2.60	E	2640	-0.26	
38	947	-1.15		1040	0.68		53747	3.56	E	11640	1.12		12507	-0.35		4160	0.84	
39	2373	0.76		1013	0.62		21507	0.23		11053	0.86		8520	-1.79		2280	-0.52	
40	1920	0.16		360	-0.80		25953	0.69		11460	1.04		13187	-0.10		2320	-0.49	
41	987	-1.10		747	0.04		22227	0.30		8160	-0.43		12733	-0.26		1147	-1.33	
42	2400	0.80		1000	0.59		29400	1.05		8900	-0.10		17000	1.28		3500	0.36	
43	2560	1.01		2080	2.93	E	15173	-0.43		6267	-1.28		5280	-2.96	E	1987	-0.73	
44	1633	-0.23		833	0.23		7433	-1.23		9567	0.19		9017	-1.61		3400	0.29	
45	1667	-0.18		533	-0.42		46800	2.85	E	5767	-1.50		7700	-2.08	E	1267	-1.24	
46	2522	0.96		899	0.37		11377	-0.82		8971	-0.07		15609	0.78		3812	0.59	
47	2600	1.07		293	-0.94		11773	-0.78		6813	-1.04		9467	-1.44		1880	-0.80	
48	2027	0.30		520	-0.45		9973	-0.96		10267	0.50		15747	0.82		2107	-0.64	
49	2747	1.27		400	-0.71		10880	-0.87		7240	-0.84		9453	-1.45		2387	-0.44	
50	711	-1.47		356	-0.81		11045	-0.85		7933	-0.54		16011	0.92		9455	4.65	E
51	2450	0.87		183	-1.18		8250	-1.14		10267	0.50		14800	0.48		4433	1.03	
52	1928	0.17		899	0.37		11087	-0.85		11131	0.89		16175	0.98		2855	-0.10	
53	2213	0.55		67	-1.43		22480	0.33		8213	-0.41		15293	0.66		3200	0.15	
54	1293	-0.69		960	0.50		50685	3.25	E	9707	0.25		21221	2.80	E	2240	-0.54	

ANNEX XI

	Chaetoceros diadema	Z score	Heterocapsa triquetra	Z score	Pseudo-nitzschia australis	Z score	Paralia sulcata	Z score	Rhizosolenia setigera	Z score	Thalassiosira punctigera	Z score
55	1833	0.04	1267	1.17	24767	0.57	14500	2.39 E	16367	1.05	3833	0.60
56	880	-1.24	< 0		3227	-1.66	827	-3.70 E	3080	-3.75 E	573	-1.74
57	293	-2.03 E	< 0		18800	-0.05	4267	-2.17 E	12747	-0.26	1200	-1.29
58	2240	0.59	1160	0.94	33947	1.52	11213	0.93	13920	0.16	1280	-1.24
59	600	-1.62	253	-1.03	11467	-0.81	5027	-1.83	11227	-0.81	3000	0.00
60	< 0		1053	0.71	17413	-0.19	6680	-1.09	14920	0.53	893	-1.51
61	1173	-0.85	800	0.16	8587	-1.11	12053	1.30	13267	-0.07	2027	-0.70
62	1692	-0.15	1282	1.20	13486	-0.60	7859	-0.57	16269	1.01	3949	0.68
63	2115	0.42	1039	0.67	15153	-0.43	10051	0.41	14256	0.29	3026	0.02
64	1231	-0.77	282	-0.97	11089	-0.85	6987	-0.96	13807	0.12	2077	-0.66
–	–	– –	–	– –	–	– –	–	– –	–	– –	–	– –
Statistical method	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	
No. of laboratories that submitted results	64		64		64		64		64		64	
No. of participants (according to design)	64		64		64		64		64		64	
No. of laboratories with quantitative values	63		60		64		64		62		64	
Arithmetical mean	1795		721		20910		9090		13280		3185	
Median	1840		780		18280		9222		14100		2800	
Assigned value	1804		728		19291		9135		13464		2997	
Mean	1806		721		19838		9114		13503		3010	
Reference value	1804		728		19291		9135		13644		2997	
Target s.d.	745		461		9669		2243		2767		1390	
Reproducibility s.d.	890		444		10439		2834		2760		1427	
Repeatability s.d.	623		205		3812		1902		1069		548	
Rel. target s.d.	41.30 %		63.32 %		50.12 %		24.55 %		20.55 %		46.38 %	
Rel. reproducibility s.d.	49.34 %		60.97 %		54.11 %		31.03 %		20.50 %		47.61 %	
Rel. repeatability s.d.	34.56 %		28.12 %		19.76 %		20.82 %		7.94 %		18.27 %	

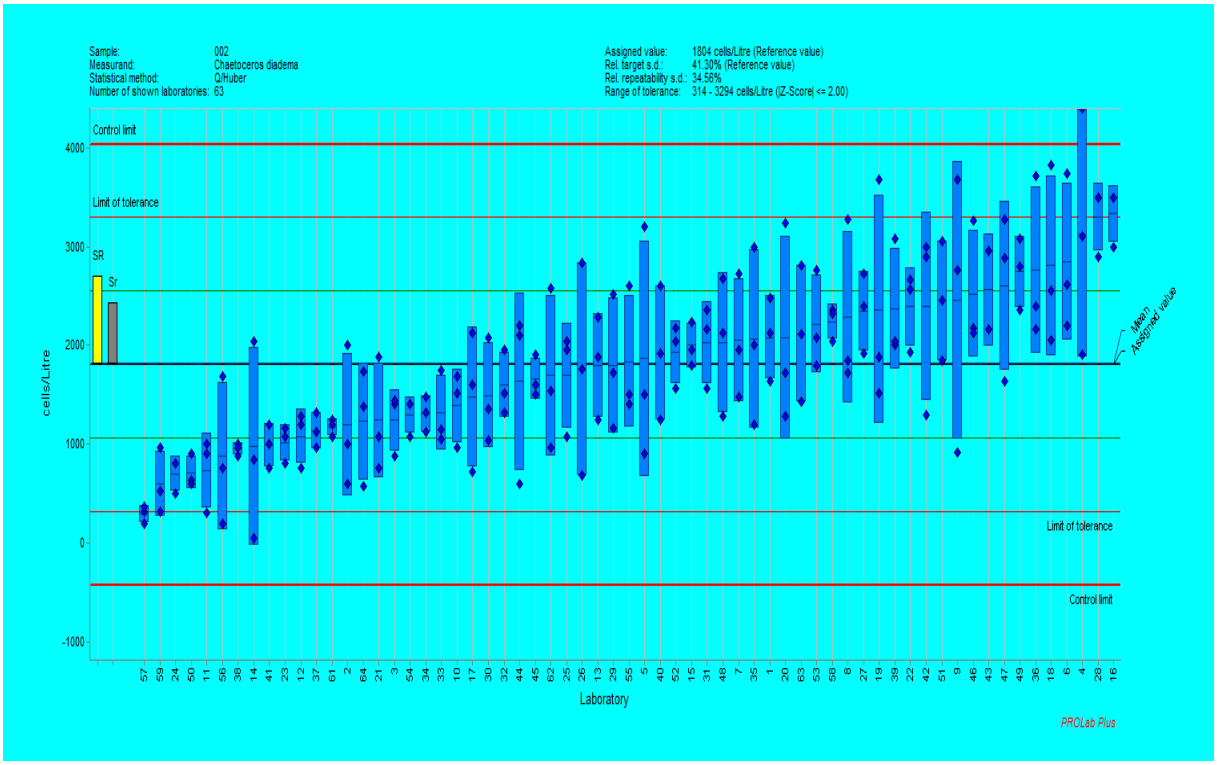
ANNEX XI

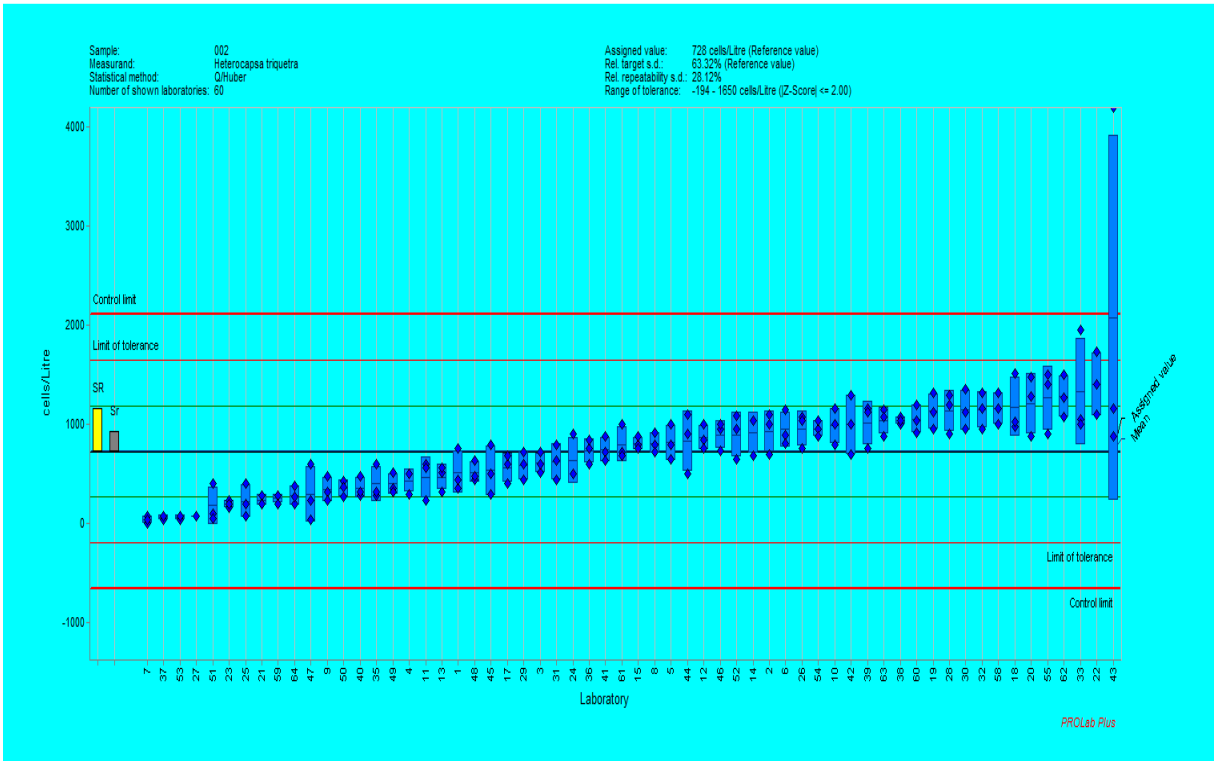
	Chaetoceros diadema Z score	Heterocapsa triquetra Z score	Pseudo-nitzschia australis Z score	Paralia sulcata Z score	Rhizosolenia setigera Z score	Thalassiosira punctigera Z score
Reference s.d.	745	461	9669	2243	2767	1390
Limit of reproducibility, R (2.80 X sR)	2492	1243	29229	7936	7727	3996
Limit of repeatability, r (2.80 X sr)	1745	573	10673	5326	2994	1533
Rel. limit of reproducibility	138.15 %	170.72 %	151.52 %	86.88 %	57.39 %	133.32 %
Rel. limit of repeatability	96.75 %	78.73 %	55.33 %	58.31 %	22.23 %	51.16 %
HORRAT	63.82	85.37	110.65	48.44	42.98	77.36
Absolute classical Horwitz s.d.	12	5	87	46	64	18
Relative classical Horwitz s.d.	0.65 %	0.74 %	0.45 %	0.51 %	0.48 %	0.60 %
Lower limit of tolerance	314	-194	-47	4649	7930	217
Upper limit of tolerance	3294	1650	38629	13621	18998	5777
Standard error	140	72	1631	443	438	223
Type F outliers	0	0	0	0	0	0
No. of laboratories after elimination of outliers type A-I except E (without laboratories that only gave states but no measured values)	63	60	64	64	62	64
Number of laboratories with replicates outside of tolerance limits	12	3	10	17	12	8
Number of laboratories with mean outside of tolerance limits	3	1	8	4	8	7
No. of measurement values and states	64	64	64	64	64	64
No. of measurement values	188	177	192	192	186	192

ANNEX XI

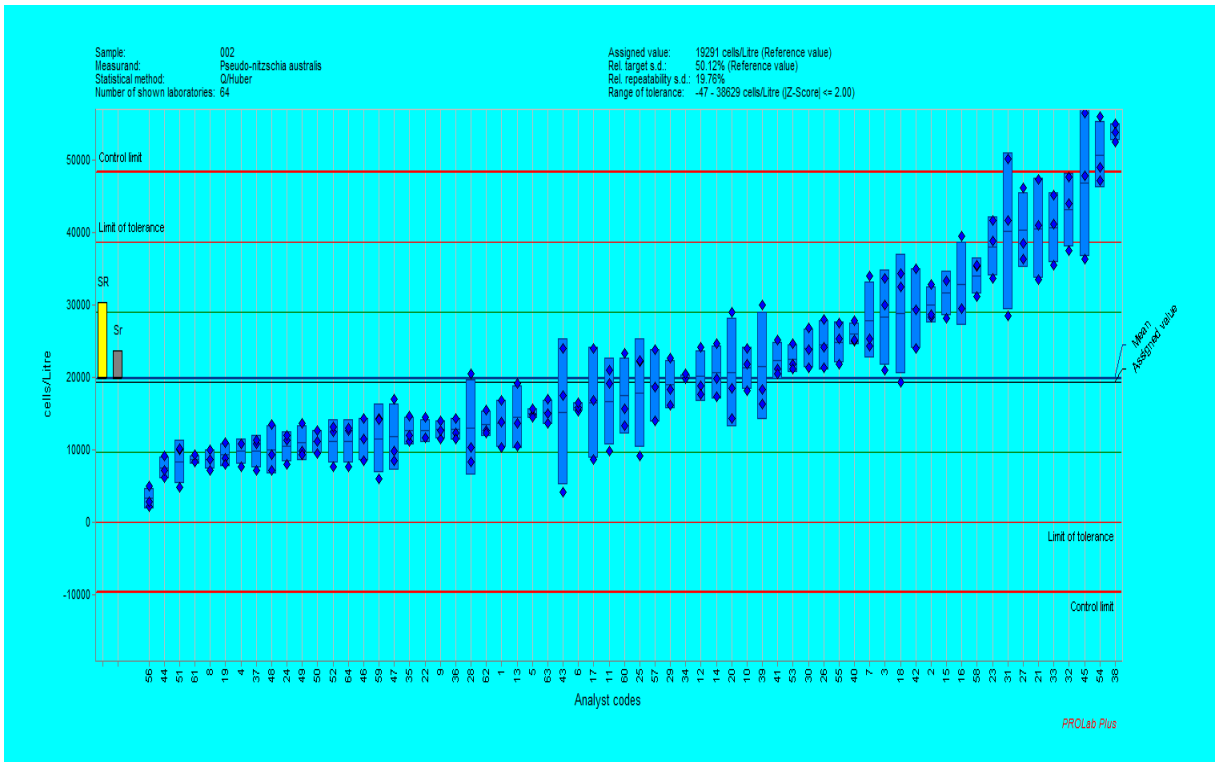
	Chaetoceros diadema Z score	Heterocapsa triquetra Z score	Pseudo-nitzschia australis Z score	Paralia sulcata Z score	Rhizosolenia setigera Z score	Thalassiosira punctigera Z score
No. of measurement values without outliers	188	177	192	192	186	192
Explanation of outlier types						
A: Single outlier	Grubbs					
B: Differing laboratory mean	Grubbs					
C: Excessive laboratory s.d.	Cochran					
D: Excluded manually						
E: score outside tolerance limits						

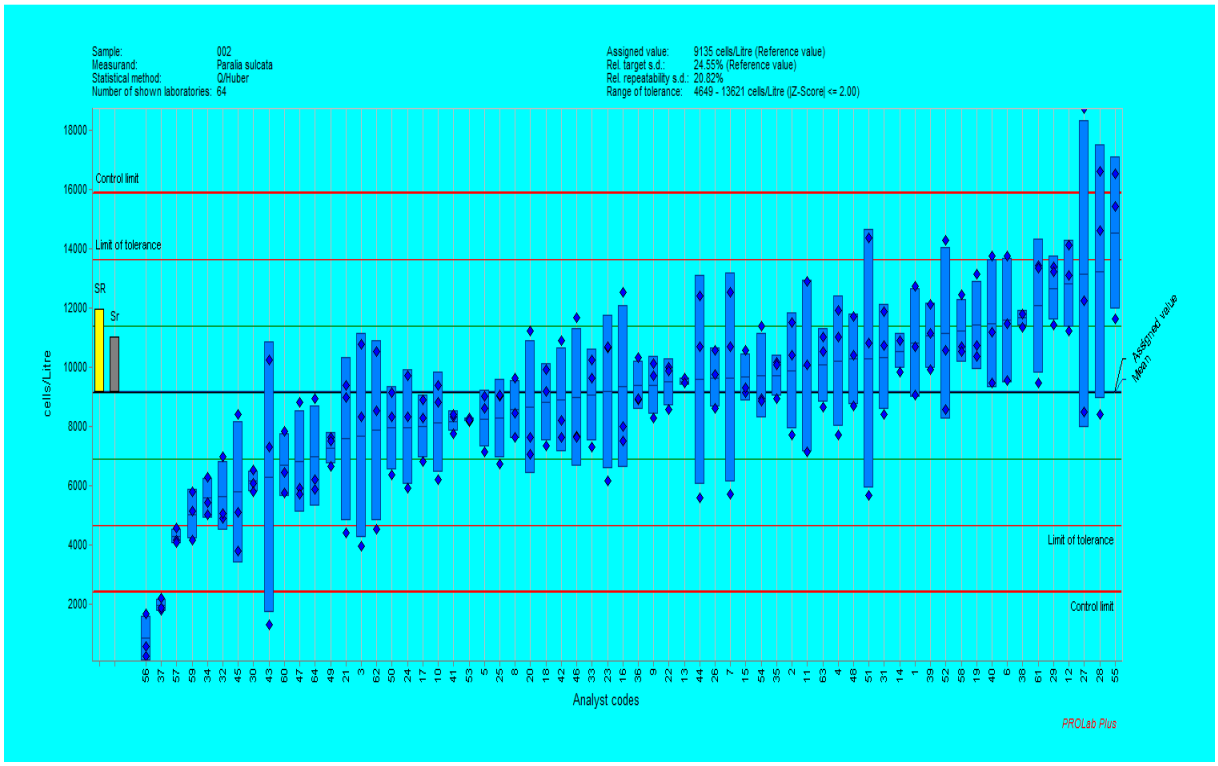
ANNEX XII: Graphical summary of results cells/L by analysts



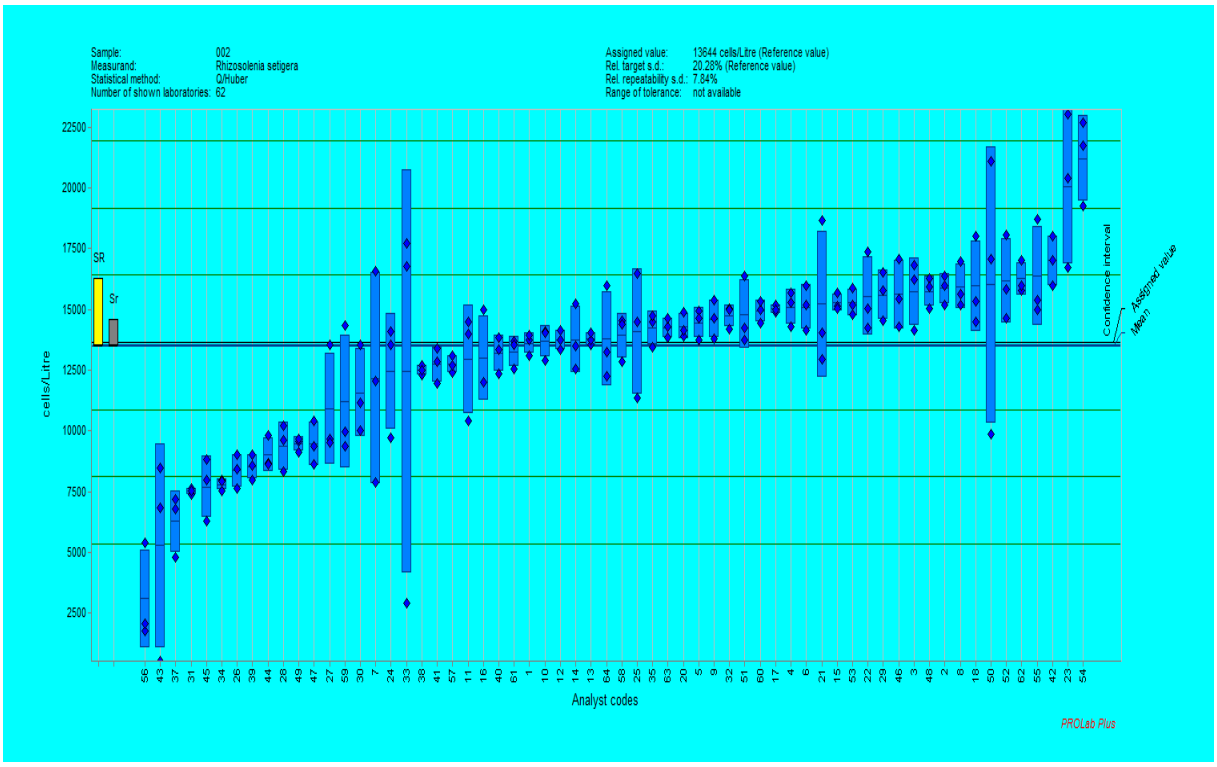


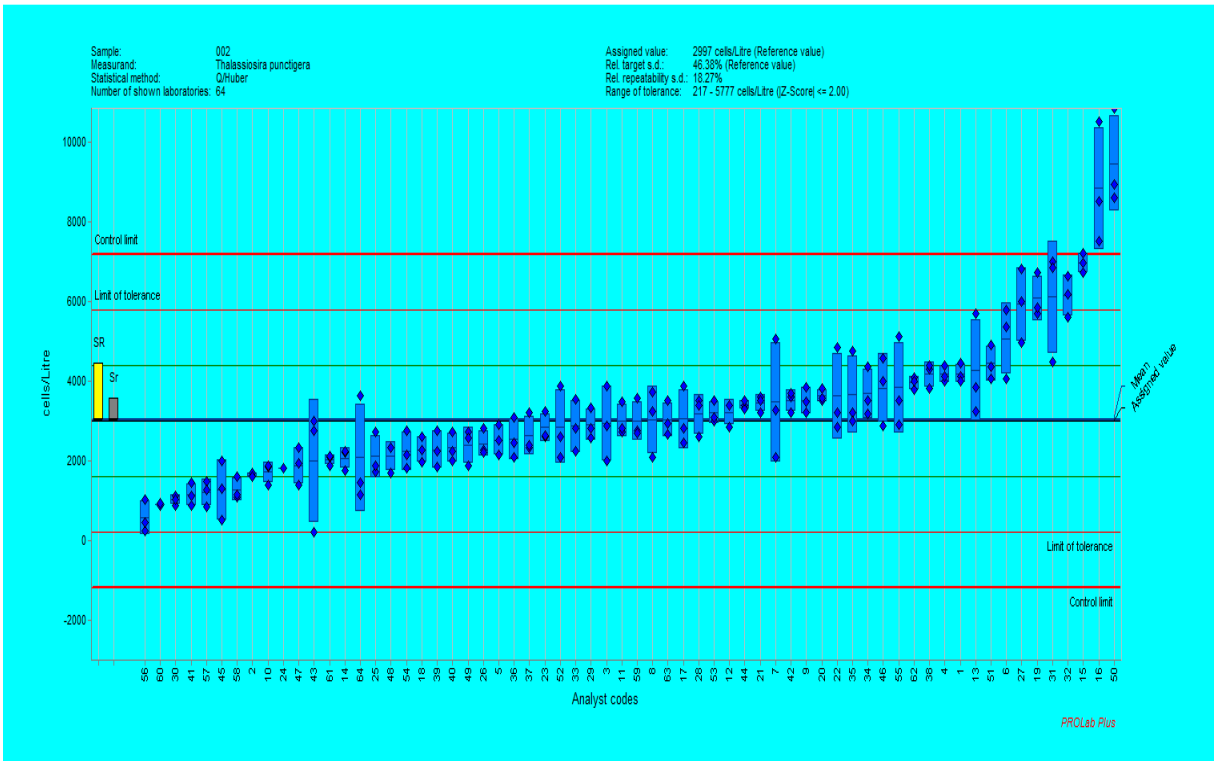
ANNEX XII


Sample: 002
Measurand: Pseudo-nitzschia australis
Statistical method: Q/Huber
Number of shown laboratories: 64
Assigned value: 19291 cells/Litre (Reference value)
Rel. target s.d.: 50.12% (Reference value)
Rel. repeatability s.d.: 19.76%
Range of tolerance: -47 - 38629 cells/Litre (|Z-Score| <= 2.00)
Control limit
Limit of tolerance
SR
Sr
Mean
Assigned value
Limit of tolerance
Control limit
cells/Litre
Analyst codes
PROLab Plus

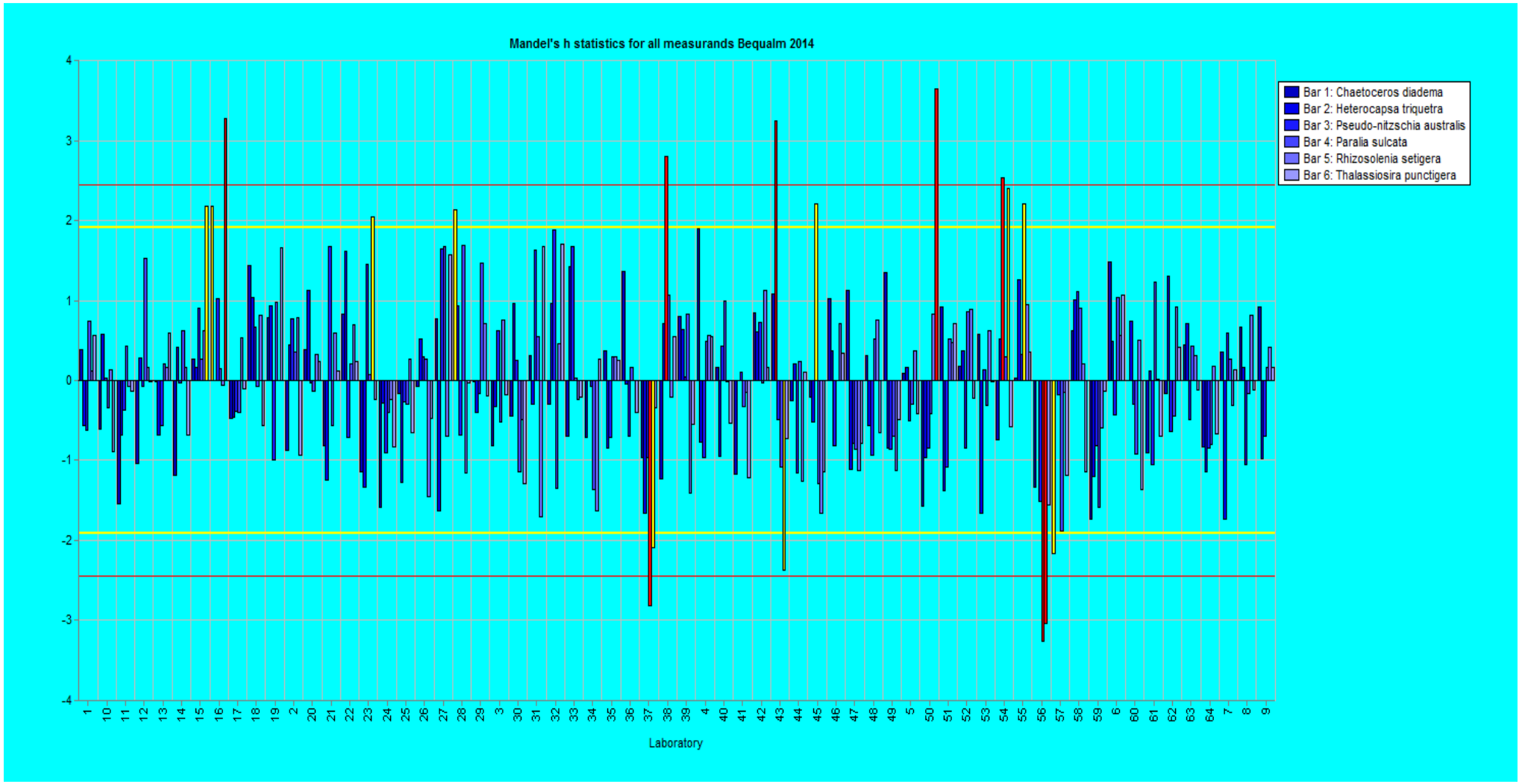

Sample: 002
Measurand: Paralia sulcata
Statistical method: Q/Huber
Number of shown laboratories: 64
Assigned value: 9135 cells/Litre (Reference value)
Rel. target s.d.: 24.55% (Reference value)
Rel. repeatability s.d.: 20.82%
Range of tolerance: 4649 - 13621 cells/Litre (|Z-Score| <= 2.00)
Control limit
Limit of tolerance
SR
Sr
Assigned value
Mean
Limit of tolerance
Control limit
cells/Litre
Analyst codes
PROLab Plus

ANNEX XII

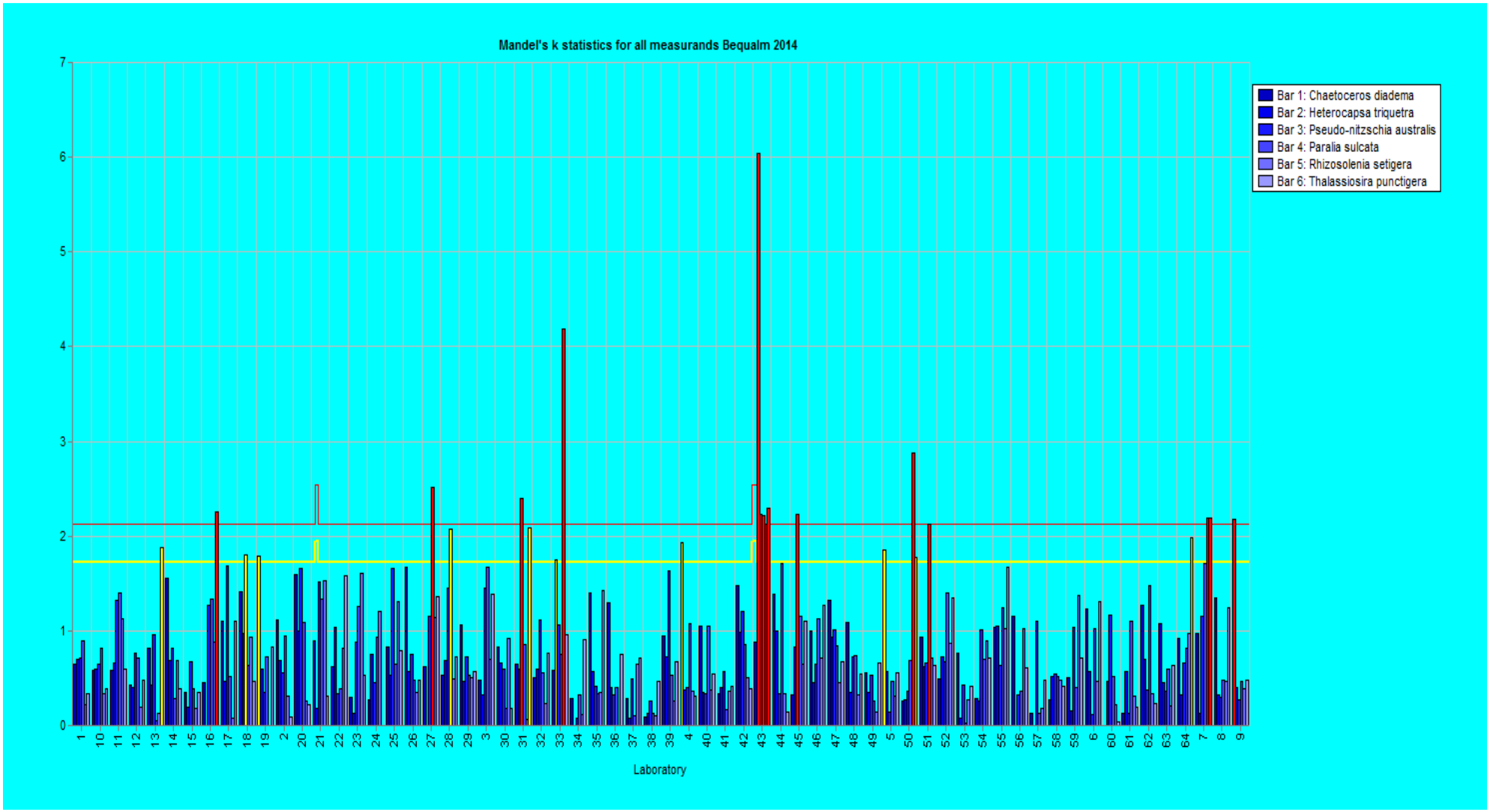

Sample: 002
Measurand: Rhizosolenia setigera
Statistical method: Q/Huber
Number of shown laboratories: 62
Assigned value: 13644 cells/Litre (Reference value)
Rel. target s.d.: 20.28% (Reference value)
Rel. repeatability s.d.: 7.84%
Range of tolerance: not available
cells/Litre
SR
Sr
Confidence interval
Assigned value
Mean
Analyst codes
PROLab Plus


Sample: 002
Measurand: Thalassiosira punctigera
Statistical method: Q/Huber
Number of shown laboratories: 64
Assigned value: 2997 cells/Litre (Reference value)
Rel. target s.d.: 46.38% (Reference value)
Rel. repeatability s.d.: 18.27%
Range of tolerance: 217 - 5777 cells/Litre (|Z-Score| <= 2.00)
cells/Litre
Control limit
Limit of tolerance
SR
Sr
Mean
Assigned value
Limit of tolerance
Control limit
Analyst codes
PROLab Plus

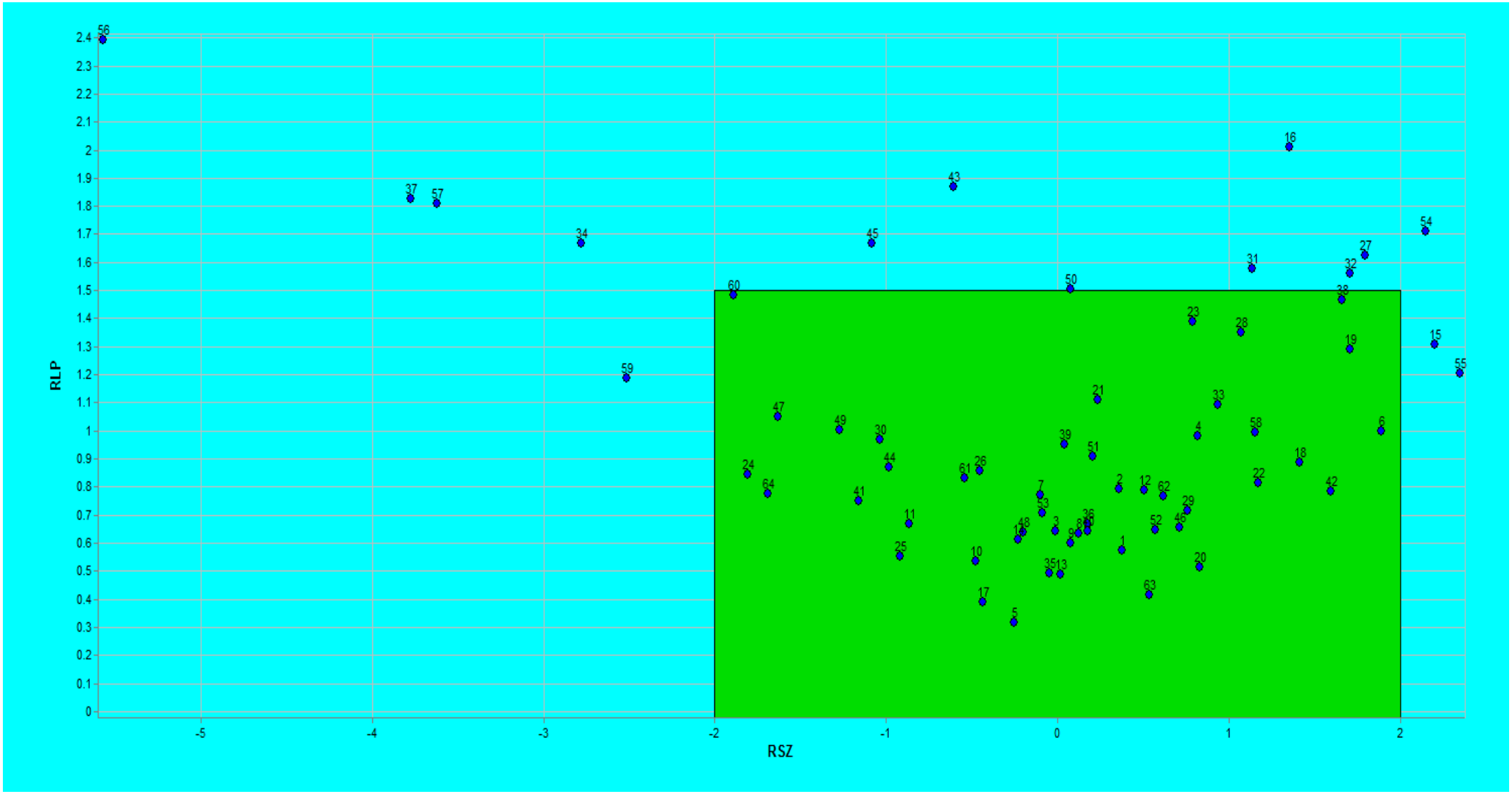
ANNEX XIII: Mandel's h and k statistics



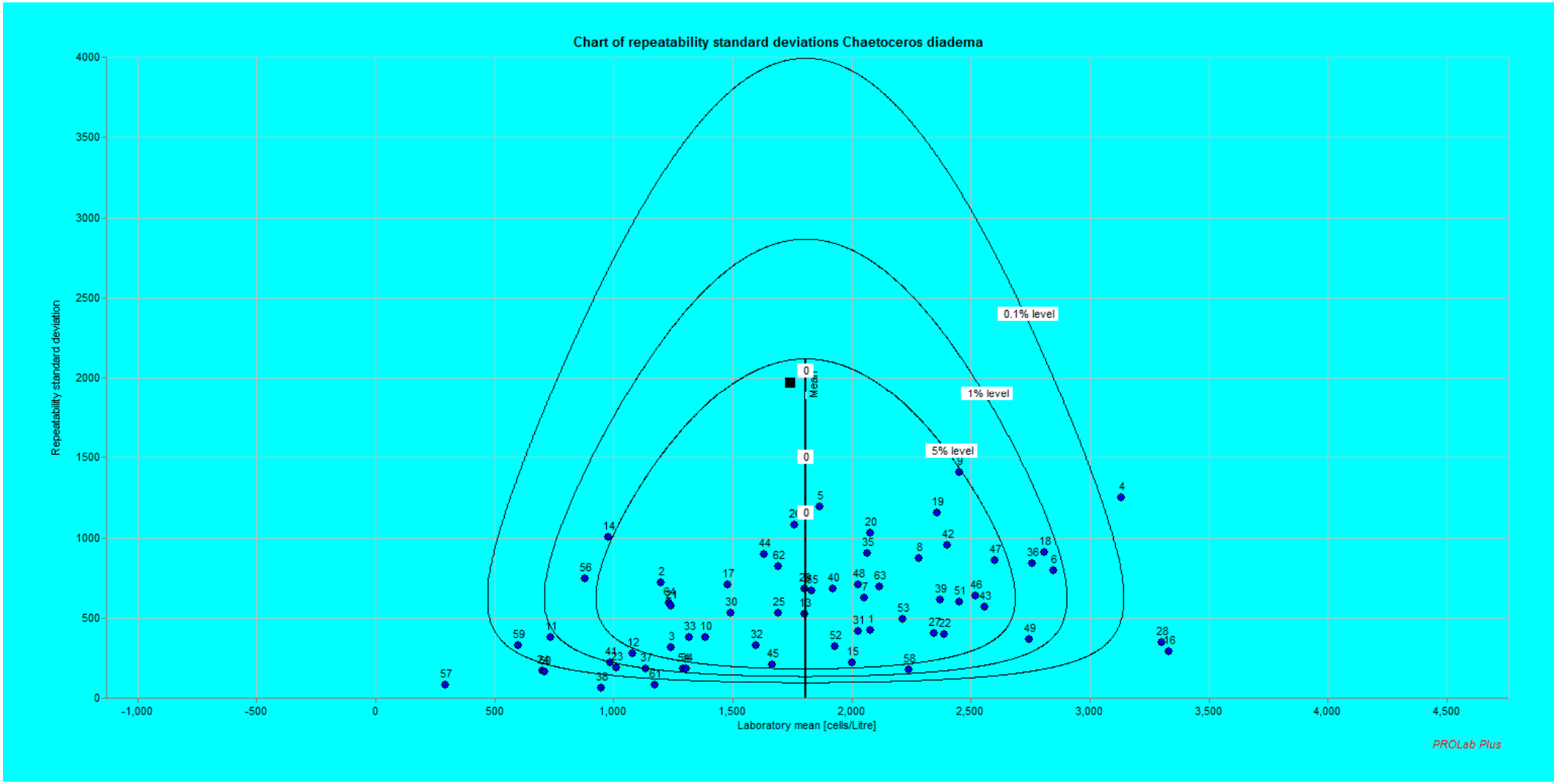
ANNEX XIII


Mandel's k statistics for all measurands Bequalm 2014
Bar 1: Chaetoceros diadema
Bar 2: Heterocapsa triquetra
Bar 3: Pseudo-nitzschia australis
Bar 4: Paralia sulcata
Bar 5: Rhizosolenia setigera
Bar 6: Thalassiosira punctigera
Laboratory

ANNEX XIV: RLP and RSZ for all measurands Bequalm 2014



ANNEX XV: Chart of repeatability standard deviations



ANNEX XV

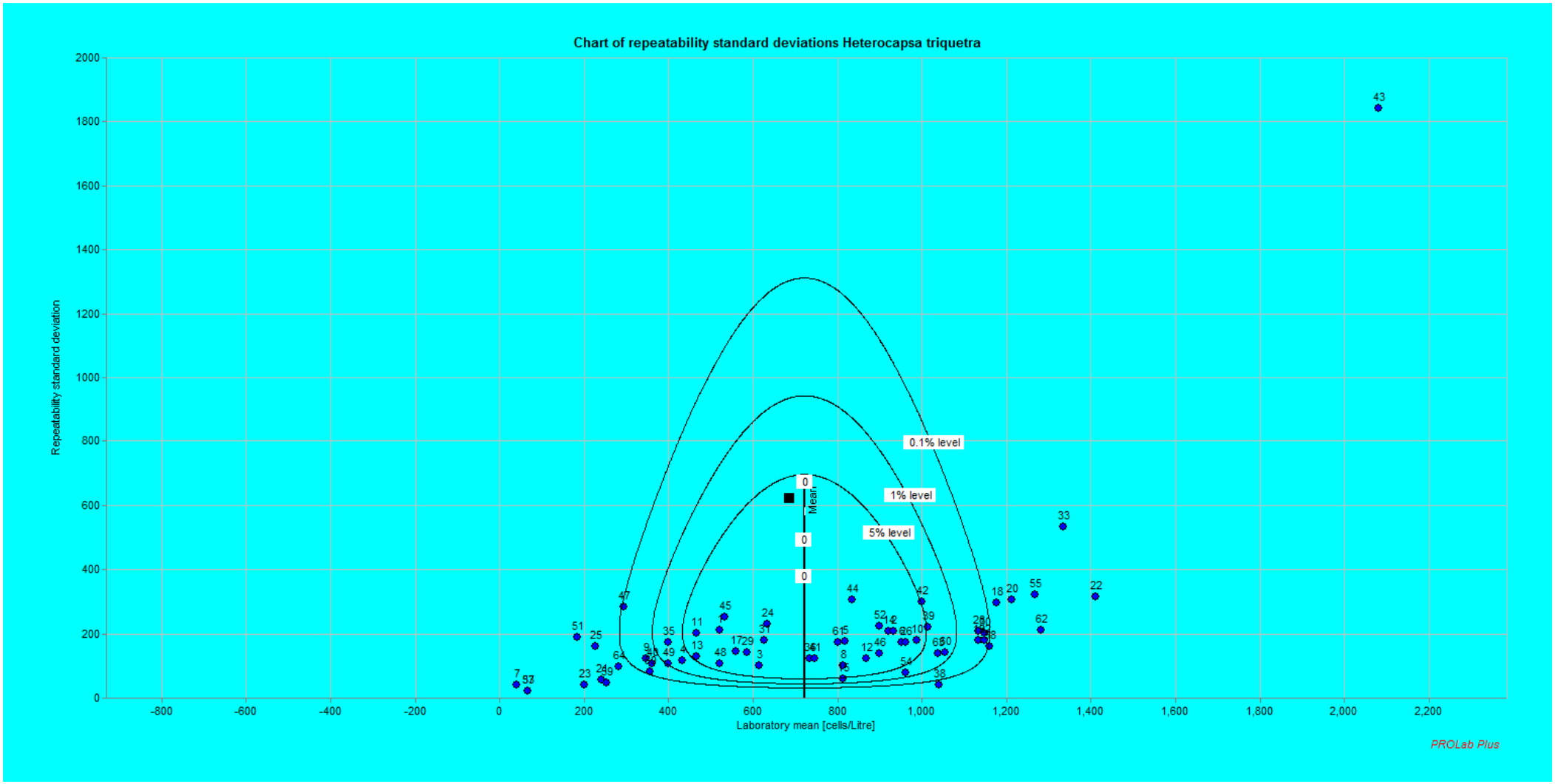


Chart of repeatability standard deviations Heterocapsa triquetra
Repeatability standard deviation
Laboratory mean [cells/Litre]
0.1% level
1% level
5% level
Mean
PROLab Plus

ANNEX XV

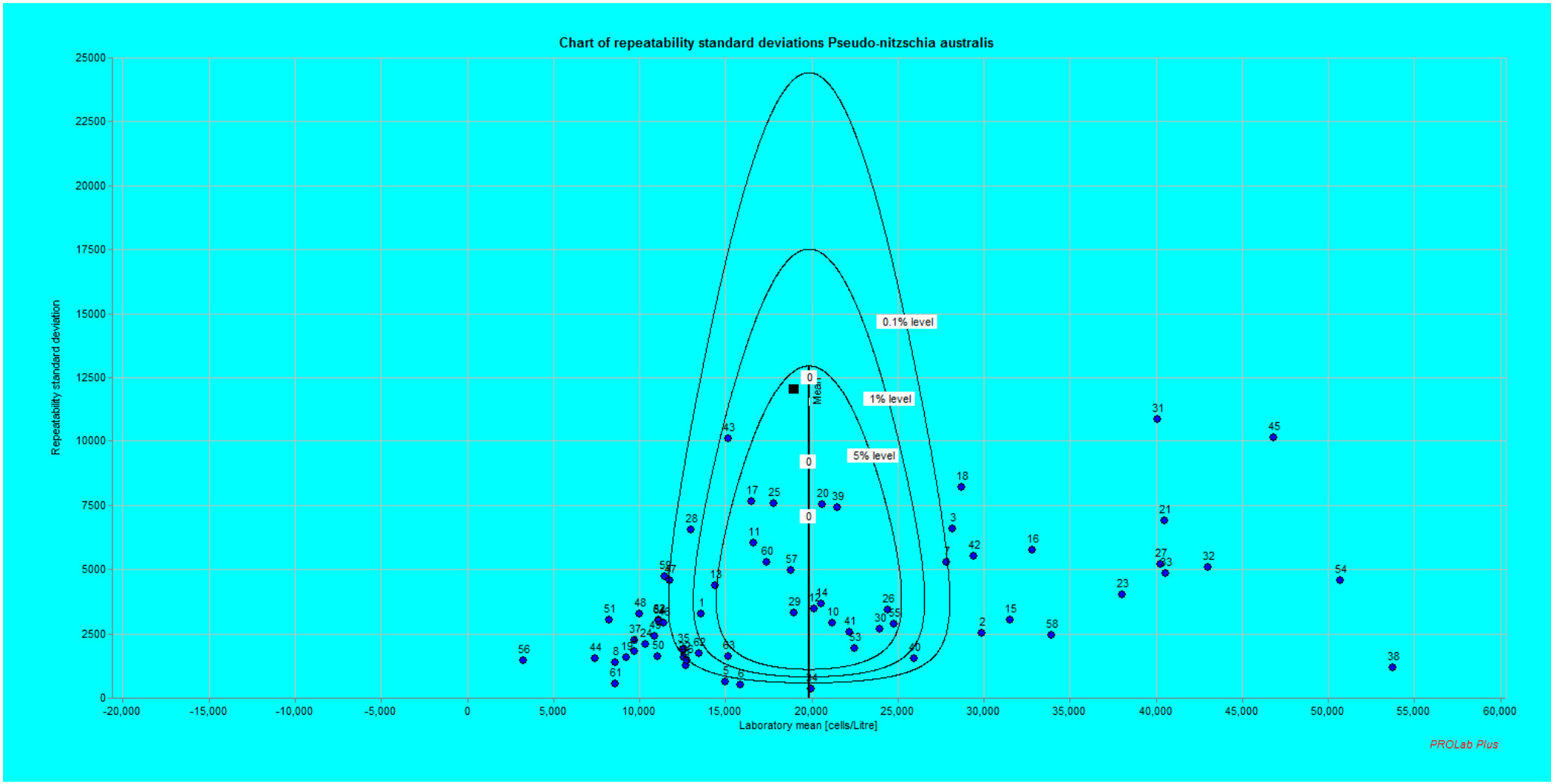


Chart of repeatability standard deviations Pseudo-nitzschia australis
Repeatability standard deviation
Laboratory mean [cells/Litre]
0.1% level
1% level
5% level
Mean
PROLab Plus

ANNEX XV

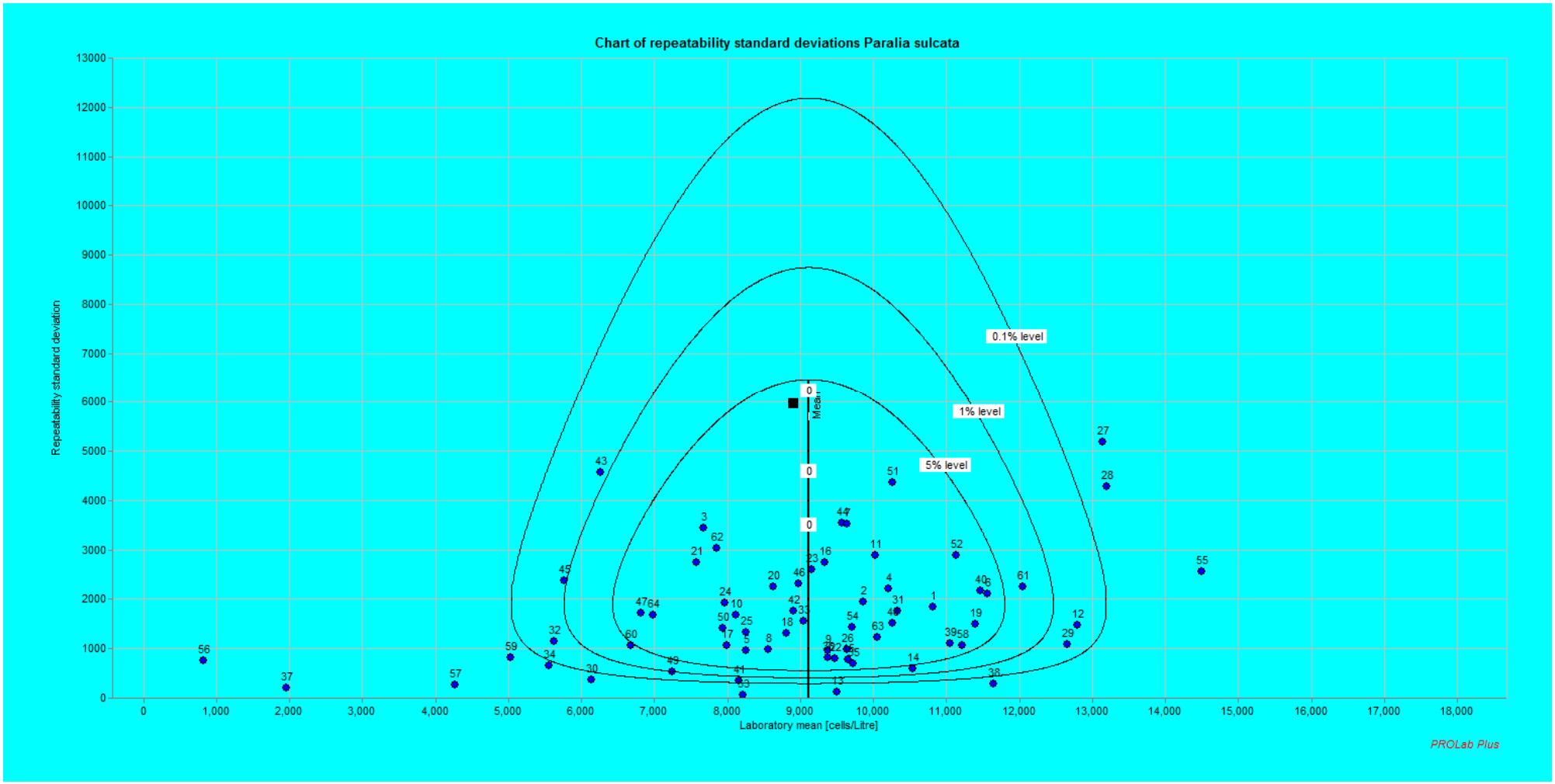


Chart of repeatability standard deviations Paralia sulcata
Repeatability standard deviation
Laboratory mean [cells/Litre]
0.1% level
1% level
5% level
Mean
0
PROLab Plus

ANNEX XV

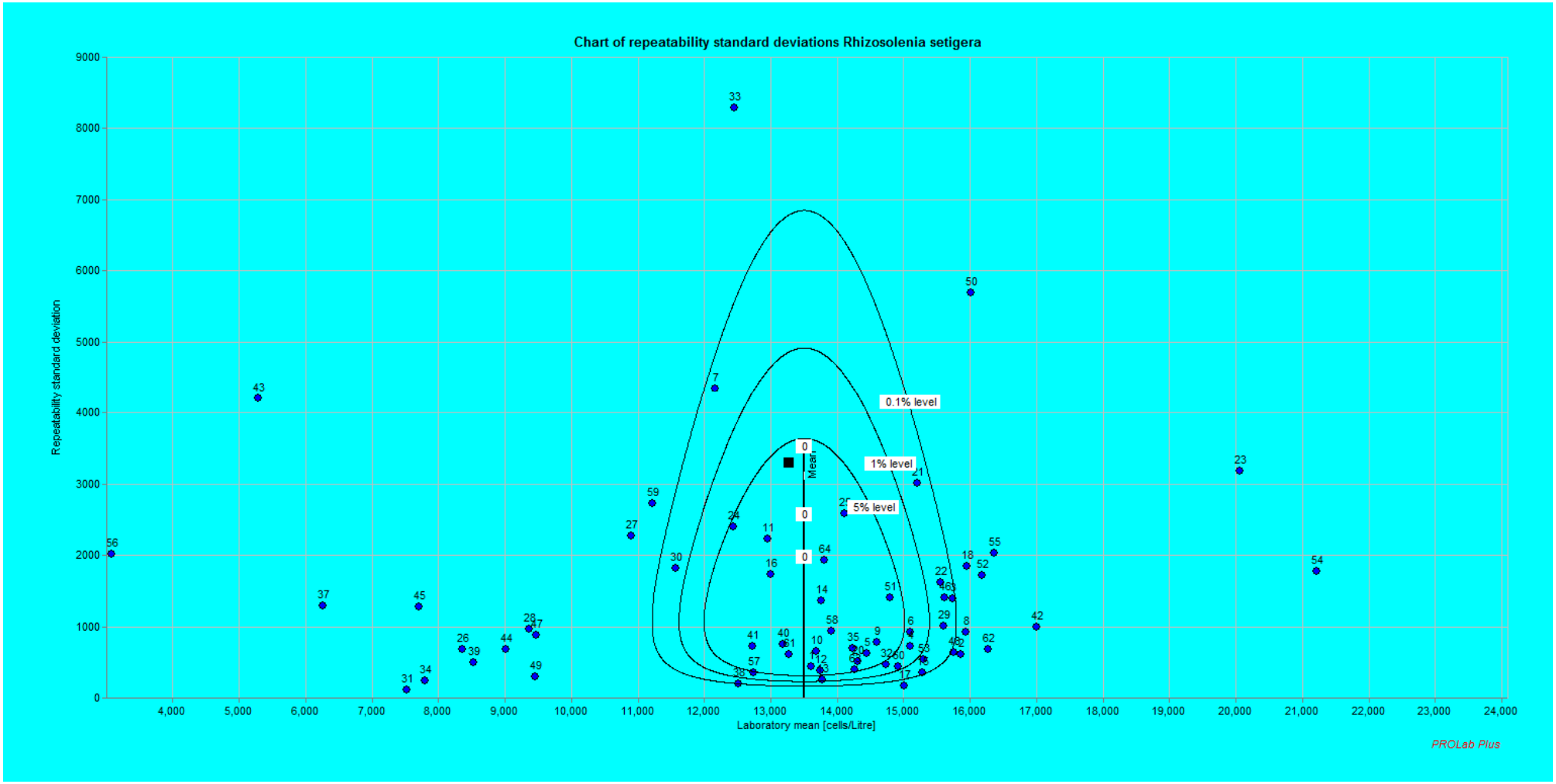


Chart of repeatability standard deviations Rhizosolenia setigera
Repeatability standard deviation
Laboratory mean [cells/Litre]
0.1% level
1% level
5% level
Mean
0
PROLab Plus

ANNEX XV

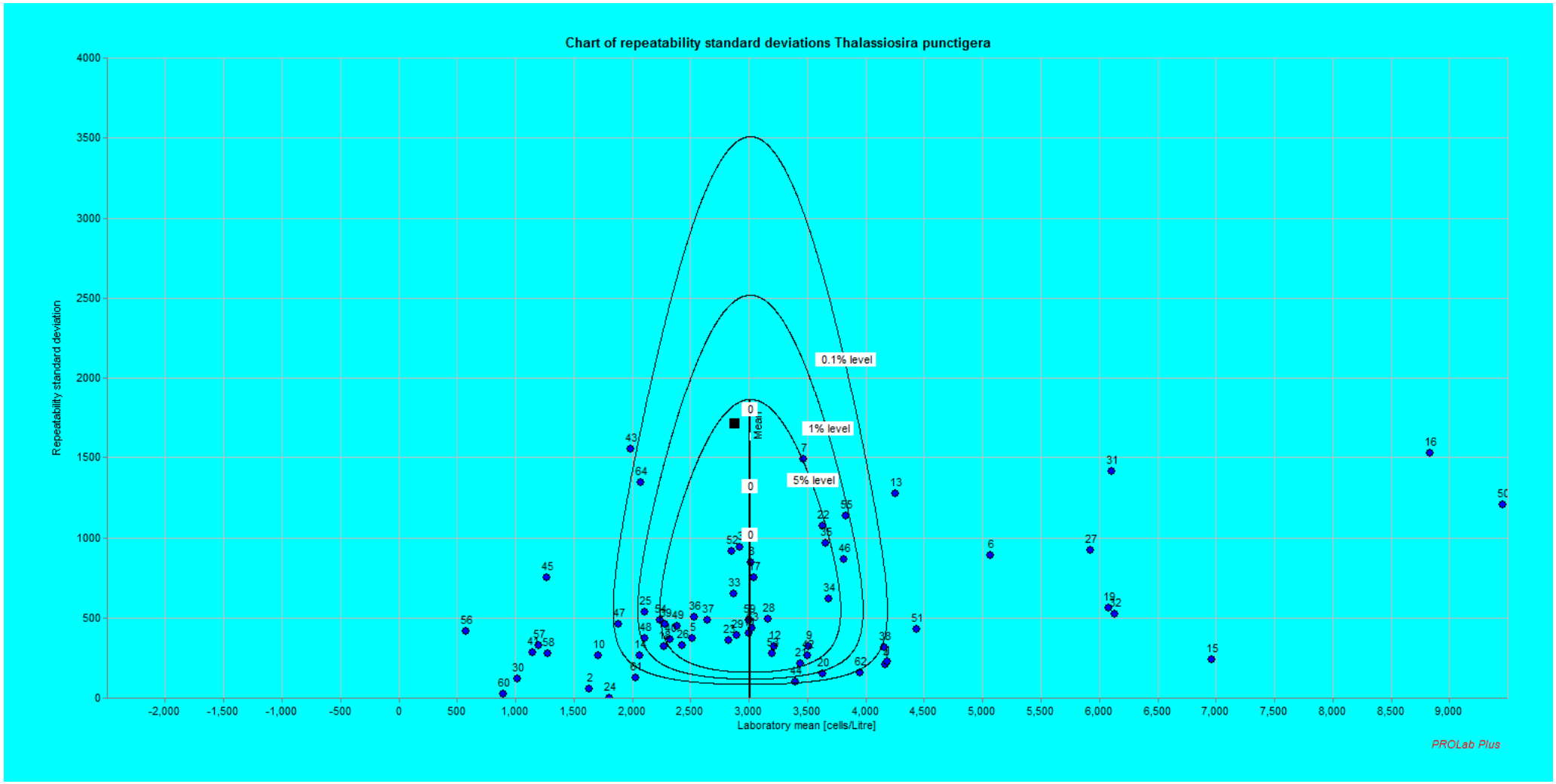
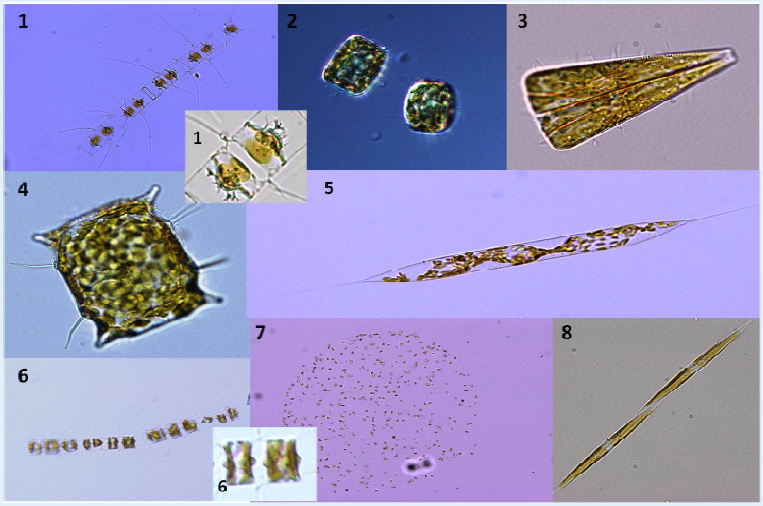


Chart of repeatability standard deviations Thalassiosira punctigera
Repeatability standard deviation
Laboratory mean [cells/Litre]
0.1% level
1% level
5% level
Mean
PROLab Plus

ANNEX XVI: Ocean Teacher HAB Quiz

Question 1
Correct
Mark 1.0 out of 1.0
Edit question

Match the following species:



2	Thalassiosira sp.
1	Chaetoceros diadema
4	Odontella sp.
6	Chaetoceros didymus
5	Rhizosolenia sp.
3	Licmophora sp.
8	Pseudo-nitzschia sp.
7	Phaeocystis sp.

Question 2
Correct
Mark 1.0 out of 1.0
Edit question

Enumerate the number of viable cells in this diatom chain: (Enter numerals not words. for example: use 5 not five)

Answer: 13

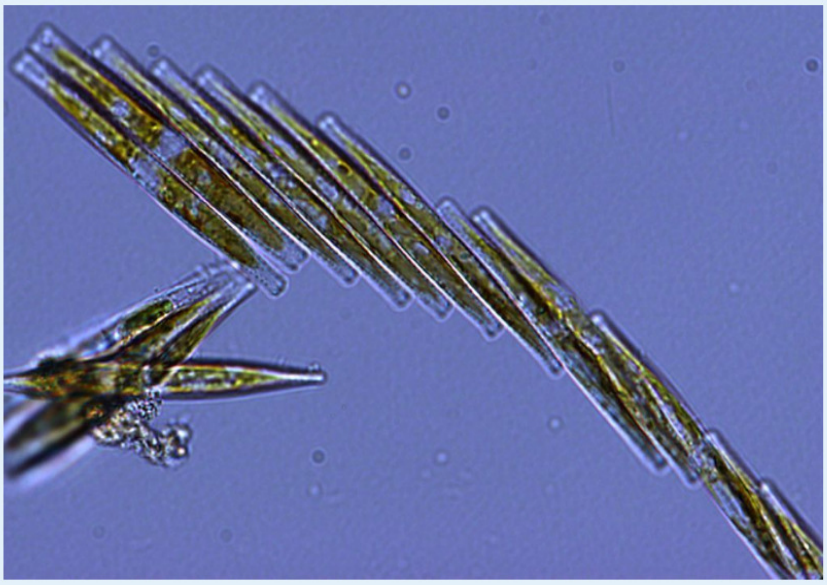
The correct answer is: 13

ANNEX XVI

Question 3
Correct
Mark 1.0 out of 1.0
Edit question

Enumerate the number of viable cells in these diatom chains (count even partially visible cells in the image): (Enter numerals not words. for example: 5 not five)

Please note: Change browser zoom level to see the full image first if necessary

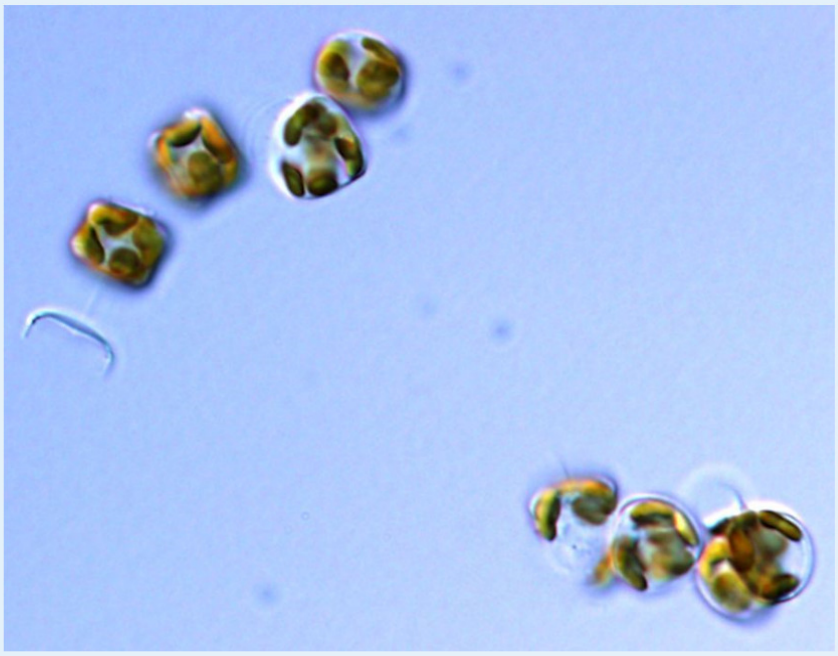


Answer: 16

The correct answer is: 16

Question 4
Correct
Mark 1.0 out of 1.0
Edit question

Enumerate the viable cells in these diatom chains. (Enter numerals not words. for example: 5 not five)



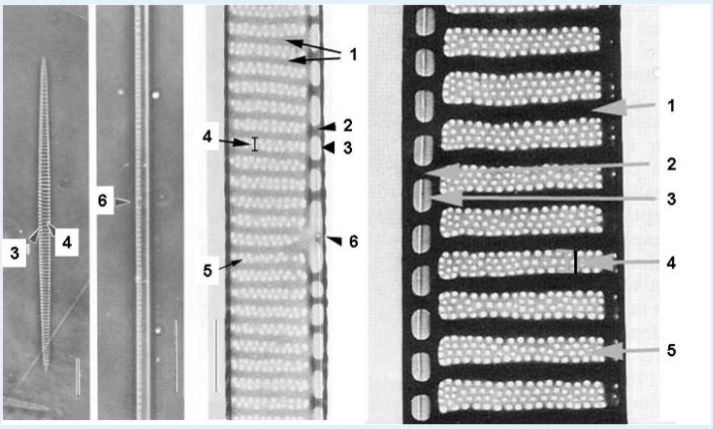
Answer: 8

The correct answer is: 8

ANNEX XVI

Question 5
Correct
Mark 1.0 out of 1.0
Edit question

Name the features indicated by the arrows

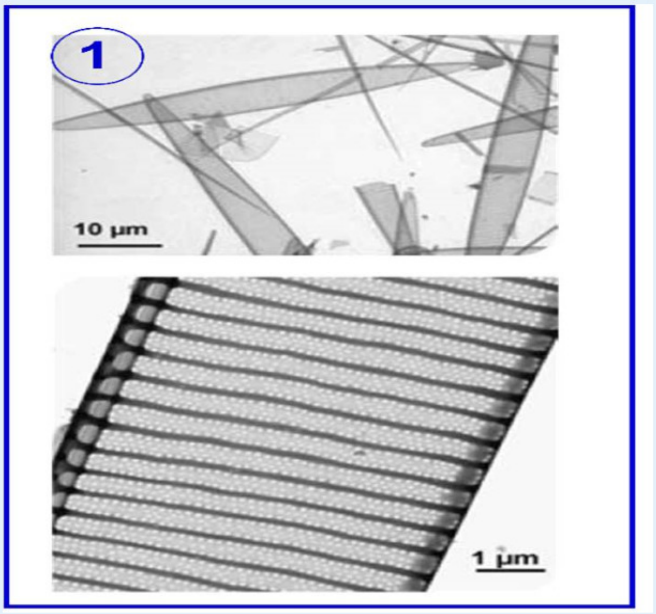


Arrow 4 points to: Stria
Arrow 1 points to: Interstria
Arrow 5 points to: Poroid
Arrow 6 points to: Central interspace
Arrow head 3 points to: Raphe slit
Arrow head 2 points to: Fibula

The correct answer is: Arrow 4 points to – Stria, Arrow 1 points to – Interstria, Arrow 5 points to – Poroid, Arrow 6 points to – Central interspace, Arrow head 3 points to – Raphe slit, Arrow head 2 points to – Fibula

Question 6
Correct
Mark 1.0 out of 1.0
Edit question

Identify this species of *Pseudo-nitzschia* (please, note that the answer has to follow the format: P. xxxx and the species names have to be spelled correctly for the programme to recognize the correct answer)



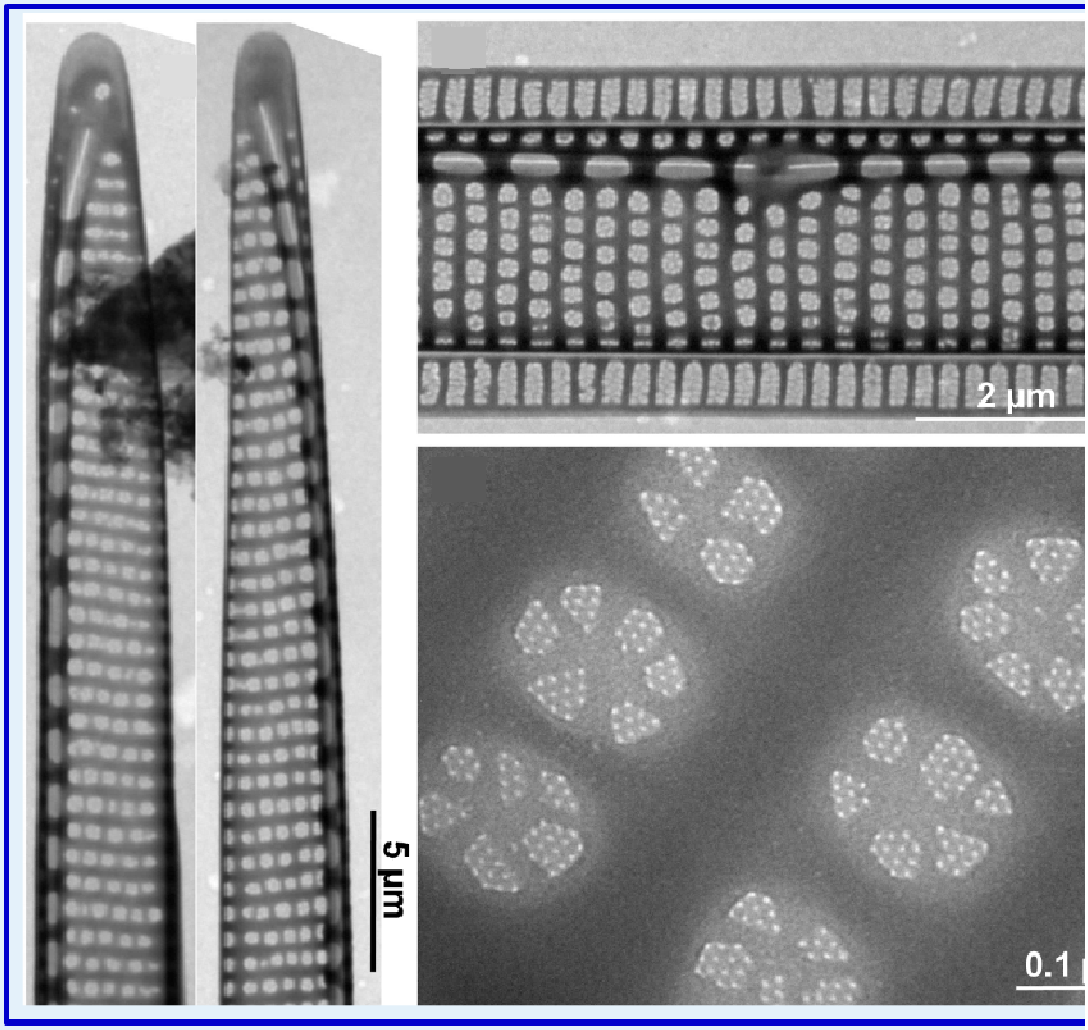
Answer: P. seriata

Correct
The correct answer is: P. seriata

ANNEX XVI

Question 7
Correct
Mark 1.0 out of 1.0
Edit question

Identify the species of *Pseudo-nitzschia* illustrated below (please, note that the answer has to follow the format: P. xxxx and the species name has to be spelled correctly for the programme to recognize the correct answer).



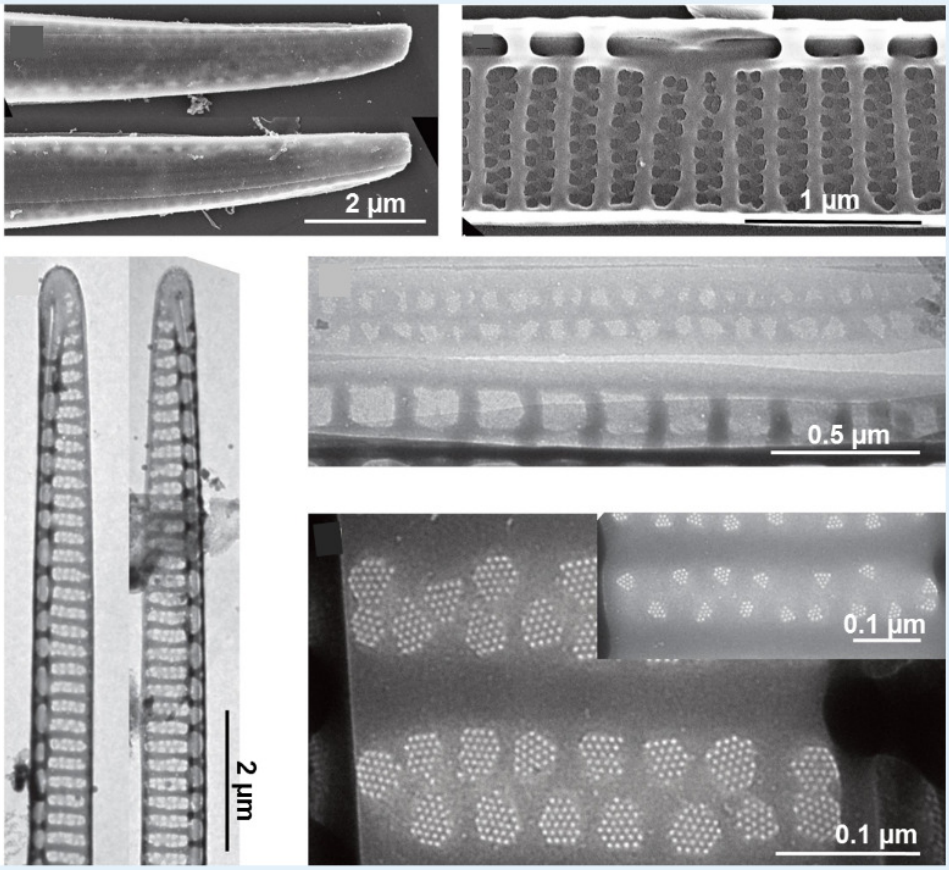
Answer: P. calliantha

Correct
The correct answer is: P. calliantha

ANNEX XVI

Question 8
Correct
Mark 1.0 out of 1.0
Edit question

Identify the species of *Pseudo-nitzschia* illustrated below (please, note that the answer has to follow the format: P. xxxx and the species name has to be spelled correctly for the programme to recognize the correct answer).



Answer: P. delicatissima

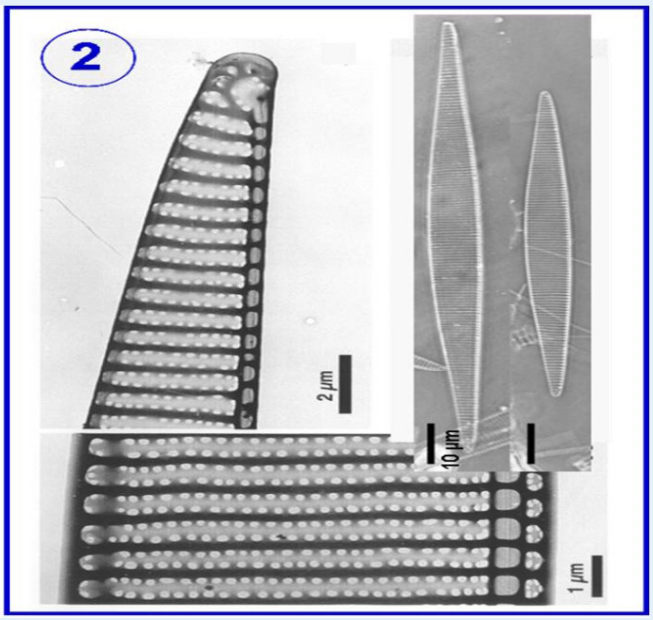
Correct

The correct answer is: P. delicatissima

ANNEX XVI

Question 9
Correct
Mark 1.0 out of 1.0
Edit question

Identify this species of *Pseudo-nitzschia* (please, note that the answer has to follow the format: P. xxxx and the species names have to be spelled correctly for the programme to recognize the correct answer)

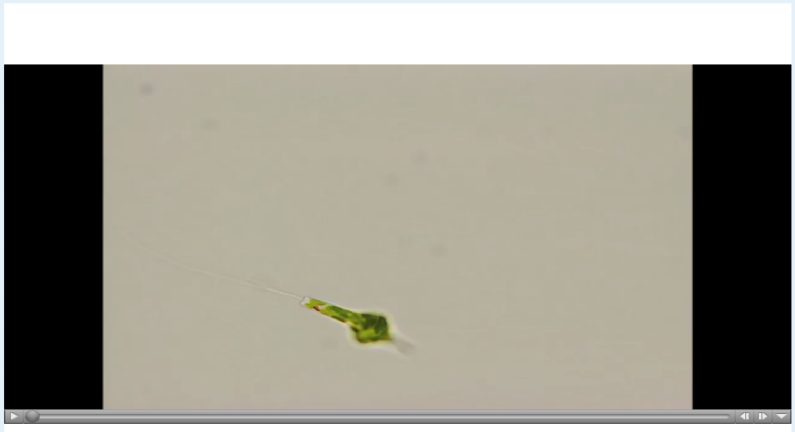


Answer: P. australis

Correct
The correct answer is: P. australis

Question 10
Correct
Mark 1.0 out of 1.0
Edit question

Watch this video (Click on the link). Identify the organism to genus level. (Correct grammar is essential in short answer questions) **Please note: Capitalise first letter of genus name and do not use full stops or other characters after the name.**



Answer: Eutreptiella

Eutreptiella has two unequal flagella
The correct answer is: Eutreptiella

ANNEX XVI

Question 11
Correct
Mark 1.0 out of 1.0
Edit question

Identification of species of Protoperidinium usually requires careful examination of the first apical plate (1') and the second anterior intercalary plate (2a). The different images show different types of plate configuration; name these configurations.

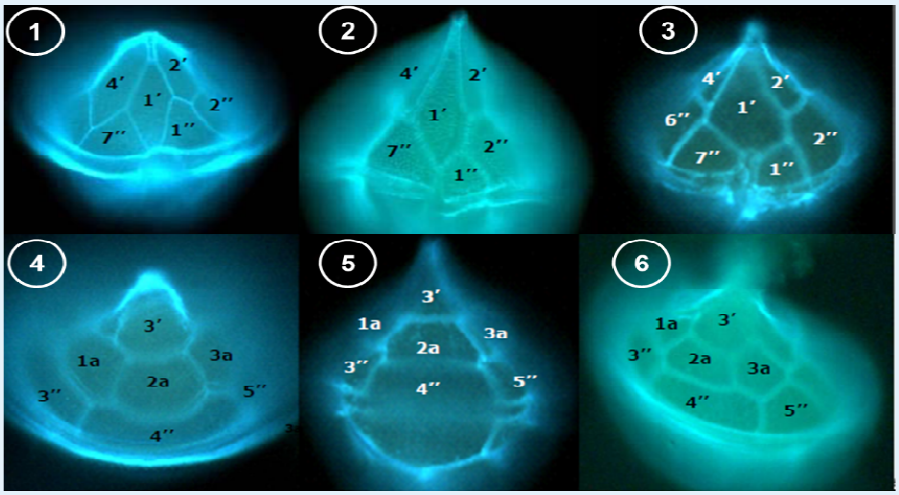
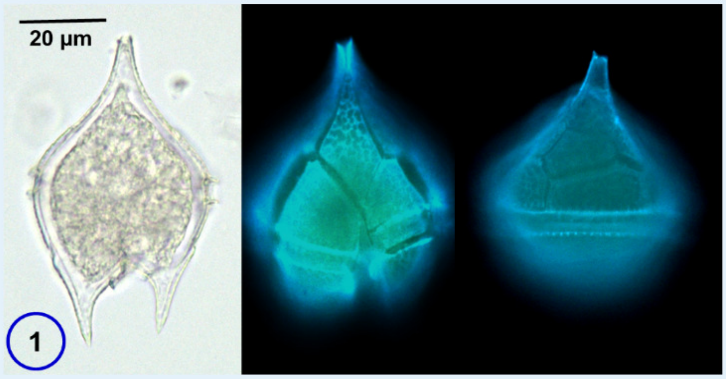
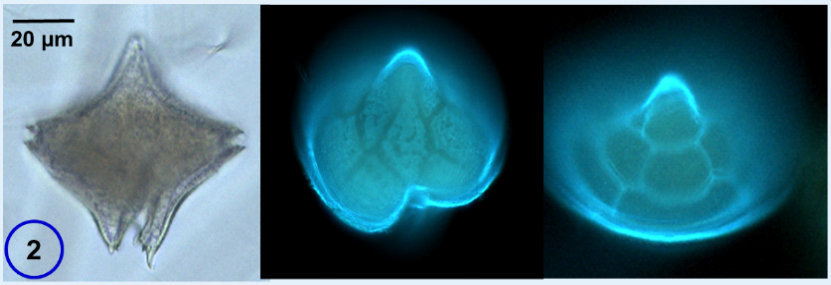


Fig.3 shows: 1' para configuration ✓
Fig.1 shows: 1' ortho configuration ✓
Fig.4 shows: 2a quadra configuration ✓
Fig.6 shows: 2a penta configuration ✓
Fig..2 shows: 1' meta configuration ✓
Fig.5 shows: 2a hexa configuration ✓

Question 12
Correct
Mark 1.0 out of 1.0
Edit question

Below are illustrated 2 different species of *Protoperidinium*; which species are illustrated?



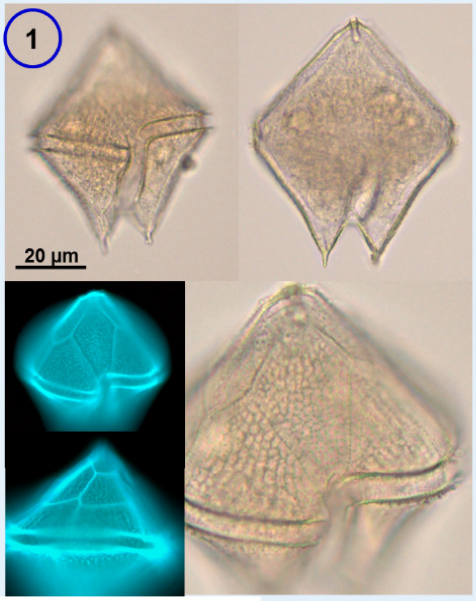


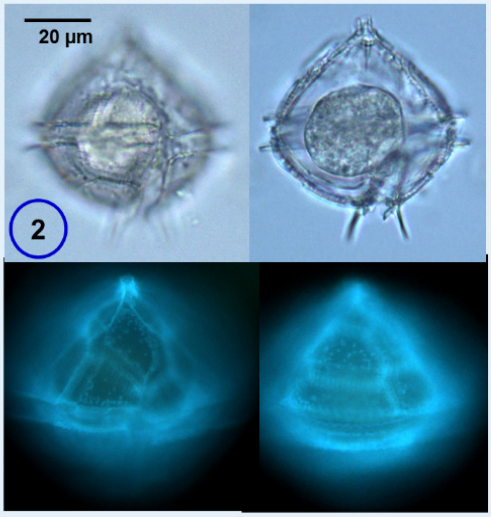
Species 1 is: P. claudicans ✓
Species 2 is: P. curtipes ✓

ANNEX XVI

Question 13
Correct
Mark 1.0 out of 1.0
Edit question

Below are illustrated 2 different species of *Protoperidinium*; which species are illustrated?





Species 2 is P. pellucidum ✓

Species 1 is P. leonis ✓

ANNEX XVII: HABs Oceanteacher quiz results

ANALYST CODE	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Grade
15	100	0	100	100	100	100	100	100	100	100	100	100	100	92.3
58	100	100	100	100	100	100	0	100	0	100	100	100	49	80.8
42	100	100	100	100	100	0	0	100	100	100	100	100	100	84.6
45	75	100	100	0	17	0	0	100	0	100	100	49	0	49.4
48	100	100	100	100	66	0	100	100	0	0	66	100	0	64.1
24	100	100	100	100	100	100	100	100	0	100	100	100	100	92.3
22	100	100	100	100	100	100	100	100	100	100	100	100	100	100
55	100	100	100	100	100	0	100	100	100	100	100	49	49	84.6
53	100	0	100	100	100	100	100	100	100	0	100	100	100	84.6
1	100	100	100	100	100	100	100	100	100	100	100	100	100	100
49	100	100	100	100	100	0	100	100	100	0	100	49	49	76.9
12	100	100	100	100	100	100	100	100	100	100	100	100	100	100
33	100	100	100	100	100	0	100	100	100	100	100	49	49	84.6
20	100	100	100	100	100	100	100	100	100	100	100	100	100	100
54	100	100	100	100	100	100	100	100	100	100	100	100	100	100
3	100	100	100	100	100	100	100	100	100	100	100	100	100	100
26	100	100	100	100	100	100	0	0	100	100	100	49	49	76.9
25	100	100	100	100	100	0	100	100	100	0	100	0	100	76.9
16	100	100	100	100	100	100	100	100	100	0	100	100	49	88.5
52	100	100	100	100	100	100	100	100	100	100	100	100	100	100
60	100	100	100	100	100	100	100	100	0	0	100	100	100	84.6
43	100	100	100	100	100	100	100	100	100	0	100	100	100	92.3
39	100	100	100	100	100	100	100	100	100	0	100	100	100	92.3
8	100	100	100	100	100	100	100	100	100	0	100	100	100	92.3
17	100	100	100	100	100	100	100	100	100	100	100	100	100	100
61	100	100	100	100	100	100	100	100	100	0	100	100	100	92.3
27	100	100	100	0	100	100	100	100	100	100	100	49	100	88.5
36	100	100	100	100	100	100	100	100	100	100	100	100	100	100
32	100	100	100	100	100	100	100	100	100	100	100	100	100	100
11	100	100	100	100	100	100	100	100	100	100	100	100	100	100
19	0	100	100	100	100	100	100	100	0	100	17	0	49	66.7
30	100	100	100	100	100	100	100	100	100	100	100	100	100	100
14	100	0	100	100	100	100	100	100	100	0	100	100	100	84.6

ANNEX XVII

ANALYST CODE	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Grade
2	100	100	100	100	100	0	100	100	100	100	100	49	100	88.5
21	100	100	100	100	66	0	100	100	100	100	66	100	100	87.2
34	100	100	100	100	66	0	100	100	100	100	100	100	100	89.7
47	75	0	0	0	100	0	0	100	0	0	100	49	0	32.7
50	100	100	100	0	100	100	0	0	100	100	100	100	100	76.9
7	100	100	100	100	100	100	0	0	100	0	100	100	100	76.9
6	100	100	100	100	100	100	100	100	100	100	100	100	100	100
10	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4	100	100	100	100	100	100	0	0	100	0	100	49	49	69.2
46	100	100	100	100	100	100	100	100	100	100	100	100	100	100
41	100	100	100	100	100	100	100	100	100	100	100	100	100	100
5	100	100	100	100	100	100	100	100	0	100	100	100	49	88.5
64	100	100	100	100	100	100	100	100	100	100	66	100	49	93.6
59	100	100	100	100	66	0	100	100	0	0	100	100	100	74.4
62	100	100	100	100	100	100	100	100	100	100	66	100	49	93.6
56	100	100	100	100	66	0	100	100	0	0	83	100	0	65.4
63	100	100	100	100	100	100	100	100	100	100	66	100	100	97.4
29	100	100	100	100	100	100	100	100	100	0	100	100	49	88.5
38	100	100	100	100	100	100	100	100	100	0	100	100	100	92.3
37	100	100	100	100	100	0	100	100	100	0	100	100	100	84.6
23	100	100	100	100	100	100	100	100	100	100	100	100	100	100
40	100	100	100	100	100	100	100	100	100	100	100	100	100	100
57	100	0	100	0	100	100	0	100	0	0	100	49	49	53.8
28	100	100	100	100	100	100	100	100	100	0	100	49	100	88.5
44	100	100	100	100	100	100	100	100	100	0	100	49	100	88.5
18	100	100	100	100	100	100	100	100	100	100	100	100	0	92.3
35	100	100	100	100	100	100	100	100	100	0	100	100	100	92.3
13	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	98	92	98	92	96	77	85	93	82	62	96	87	81	88