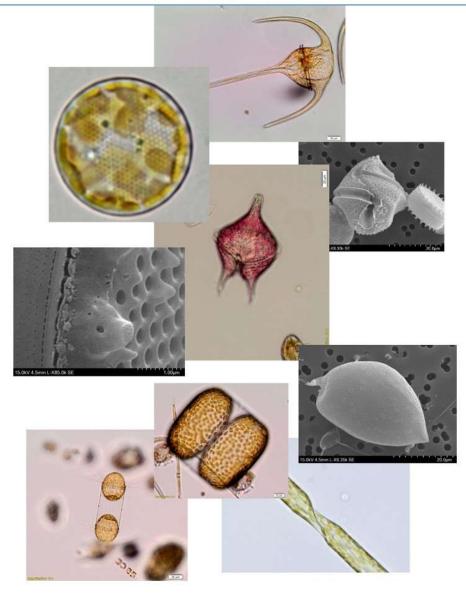


INTERNATIONAL PHYTOPLANKTON INTERCOMPARISON (IPI) Proficiency testing in the abundance and composition of marine microalgae 2019 report



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1. Summary of results

• In 2019, 98 analysts in 52 laboratories from across the world participated in the IPI intercomparison exercise. European countries accounted for 67% of the total participation, 19% from central and South America, 10 % from African countries and 4% from Oceania.

• Ten species were used in the IPI2019 test. There were five dinoflagellates and five diatoms in the samples. The dinoflagellates were Akashiwo sanguinea (K.Hirasaka) Gert Hansen & Moestrup, 2000, Prorocentrum micans Ehrenberg, 1834, Gonyaulax spinifera (Claparède & Lachmann) Diesing, 1866, Azadinium spinosum Elbrächter & Tillmann, 2009 and Heterosigma akashiwo (Y.Hada) Y.Hada ex Y.Hara & M.Chihara, 1987. Diatoms include Pseudo-nitzschia seriata complex (Cleve) H.Peragallo, 1899, Chaetoceros danicus Cleve, 1889, Corethron hystrix Hensen, 1887, Chaetoceros curvisetus Cleve, 1889 and Thalassiosira tenera Proschkina-Lavrenko, 1961.

• The robust average and confidence limits for each test item was calculated using the robust algorithm in annex C of ISO13528:2015 which takes into account the heterogeneity of the samples and the between samples standard deviation from the homogeneity and stability test. ISO 13528:2015 is only valid for quantitative data. We have used the consensus values from the participants.

• All measurands passed the expanded criterion for homogeneity according to ISO13528:2015 and the stability test according to ISO13528:2015.

• There were a very small number of warning and action signals across measurands. 18 Red flags (1.8%), 23 (2.3%) yellow flags and 12 (1.2%) non-identification flags from 980 scores is evidence of good performance overall.

• Six analysts failed the test (see annex XI). One analyst (70%) is just below the requirement with three failed test items and 4 analysts (60%) failed 4 items need some improvement. One analyst (20%) score failed 8 out 10 items requires substantial training and improvement in the next round.

• There were no significant issues with the qualitative aspects of this exercise and the number of non-detections 2.04% (2.1% in 2018) and mis-identifications 1.73% (5.9% in 2018) in the samples were relatively lower in comparison with previous years.

• The hardest species to recognize in this test was *Gonyaulax spinifera* which was erroneously classified by 11 analysts. Six analysts confused this species with *lingulodinium polyedrum* which is similar in shape and size.

• The most undetected species in the samples was *Akashiwo sanguinea* which had a relatively low cell density. Six analysts did not detect this organism compared with three analysts for *Heterosigma akashiwo*.

• Overall, from 980 possible correct identifications, there were a total of 950 correct answers at genus level that is 97.2% correct, 20 (2.04%) mis-identifications and 10 (1.02%) non-detections mainly on one species. This indicates a high level of taxonomic proficiency amongst participants

• Oceanteacher test: 74.2% analysts performed above the proficiency threshold of 90% and 20.6% of all analysts between 80-90%. 4.1% above 70% and only 1.1% requiring improvement. The consensus is largely rather good among participants and the scores suggest a high degree of proficiency.

• The key difficulties analysts found throughout the 2019 test relates to counting more than taxonomical nomenclature or classification. The facility index of Q3 and Q10, the two numerical questions in the test amounted to 71.6% and 81.84% and this compared unfavorably with most other questions in the exam, with scores between 90-98%, except for Q1 and Q2 with slightly lower marks (84-86%).

2. Introduction

The Proficiency testing scheme IPI has been designed to test the ability of analysts to identify and enumerate correctly marine phytoplankton species in lugol's preserved water samples using the Utermöhl method. As in previous years, samples have been produced using laboratory cultures.

Ten species were used in the IPI2019 test. There were five dinoflagellates and five diatoms in the samples. These were the dinoflagellates Akashiwo sanguinea (K.Hirasaka) Gert Hansen & Moestrup, 2000, Prorocentrum micans Ehrenberg, 1834, Gonyaulax spinifera (Claparède & Lachmann) Diesing, 1866, Azadinium spinosum Elbrächter & Tillmann, 2009, Heterosigma akashiwo (Y.Hada) Y.Hada ex Y.Hara & M.Chihara, 1987 and the diatoms Pseudo-nitzschia seriata complex (Cleve) H.Peragallo, 1899, Chaetoceros danicus Cleve, 1889, Corethron hystrix Hensen, 1887, Chaetoceros curvisetus Cleve, 1889 and Thalassiosira tenera Proschkina-Lavrenko, 1961.

The collaboration between the Marine Institute in Ireland and the IOC UNESCO Centre for Science and Communication of Harmful algae in Denmark dates back to 2011. This collaboration involves the use of algal cultures from the Scandinavian Culture Collection of Algae and Protozoa in Copenhagen, the elaboration of an online marine phytoplankton taxonomy assessment and the organization of an annual training workshop to discuss the results of the intercomparison exercise and to provide guidance on phytoplankton taxonomy.

This is a three full day training workshop which is held in Hillerød, Denmark in rooms equipped with microscopes and using live cultures (see workshop agenda Annex IV). this workshop has become an important forum for taxonomists working on phytoplankton monitoring programmes to convene and discuss taxonomical matters, new advances and finds, nomenclatural changes, samples from different locations and listen to relevant stories from other laboratories about harmful algal events in their regions of relevant ecological importance.

The taxonomic assessment is set up in the online platform 'Ocean Teacher Global academy' hosted by the IODE (International Oceanographic Data and information Exchange) office based in Oostende, Belgium, a project office of the IOC.

In 2019, 98 analysts in 52 laboratories from across the world participated in the IPI exercise. European countries accounted for 67% of the total participation, 19% from central and South America, 10 % from African countries and 4% from Oceania (Figure 1).

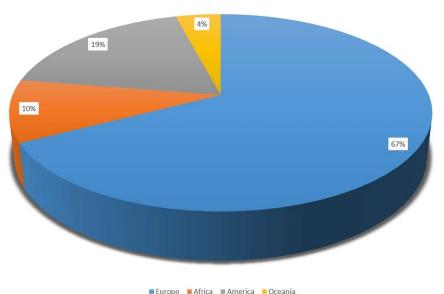




Figure 1: Participants by continent IPI2019

21 countries are represented in this intercomparison exercise. The list of participating laboratories can be found in Annex V and a breakdown of participation from each country in figure 2.

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-19-MI1. PHY standing for phytoplankton, ICN for intercomparison, 19 refers to the year 2019, 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 2019.

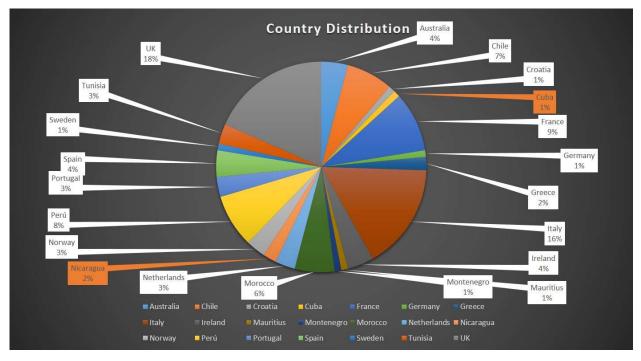
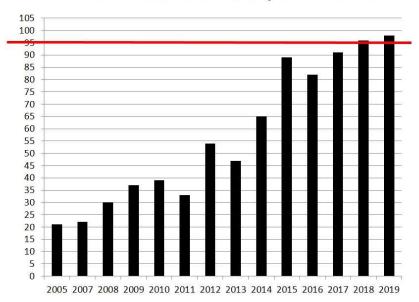


Figure 2: Participants by country IPI 2019

As figure 3 indicates the number of IPI participants has increased significantly since 2011 and the influence of the test has also been widened to many regions across the globe (figure 2). This year we reached the highest number of analysts (98) and the largest number of laboratories (52). Many laboratories participate regularly and since 2005 approximately 100 laboratories have partaken of this exercise since 2005 and several analysts have more than 10 contributions. In 2019 we had for the first time participants from Central America; Cuba and Nicaragua (highlighted in figure 2).

Last year, we introduced a new registration system to the IPI intercomparison. We developed the website <u>www.iphy.org</u> to provide a structured and user-friendly single point source of information relating to the IPI. Here, laboratories can find information about the IPI scheme, find the schedule for the year and register analysts.

As part of the registration process, we asked laboratories if bio-volume measurements were to be introduced, whether there would be interest in this new measurement. 53 analysts or 54% of the total for 2019 responded that they would be interested in participating in bio-volume measurements. This compares with 58% (57 analysts) in 2018 and 32% (29 analysts) when asked the same question in 2017. This is an area that we are interesting in and something that we would like to develop further as Bio-volume measurements could be easily integrated into the IPI programme.



IPI Number of Participants 2005-2019

Figure 3: IPI participation for the past 14 years

Also, since 2018 we have changed how we produce our materials. The main variation introduced during the production process was the preservation of materials using 10 ml brown glass ampoules under nitrogen gas and the automation of the homogeneity of the materials using the 'inversina', a bio-engineered mixer that uses the Paul-Schatz inversion method. Materials produced in this way are very stable for long periods of time. This is discussed at length in the materials and methods section of this report.

3. Materials and Methods

3.1 Sample preparation, homogenization and inoculation

The seawater used in this study was collected at Ballyvaughan pier, Galway bay, Ireland, filtered through 47mm GF/C Whatmann filters (Whatmann[™], Kent, UK), autoclaved (Systec V100, Wettenberg, Germany) and preserved using neutral Lugol's iodine solution (Clin-tech, Dublin, Ireland).

The materials were produced from a number of strains. A stock solution for each of the species was prepared using 50ml glass screw top bottles (Duran®, Mainz, Germany). Then, a working stock to the required cell concentration was prepared using a measured aliquot from each stock solution into a 2l Schott glass bottle. The stock solution containing all the species were homogenized using the 2L Inversina (Bioengineering AG, Wald, Switzerland), which uses the Paul-Schatz rotation method and sub-divided into five replicate working stocks containing 400 ml each. These working stocks were homogenized again before inoculation for 3 minutes at speed setting number 4 or roughly 73 rpm.

5 ml amber glass ampoules (Wheaton, New Jersey, USA) were used to store the inoculum. 3ml aliquots of the homogenized materials were inoculated into each ampoule containing 100µl of neutral lugol's iodine. This was carried out using an automatic eppendorf multipipette Xstream (0-10ml) (Eppendorf, Hamburg, Germany), set to dispense accurately 3 ml per sample. Once all the samples were inoculated, ampoules were purged with nitrogen gas to stop oxidation and sealed using a flame torch. The ampoules were submerged into a water bath to test that they were sealed properly.

Each ampoule was labeled with a sequential number and each box of ampoules was also labeled to differentiate sample sets produced from different working stocks (IPI2019-1 to IPI2019-5) and store in the fridge (2-5 °C) in the dark until further transport to the participating laboratories.

Samples were couriered on a one-day delivery across the world, in order for all laboratories to have approximately the same arrival time. We generally courier to laboratories further away from Europe first. If samples are delayed or don't arrive in time, an extra time allowance can be agreed.

Participants must carry out a preparatory step before the samples can be analysed. Analysts had to accurately pipette or dispense 47 ml of seawater including lugol's iodine (if necessary) into the

sterilin tubes, open the ampoule by the break-line carefully and pipette out its contents including a rinsing step into the sterilin tube. Once the sterilin tube is inoculated with the 3ml ampoule, the tube is ready for homogenization and analysis.

3.2 Culture material, treatments and replicates.

All the cultures used in this study have been collected during the 2018 Heincke survey HE516 in July 2019 in the English Channel, Celtic Sea and West of Ireland except for the diatom cultures which were isolated from samples collected from coastal locations in the South and West coasts of Ireland. Most species were identified through light microscopy techniques using an inverted microscope Olympus IX-51 and a compound research Olympus microscope BX-53 (Olympus, Southend-on-Sea, UK) and a bench-top SEM Hitachi FlexSEM 1000 (Hitachi, Maidenhead, UK) except for the *Pseudo-nitzschia seriata complex* which we weren't able to confirm to species level using our specific gene probes in our Lightcycler 480 (Roche, Dublin, Ireland) and could not fully identify to species level.

The cultures are checked by light microscopy in relation to their condition, shape, size and quality of their fixation using lugol's. Chain formers are also examined for their ability to stay in chains after preservation. At this point some other preliminary cultures may be discarded if they don't achieve the desired standard for the test. Images under the LM and SEM are taken of all the potential candidate species at high magnification as a record for the species in the test.

A total of 1000 ampoules were produced for this study. Each participant was sent a set of four replicates. 98 analysts were sent a total of 392 ampoules in 52 laboratories. Each sample set consisted of a padded brown envelope labeled with the analyst code and this contained 4 ampoules, 4×50 ml skirted centrifuge tubes, 4 plastic droppers and one 1.5 ml eppendorf microtube containing 1 ml of neutral lugol's iodine.

3.3 Cell concentrations

Preliminary cell counts from individual stock solutions were carried out using a 1 ml glass Sedgewick-Rafter cell counting chamber (Pyser-SGI, Kent, UK) to establish the approximate cell concentration for each species.

These approximate cell concentrations were used to decide the volume of the aliquot for each species and the final concentration required for the working stock. Microscopic analysis of an

aliquot of all the working stocks together, allow us to preview how the final samples will appear before a final decision is made on cell concentrations and number of species to be inoculated.

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

The instructions and forms required for this test are available at <u>www.iphyi.org</u> for download in the menu item IPI documents and are also sent via e-mail to all registered participants including their unique identifiable laboratory and analyst code. Here you can find a counting guide in pdf format to advise in the identification and counting of the species. Also, a short video is uploaded onto our website in the IPI documents under sample preparation, showing how to prepare the samples prior to analysis.

Form 1 (Annex I) is required to confirm the receipt of materials; the number and condition of samples and the correct sample code. Form 2 (Annex II) in Excel format is required to record the species composition in the samples and to calculate their abundance. All participants are asked to read and follow the instructions for the test (Annex III) before commencing.

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 2018.6.19.0, dedicated software for the statistical analysis of intercalibration and proficiency testing exercises from Quodata, Minitab® Statistical Software Vr16.0 and Microsoft office Excel 2016.

We followed the standard ISO normative 13528:2015, which describes the statistical methods to be used in proficiency testing by inter-laboratory comparisons. Here, we use this standard to determine and assess the homogeneity and stability of the samples, how to treat 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 transformation is the use of iteration to arrive at robust averages and standard deviations for each test item. This process allows for outliers and missing values to be dealt with, and it also allows for the heterogeneity of the samples to be taken into consideration when calculating these values.

3.7 IPI Ocean teacher online HAB quiz.

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

First time participants had to register in the following web address:

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 given permission by the site administrator to access the test. The test opened on the 8th October 2019 and closed on the 30th of November 2019, just over a month and a half to complete this assignment. The course itself was found under the courses tab in the main menu page. Analysts could link to the International Phytoplankton Intercomparison and quiz IPI 2019 HAB quiz content from here.

The test itself consisted of 12 questions (see Annex XVII). Question types used in the quiz were; 'matching type' (Q2, 3, 4, 9,10, 11 & 12) which have dropdown menus including a selection of answers which analysts must choose from and 'multiple choice' (Q 1, 5, 6, 7 & 8) where the participant must fill in the right option from those given. All questions had equal value and the quiz had a maximum grade of 100% for a perfect score. In the multiple choice type questions we have introduced penalties for wrong answers, where an incorrect choice incurs a percentage deduction. The amount of this deduction depends on the number of possible answers and ranges from 5% to 25% per wrong answer. The online quiz can only be submitted once. After submission, no changes can be made. However, analysts can login and out as many times as they wish throughout the allocated time period and make changes to the quiz. The changes are saved and can be accessed at a later stage, as long as the participant don't press submit.

4. Results

4.1 Homogeneity and stability study

The procedure for a homogeneity and stability test is recorded in annex b of ISO13528:2015. 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 2018.6.19.0 and the reports for homogeneity and stability are given separately for each measurand. The top of the report gives you information on the measurand, mean and analytical standard deviation for the homogeneity analysis and the homogeneity and stability mean comparison in the stability analysis. The reports, also show the target standard deviation for each measurand, which in this case was calculated manually using the consensus results of the participants and taking into consideration the heterogeneity of the samples, as will be explained later.

The middle part of the report gives you the results of the different tests. ProLab Plus calculates whether the data has passed the criteria for the F-test and ISO13528:2015 test for homogeneity and significant heterogeneity. 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 that were analyzed and calculates the heterogeneity standard deviation (SD between samples) and the analytical standard deviation (SD within samples). The stability test graph shows the 10 homogeneity sample results and the 3 stability test sample results, thirteen in total and compare their mean values (Annex VII).

According to ISO 13528:2015, the heterogeneity standard deviation (s(sample)) between the proficiency test items should not exceed 30 % of the standard deviation for the proficiency assessment. If the homogeneity test fails, the heterogeneity standard deviation has to be taken into account when calculating the standard deviation for the measurand. The consensus values new heterogeneity standard deviation (STD) was used for all measurands as the items failed the adequate homogeneity criterion (table 1). However, no significant heterogeneity was found according to the

expanded criterion. Hence, the proficiency test items cannot be considered fully homogeneous but not significantly heterogeneous (Table 1).

ISO13528	Cochran outliers	F-test	ISO 13528:2015 test for adequate homogeneity	ISO 13528:2015 - test for significant heterogeneity	Stability test ISO 13528:2015	Stability test - expanded criterion
Akashiwo sanguinea	no outliers found	Ok	Not OK	Ok	Not OK	Ok
Azadinium spinosum	no outliers found	Ok	Not OK	Ok	Ok	Ok
Chaetoceros curvisetus	no outliers found	Ok	Ok	Ok	Ok	Ok
Chaetoceros danicus	no outliers found	Ok	Not OK	Ok	Ok	Ok
Corethron hystris	no outliers found	Ok	Ok	Ok	Ok	Ok
Gonyaulax spinifera	no outliers found	Not OK	Not OK	Ok	Ok	Ok
heterosigma akashiwo	no outliers found	Ok	Not OK	Ok	Not OK	Ok
Prorocentrum micans	no outliers found	Ok	Not OK	Ok	Not OK	Ok
Pseudo-nitzschia seriata group	no outliers found	Not OK	Not OK	Ok	Not OK	Ok
Thalassiosira tenera	no outliers found	Ok	Ok	Ok	Ok	Ok

Table 1: IPI2019 Homogeneity and stability results according to ISO13528:2015

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 13528:2015. The robust estimates for this exercise have been derived by iterative calculation, that is, by convergence of the modified data (Annex IX) for each measurand.

In relation to missing values, the standard proposes that participants must report 0.59 n replicate measurements, so in the case of three replicates, at least two replicate results from each measurand must be obtained from each participant for the data to be included in the statistical calculations. If this rule is not fulfilled results from these participants won't be included in the calculation of statistics that affect other laboratories but they may be used for the calculation of their own, for example z-scores.

4.3 Analysts' Data

The table of participants' results can be found in Annex VIII at the end of this report. The average count for each measurand was used to calculate the robust averages and standard deviations by iteration. These values were then 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 ISO13528:2015. The calculations are generated by iteration and can be found for each measurand in this report in annex IX.

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

Where;

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

4.4 Assigned value and its standard uncertainty

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

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

Where;

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

 s^* = robust standard deviation for the test

p = number of analysts

Species	Akashiwo sanguinea		Gonyaulax spinifera		Heterosigma akashiwo		Corethron hystris	Chaetoceros curvisetus	Pseudo-nitzschia seriata group	Thalassiosira tenera
Robust mean x*	119	2756	5837	7773	11357	16840	2144	8264	64108	11288
Robust Stdev s*	70	1015	1583	3577	6410	2639	551	3594	23417	2902
Standard Ux	9	128	200	454	822	333	70	459	2957	366
n=	92	98	98	97	95	98	98	96	98	98
if Ux < 0.3xSTdev	21	304	475	1073	1923	792	165	1078	7025	871
then Ux is negligible	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
The equation is satisfied in all cases										

Table 2: Assigned values and standard uncertainties for the test.

If U_x is less than 0.3 times the standard deviation for the test, then this uncertainty is negligible for the test material. In our case, all our test materials satisfy the equation (Table 2).

4.4 Calculation of performance statistics

The performance statistics for the exercise have been calculated using ProLab Plus. 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/Hampel), 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.

4.4.1 Z-scores

The z-scores derived using the robust averages and standard deviations can be found in Annex X. Any results in blue are within the specification of the test (2SD). The yellow triangles indicate warning signals (outside 2SD), red triangles indicate action signals (outside 3SD). If the analyst failed to identify one or various species in the samples, no triangle will appear for that score. All qualitative scores are included for the final evaluation of analysts.

There were a very small number of warning and action signals across measurands. 18 Red flags (1.8%), 23 (2.3%) yellow flags and 12 (1.2%) non-identification flags from 980 scores is evidence of good performance overall. Six analysts failed the test (see annex XI). One analyst (70%) is just below the requirement with three failed test items and 4 analysts (60%) failed 4 items need some improvement. One analyst (20%) score failed 8 out 10 items requires substantial training and improvement in the next round.

4.5 Combined performance scores

Mandel's h and k statistic present measures for graphically surveying the consistency of the data for all measurands in the test (Annex XIV). Mandel's h statistics determines the differences between the mean values of all the laboratories and measurand combinations and it may point out at particular patterns for specific laboratories. In this graph, laboratories may have positive or negative values. Laboratories with large all-positive values or all-negative values for all measurands may indicate laboratory bias. The k statistics only produce positive results, zero is the baseline and it looks at repeatability precision between measurands. Generally, analysts with larger values tend to have poorer repeatability precision between replicates than the consensus mean values.

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

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

4.5.2 Lischer plots

The plots of repeatability standard deviations are used to identify analysts whose average and standard deviations are unusual from the consensus. They assume that the data is normally distributed and the null hypothesis is that there are no differences between the analyst means and standard deviations using the Lischer plot technique (Annex XVI) for each measurand.

4.6 Qualitative data

The performance of analysts on the correct identification of species was generally very good (Table 3). *Prorocentrum micans* was recognized by all 98 analysts to species level correctly and the easiest to identify dinoflagellate. *Pseudo-nitzschia* was also detected by all analysts to genus level. There were a small number of mis-identifications and non-identifications across the measurands. The most non detected organisms were *Akashiwo sanguinea* (6) related probably to its low cell density and *Heterosigma akashiwo* (3), a difficult raphidophyte. The most mis-identified species were *Gonyaulax spinifera* which was confused with *Lingulodinium polyedrum* by 6 analysts followed by *Scrippsiella spp*. (4 analysts).

Species	Identification	Species	Identification	Species	Identification
Prorocentrum micans	98	Chaetoceros danicus	92	Thalassiosira sp.	77
Total analysts	98	Chaetoceros sp. (Phaeoœros)	3	Thalassiosira pacifica	5
Species	Identification	Chaetoceros danicus/similis	2	Thalassiosira tenera	4
Gonyaulax spinifera	70	Atheya sp.	1	Thalassiosira rotula/gravida	4
Gonyaulax sp.	16	Total analysts	98	Thalassiosira eccentrica	4
Gonyaulax polygramma	1	Species	Identification	Thalassiosira anguste-lineata	1
Lingulodinium polyedrum	6	Chaetoceros curvisetus	31	Coscinodiscus sp.	2
Scrippsiella sp./spinifera/trochoidea	4	Chaetoceros sp. (Hyalochates)	49	Actynoptychus/Actynocyclus	1
Alexandrium/scrippsiella	1	Chaetoceros socialis	2	Total analysts	98
Total analysts	98	Chaetoceros diadema	1	Species	Identification
Species	Identification	Chaetoceros debilis	2	Pseudo-nitzschia seriata group	75
Azadinium spinosum	41	Chaetoceros didymus	1	Pseudo-nitzschia fraudulenta	11
Azadinium /Heterocapsa	34	Chaetoceros coronatus/debilis	2	Pseudo-nitzschia seriata	4
Azadinium sp.	17	Chaetoceros curvisetus/brevis	1	Pseudo-nitzschia delicatissima group	3
Heterocapsa sp.	3	Chaetoceros brevis	1	Pseudo-nitzschia caliantha	1
Heterocapsa rotundata	1	Chaetoceros danicus	1	Pseudo-nitzschia australis	1
Amphidoma languida	1	Chaetoceros brevis/curvisetus,	1	Pseudo-nitzschia multiseries	2
not detected	1	Chaetoceros sp. (phaeoœros)	4	Pseudo-nitzschia pungens/seriata	1
Total analysts	98	not detected	2	Total analysts	98
Species	Identification	Total analysts	98	020	
Heterosigma akashiwo	93	Species	Identification	Species	Identification
Heterosigma sp.	1	Akashiwo sanguinea	91	Corethron Hystris	77
Fibrocapsa japonica	1	Gymno/Gyrodinium	1	Corethron criophilum	20
not detected	3	not detected	6	Corethron criophilum/hystris	1
Total analysts	98	Total analysts	98	Total analysts	98

Table 3: Qualitative data by measurand.

Chaetoceros curvisetus was widely recognized to the highest taxon by 31 analysts and it was named as 6 other possible species by a number of analysts. Most analysts used the 'hyalochates' term to describe the species which is also correct. *C.danicus* was correctly classified by the majority to species level.

The majority of identifications were straightforward and no major difficulties were found with any of the measurands. The dinoflagellate *Azadinium spinosum* was the smallest species in the sample but it was mostly well identified to species level by 41 analysts, although 34 analysts decided to be more conservative and use the couple '*heterocapsa*/*Azadinium*' which is also fine. All were given as correct identifications.

Thalassiosira tenera, a small ($\leq 20\mu$ m) and difficult to identify non-chain forming diatom of the Thalassiosiraceae family was mostly identified correctly by all except for 3 analysts. Most analysts (77) identified to genus level with a few (4 analysts) identifying correctly to species level, even though the name had not been included in the list of possible species in Form 2 by error.

Also, for the diatom *Pseudo-nitzschia seriata complex* we expected analysts to identify correctly to group level which all did (98). The main consensus species name given outside this was *P.fraudulenta* (11) and *P.seriata* (4). However, we tested our strain with qPCR probes for these two species including *P.australis, P.pungens and P.multiseries* and we did not obtain a positive result for any of them.

Corethron was identified correctly by all analysts and there was consensus to species level to *C.hystris*, although 20 analysts opted for *C.criophilum*. The differences between the two must be found in the barbed spines at one end of the heterovalve and this requires high resolution microscopy.

4.7 Ocean Teacher 2019 online HAB quiz

The online HAB assessment was set up in the OTGA website (<u>http://classroom.oceanteacher.org/</u>) and consisted of 12 questions; Annex XVII shows the questions and correct answers for the test and annex XVIII show the final grades. 97 analysts completed and submitted the quiz.

There were two type of questions in this assessment; matching (Q2, 3, 4, 9,10, 11 & 12) and multiple choice (Q 1, 5, 6, 7 & 8). Multiple choice type questions carried penalties for choosing the wrong answer and this penalty was proportional to the number of possible erroneous answers. For instance, in Q1 a wrong choice could deduct 25% of the total for that question. In Matching questions there were no penalties but there were many more possible answers to choose from.

Q1, 4, 5, 7 and 8 were multiple choice type questions. For each question a plate of images were shown and analysts were asked to pick the right answers from the list. Q1 depicted a number of dinoflagellates and participants were asked to choose the images that represented dinoflagellates bearing an Ocellus (a light sensing organelle). The right answers were C and F, both Warnowiids (*Nematodinium* and *Warnowia*). 91 (93.81%) and 94 (96.90%) selected both C and F, the correct answers however, 20 analysts selected image A (*Cochlodinium*) which was incorrect. Q4 presented a plate of 9 species of the order Prorocentrales to be identified. Image 1 (*P.micans*) a very easily recognized species of this genus was identified correctly by 96 analysts from 97. Also, image 2 (*P.donghaiense*), image 4 (*P.compressum*) and image 5 (*Mesoporus perforatus*) were distinguished by most analysts. The most difficult identifications were image 7 (*P.cordatum*) and image 8 (*P.gracile*) which were confounded for *P.triangulatum* for image 7 and *P.arcuatum* in image 8.

Q5 showed various unarmoured dinoflagellates and analysts were asked to pick the genus *Akashiwo*. All analysts recognized this dinoflagellate correctly. In Q7, the plate showed various phytoplankton species and analysts were asked to tell us which ones did not depict a diatom. These were image A (*Prorocentrum*) a dinoflagellate, image C (*Dytiocha*) a silicoflagellate and image E (*Actiniscus*) a dinoflagellate. Only 84 analysts recognized image A as a dinoflagellate from 97. Image C (93) and E (92) were vastly identified perfectly with a small number of other answers. Q8 represented images of single cells of the genus *Thalassiosira* from SEM and light microscopy and analysts were asked to choose the correct genus from a selection. 93 analysts correctly identified the images to belong to the genus *Thalassiosira* based on the details of the images.

Q6 was similar to Q8 with only one correct answer. In this question we showed a video clip of a *D.acuta* cell undergoing a biological process and analysts were asked to choose from a range of options. The correct selection was the rotation of the nucleus or nuclear cyclosis. 91 analysts were correct, 2 selected 'digestion of *M.rubrum*', another 2 'vermifore parasitic stage' and two more 'cytoplasmic streaming'.

Q2 and Q12 were similar type questions. Q2 illustrated a plate of the genus *Tripos* and analysts were asked to select the correct ones from the drop–down list. Image B (*T.fusus*) was replied accurately by all analysts. Image A (*T.azoricus*) was erroneously recognized by 16 analysts, 12 of which preferred *T.muellerii* and 4 *T.arietinus*. Image D (*T.massiliensis*) was mistaken with image E (*T.macroceros*), with 73 and 78 correct answers respectively. Image C (*T.lineatus*) was confused with *T.furca* by 11 analysts.

Q12 illustrated six *Dinophysis* species. Images D, E and F were identified perfectly by all analysts. Image A and B by 95 and 91 analysts respectively. Image B (*D.acuminata*) was mistaken with *D.ovum* by 6 analysts.

Q3 and 10 were questions where the answer was a numerical value. In Q3 image 1 depicting a chain of the diatom Detonula, the answers 6, 7 or 8 were all given as correct, because in the actual wording of the question it wasn't specified whether not complete cells of the chain were supposed to be counted in or out. In image 2 (Corethron) 57 analysts (58.76%) selected 1 cell for 40 (41.23%) selecting 2 cells, the right answer was one cell as these diatoms are heterovalvate. There was no difficulty enumerating images 5 and 6, with mostly correct scores. However, there was difficulty with image 4 (Eucampia) where some cells weren't perfectly complete in the chain, in this case, we have taken the same approach as in image 1 and have given the answers 6, 7 or 8 as correct. In Q10 image 1, we came across a similar problem to Q3 image 1, where one cell appears to be halfway inside the image and some analysts opted for not counting this cell as it wasn't specified in the wording of this particular question. In this case, we gave 4 or 5 cells as the correct answer. Something similar to the Eucampia image in Q3 occurred with image 3 in Q10, where it is not clear how many cells should be counted in this Chaetoceros chain, here we have included as correct the answers 14 to 17 cells as correct but we have left out any other options. Image 4 (Dissodinium) was counted as one cell by 78 analysts and as 4 cells by 18 analysts. This also happens with image 2 (Polykrikos) where 83 analysts selected 2 cells for 12 analysts opting for 1 cell.

Q9 and Q11 depicted a pair of dinoflagellates and using LM, SEM and schematic representations to show the taxonomical differences of these small armoured dinoflagellates, which would be otherwise very difficult to recognize. Q9 depicted the species *Vulcanodinium rugosum* and *Gonyaulax spinifera*. All analysts recognized *G.spinifera* but only 88 recognised *V.rugosum*, 6 analysts selected *Scrippsiella spinifera*, 2 *A.spinosum* and 1 *Pfiesteria*. In Q11, images 1-4 depicted *Amphidoma languida* (95 analysts) and 5-8 *Scrippsiella acuminata* (94 analysts). Most analysts selected the right options with a handful of erroneous identifications.

5. Discussion

We are following the statistical methods laid out in ISO13528:2015 to calculate the performance statistics for the test. The results of the exercise have been processed using the consensus values of all the analysts to form the basis of their final Z-scores. Since 2014, we are using the statistical software programme ProLab Plus to calculate the descriptive statistics for the test and the performance characteristics including the graphical representation of all the results. The preferred statistical method since 2017 is the Q/Hampel uncorrected Z-score algorithm and before that we used the Q/Huber algorithm.

Homogeneity and stability test

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

Our experience since 2013 from running these homogeneity tests is that our samples are neither fully homogeneous nor significantly heterogeneous. However, this year using the new Inversina homogenizer instrument according to Table 1 most of our materials satisfy at least some of the ISO13528:2015 requirements for homogeneity and stability. All the materials passed the test for significant heterogeneity which allows the standard deviation to be greater than 30% of that of the test. Also, all materials passed the stability assessment according to the expanded criterion.

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

sufficient to take into account the heterogeneity of the samples. In this test, we have added the 'between sample standard deviation' from the homogeneity test for all the measurands (see table 2) to the consensus values as a precaution. In any case, the practical effect of adding the 'between sample SD' from the homogeneity test is to widen slightly the confidence limits for each test item.

Calculation of performance statistics

The consensus values from the participants + the 'between samples standard deviation' from the homogeneity test (Annex VIII) were used to calculate the performance statistics for the test. These values are derived by 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 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 requirement for the test for most analysts with a small 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 was good for most analysts with perfect scores in all measurands. In this exercise, 18 red flags (1.8%) slightly higher than in 2018 with 13 Red flags (1.36%), 23 (2.34%) yellow flags slightly lower than in 2018 with 31 (3.26%) and 12 (1.22%) non id flags also lower than 22 (2.3%) in 2018 from 980 results is evidence of good performance overall. Six analysts did not pass the test with a score below 80% from seven in 2018.

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 considered a test item or measurand. The individual scores for each measurand are analysed individually but also can be used to calculate combined effects for a particular laboratory or analysts such as correlation between results for different measurands. Graphical methods for this include histograms, bar plots and repeatability standard deviations plots.

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

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

The chart of RLP against RSZ (Annex XV) for all measurands combined indicates systematic laboratory bias. RSZ is based on the standardized sum of all the z-scores for each analyst and it can be interpreted as a single Z-score: that is an evaluation across all samples and measurands. If the RSZ value is within the tolerance limits (2SD), there are no significant systematic deviations of the measurement values for that analyst compared to the rest. The RLP is the mean length of all the Zscores 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 shows a systematic bias towards over or underestimating most of their counts in the samples, suggesting some kind of methodology bias.

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

Qualitative data

There were no significant issues with the qualitative aspects of this exercise and the number of nondetections 2.04% (2.1% in 2018) and mis-identifications 1.73% (5.9% in 2018) in the samples were relatively lower in comparison with previous years. The hardest species to recognize in this test was *Gonyaulax spinifera* which was erroneously classified by 11 analysts. Six analysts confused this species with *lingulodinium polyedrum* which is similar in shape and size, however *G.spinifera* has a prominent apical horn and the cingulum is widely excavated and offset compared to *L.polyedrum* which is pentagonal in shape and has a median cingulum with a slight offset. If there is any consolation for these analysts, is that both these species do produce yessotoxins and from a monitoring perspective, both are considered toxic species. The prominent horn in *G.spinifera* is probably the reason that four analysts selected *Scrippsiella* instead. However, *Scrippsiella's* cingulum is not offset and bear no small antapical spines.

Somewhat not surprisingly, all analysts classified *Prorocentrum micans* perfectly to species level. A cosmopolitan species which seems to appear regularly in samples all over the world.

The most undetected species in the samples was *Akashiwo sanguinea* which had a relatively low cell density. Six analysts did not detect this organism compared with three analysts for *Heterosigma akashiwo*. Interestingly, *H. akashiwo* which loses its shape when preserved with lugol's was perfectly identified by most analysts, perhaps because of the characteristic 'mishapen' form it takes upon preservation. This has been likened to 'a bunch of grapes'.

Azadinium spinosum was the smallest organism in the whole sample but analysts were able to classify this organism quite well. 41 analysts did to species level and 17 to genus level and another 34 used the couplet Azadinium/heterocapsa. Amphidoma, a very closely related genus to Azadinium was also given as correct identification, because the differences in terms of the overall shape and size, the presence of a pyrenoid are dramatically small between these genera and confirmation using light microscopy alone would be quite tricky.

The diatoms identification standard was very high. Most analysts confirmed the two *chaetoceros* species, the *Thalassiosira* and *Pseudo-nitzschia* species correctly except for a very minor number of nondetections (two for *C.curvisetus*), three mis-identifications for *Thalassiosira* and one for *C.danicus*. *Chaetoceros curvisetus* was described as 'hyalochate' type by 49 analsyts and 31 described it perfectly to species level, which indicates good consensus for this species. Generally, this is not the case and analysts do not tend to agree at species level, presenting a number of possible of candidate names. In reality, it is quite difficult to identify many species of *Chaetoceros* and the term 'hyalochate seems the most appropriate. This is somewhat similar to *Pseudo-nitzschia* which can only be identified to genus level, here we preferred the option of at least separating the identification to 'seriata' group or 'delicatissima' group level. In this context, we consider 'delicatissima' group identifications as erroneous for the purpose of this test. Attempts to classify this species to species level did not yield a large consensus but *P.fraudulenta* won the popular vote.

Thalassiosira tenera was perfectly identified by most analysts, the counting guide was used here to good effect to provide images in SEM and high resolution LM to be able to identify this small and non-chain forming *Thalassiosira*. The species name did not appear in the list of possible names in form 2, which was an error on our part and that explains why analysts would have chosen names in the list of other non-chain forming *Thalassiosira* species like *pacifica* or *eccentrica*.

Overall, from 980 possible correct identifications, there were a total of 950 correct answers at genus level that is 97.2% correct, 20 (2.04%) mis-identifications and 10 (1.02%) non-detections mainly on one species. This indicates a high level of taxonomic proficiency amongst participants.

Online taxonomic assessment or HAB quiz

The online taxonomic assessment is produced from scratch in the web platform Oceanteacher and designed to entice participants to study the taxonomic literature. The level of taxonomic proficiency required to perform well is high. The online assessment allows us to assess participants' taxonomic ability and compare those skills across laboratories. The technical expertise should be universal but teaching tools, resources and references may be different from one place to another. 74.2% analysts approximately performed above the proficiency threshold of 90% and 20.6% of all analysts between 80-90%. 4.1% above 70% and only 1.1% requiring improvement. The consensus is largely rather good among participants and the scores suggest a high degree of proficiency.

The key difficulties analysts found throughout the 2019 test relates to counting more than taxonomical nomenclature or classification. The facility index of Q3 and Q10, the two numerical questions in the test amounted to 71.6% and 81.84% and this compared unfavorably with most other questions in the exam, with scores between 90-98%, except for Q1 and Q2 with slightly lower marks (84-86%).

Selecting the correct amount of cells depicted in a photograph appears at first glance a simple task for all involved. However, when faced with awkward choices when contemplating the intricate life history of certain dinoflagellates, like a *Dissodinium* secondary cyst stage containing four dinospores

in Q10 image 4 or two *polykrikos* cells joined together in Q10 image 2, the correct answer is not as straightforward. The approach here is to write the answer we consider correct in OceanTeacher (OT) and allow the analysts to tell us differently. The consensus response becomes the standard answer. In the case of *Polykrikos* 85% selected 2 cells and in the case of *Dissodinium* 80% selected one cell. Although, the consensus sometimes is not always as clear, for example in Q3 image 2 (*Corethron*) 59% selected one cell and 41% two cells. Whether the consensus is the most accurate result in all these cases is debatable but at least it demonstrates what the majority is thinking.

Taxonomic courses barely discuss counting phytoplankton and are based predominantly in teaching how to identify species. This shows that counting can be a problematic area and can cause large inconsistencies among participants. If we use the example of *Corethron*, which was one of our target species in the samples, we realize that all analysts actually counted 'one cell' in the samples rather than 'two cells' as in Q3 image 2, this doesn't tally with the OT results where 41% of the analysts decided that there were two cells in the image. This suggests that analysts that selected 'two cells' in the OT test, used a different approach when analyzing the samples.

Equally, there are differences between analysts when enumerating cells in diatom chains that do not appear fully intact, where part of one cell can be missing or the chloroplasts only cover a portion of the cell like in Q3 image 4 or Q10 images 3 and 5. Discussion among analysts should include what should be counted and give clear guidelines on this. Our counting guide, gives some advice on this area and this debate should be continued among laboratories and technicians to improve our counting skills and to obtain better estimates.

ANNEX I: Form 1 return slip and checklist



IPI PHY-ICN-19-MI1 FORM 1: CHECKLIST CONFIRMATION

Please ensure to complete the table below upon receipt of sample 91 387201 or scan and e-mail to <u>rafael.salas@marine.ie</u>	es, then fa	c to + 353				
Analyst Name:						
Laboratory Name:						
Analyst Code Assigned :						
Contact Tel. No. / e-mail						
CHECKLIST OF ITEMS RECEIVED (Please circle the relevant answer)						
Please enter the sample codes here:	YES	NO				
Set of Instructions	YES	NO				
Envelope containing 4 x ampoules, droppers, lugols iodine and 4 x 50ml sterilin tubes	YES	NO				
Enumeration and identification result log sheet (Form 2)	YES	NO				

I confirm that I have received the items as detailed above and that the materials were received in good working conditions.

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

SIGNED:	

DATE:	

ANNEX II: Form 2 Enumeration and identification results log sheet



IPI 2019 Phytoplankton Inte	ercompa	arison Ex	cercise							
		1								
Analyst Name:										
Laboratory Code:										
Analyst Code :										
Settlement date:										
Volume Chamber (ml)										
Analysis date:										
Sample No:										
Organism	Cell	Cell	Cell	Multiplication		Number cells/L	Number cells/L	Number	Average	
Akashiwa sanguinas	count	count	count		facto	r I	Cells/L	Cells/L	cells/L	_
Akashiwo sanguinea										
Azadinium spinosum										
Chaetoceros sp. (Hyalochates) Chaetoceros danicus										
Corethron hystrix										
Gonyaulax spinifera										
Heterosigma akashiwo										
Prorocentrum micans										
Thalassiosira sp.										
Pseudo-nitzschia delicatissima con										
										#DIV/0!
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										#DIV/0!
Comments:										
Form 2: Results logsheet										

ANNEX III: Test instructions



IPI Phytoplankton Proficiency Test PHY-ICN-19-MI1 Vr1.0 Instructions

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

- 1. Introduction
- 2. Deadlines, checklists and forms
- 3. Test method
- 4. Equipment
- 5. Sedimentation chambers and sample preparation
- 6. Counting procedure and strategy
- 7. Samples
- 8. Counting Guide IPI2019
- 9. Online HABs taxonomic assessment in Oceanteacher

1. Introduction

- The Marine Institute, Galway, Ireland, conducts an annual International Phytoplankton Intercomparison (IPI) (formerly BEQUALM) on the abundance and composition of marine phytoplankton in water samples since 2005. First, under the auspices of the BEQUALM-NMBAQC umbrella and since 2011, in collaboration with the IOC Science and Communication Centre on Harmful Algae of UNESCO, in Copenhagen, Denmark. The design and organization of this exercise continued under the Marine Institute- IOC - BEQUALM banner until 2015.
- Since 2016, the programme BEQUALM no longer exist and the intercomparison exercise has changed its name to IPI (International Phytoplankton Intercomparison) with the continued collaboration of the IOC Science and Communication Centre on Harmful Algae and in association with NMBAQC in the UK.
- The web platform <u>www.iphyi.org</u> was created to be a single point source of information about the IPI scheme. Registration to the exercise must be completed through this website and all the information required is contained here. Documents required to participate in this exercise can be downloaded directly from this site including instructions and forms required to complete the test, but also other reference documents like past intercomparison reports and also educational workshop presentations as slide shows. There are also a few additional video clips to guide you on how to set up your samples for analysis.
- Information about this scheme can also be found through our partners, the IOC (http://hab.iocunesco.org under the heading 'activities and training courses') and associates in the NMBAQC website (www.nmbaqcs.org) under scheme components and phytoplankton, you'll find information on the current timetable schedule for the exercise, the list of participants, previous reports and the workshop agenda from the previous exercises to give you an idea of the range of activities within this intercomparison exercise. There is also information of the other NMBAQC schemes.
- The purpose of this exercise is to compare and evaluate the performance of testing laboratories and to monitor the laboratories continuing performance over time on the composition and abundance of marine microalgae in preserved marine samples. We work mainly with laboratories engaged in national official/non-official phytoplankton monitoring programmes, water framework directive, marine strategy framework directive and others (environmental

agencies, consultancies, private companies) working in the area of analysis of quality assurance in marine phytoplankton. Phytoplankton analysts should participate annually in an external and independent proficiency testing scheme to test their ongoing competency.

- The Marine Institute is accredited to ISO 17025 for toxic marine phytoplankton abundance and composition since 2005 and recognises that regular quality control assessments are crucial to ensure a high quality output of phytoplankton data. We are programmed to apply for the accreditation of this Proficiency Testing scheme under ISO 17043 for 2020. All our work is carried out following the technical and managerial requirements for PT schemes (ISO17043:2010) and the data is statistically analysed using the statistical methods as laid out in ISO13528:2015 'Statistical methods for use in PT by interlaboratory comparisons'. We use the statistical database software ProLab Plus from QuoData to do the statistical evaluation of the participants' data.
- 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.
- In 2018 for the first time, we have changed the way we prepare the samples for this intercomparison. These changes will have implications in the way participants must prepare their samples for analysis, so read carefully the following notes.
- In previous years, we have prepared a '**master mix'** by mixing manually using the Paul-Schatz rotation (figure of eight movement) a Schott glass bottle containing the species of interest. Then, a 5ml aliquot from this manually homogenised '**master mix'** was pipette into each pre-prepared sterilin tube containing an accurate volume of sterile seawater + lugol's iodine (45ml).
- From now on, we are using an Inversina 2L tumbler mixer by Bioengineering to homogenize the Master mix (see video <u>https://youtu.be/LTQ_mzolXIU</u>) to improve sample homogenization and an automated multi-pippetor (Xstream, eppendorf) delivers the aliquots with accuracy into 10ml brown glass ampoules where the samples are finally stored at 2-5 °C until they need to be transported. Using this technique, the degradation of the samples is practically zero over 24 months.



Figure 1: Sample set per participant including sealed vials, lugol's iodine, plastic droppers and 50ml sterilin tubes.

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

2. Deadlines, checklists and forms

- Upon sample receipt, analysts should ensure that they received everything listed in form 1; checklist confirmation (See fig. 1). Make sure that all the samples are intact and sealed properly and check that you have received Form 2; Enumeration and identification results log sheet (Excel workbook).
- Please complete Form 1: checklist confirmation form and send it back to me by fax to (+353 91 387201) or scan it as a pdf file and send it to me via e-mail to <u>rafael.salas@marine.ie</u>. If you send the form via e-mail, please name the file as Form 1 followed by the exercise code and your full name **i.e. Form 1: IPI19 Rafael Salas**. This validates the traceability of the samples from origin to the laboratories and ensures that the materials arrived to the performing laboratories in good working conditions.
- Analysts must complete and send their test results before or on 31/10/2019 to <u>rafael.salas@marine.ie</u> or fax (+353 91 387201) or post to Rafael Salas, Marine Institute, Phytoplankton laboratory, Rinville, Oranmore, Co. Galway, Ireland. 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.

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 don't arrive.

- Form 2 is an Excel workbook named 'Enumeration and identification logsheet' for analysts to input their results. At the top of the form, first fill in your name, analyst and laboratory code. Fill in all the information relevant to the analysis of your samples, for example the settlement date, chamber volume used in 'mls', the analysis date and the sample number in the corresponding cells.
- Under the column 'organism' a drop down menu appears 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 inoculated in the samples and provide a cell count. If is not in the list, is not in the sample.
- The number of rows under the column name 'organism' is arbitrary and independent of the number of species in the samples. There are 14 rows but this doesn't necessarily mean that you need to enter 14 names or that there are 14 species in the samples. The number and type of species inoculated in the samples is different from year to year.
- In the comments box, you can write information about the test method you used, any deviation from the Utermöhl test method and how you performed your calculations if you think is necessary.
- Once you have completed your samples and have reviewed your calculations in form 2, please send your form 2 back to me by fax to (+353 91 387201) or scan, pdf and send it via e-mail to <u>rafael.salas@marine.ie</u>. If you send the form via e-mail, please name the file as Form 2 followed by the exercise code and your full name **i.e. Form 2: IPI19 Rafael Salas**.

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 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: <u>Sedimentation chambers</u> 25ml volume if possible but other volume chambers can be used.

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

5. Sedimentation chambers and sample preparation

Sedimentation chambers consist of a clear plastic cylinder, a metal plate, a glass disposable cover-slip base plate and a glass cover plate (Fig 2). Three sedimentation chambers are required.

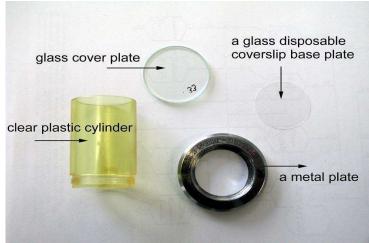


Fig 2: Sedimentation counting chamber

- 5.1 **Storage of ampoules:** If you are not analysing the samples straightaway or if you are analysing them in different dates, please ensure the samples are kept in a fridge at 2-5°C away from direct sunlight and in an upright position.
- 5.2 **Temperature adaptation:** Vials must be adapted to room temperature before aliquoting and sedimentation takes place. This reduces the risk of air bubbles inside the sedimentation chambers due to temperature differences between room and sample.

5.3 Preparation of samples for analysis from ampoules:

- 5.3.1 Please follow the link here to watch a video on how to prepare your sample for analysis from an ampoule. <u>https://youtu.be/2WgRNGDn4MU</u>
- 5.3.2 The sterilin tubes should be prepared in advance of opening the ampoule.
- 5.3.3 Measure accurately 47ml of sterile seawater containing a few drops of lugol's iodine. The ampoules are already preserved in lugols, but when the sample is aliquot into the tube, it is going to be diluted and pale in colour, so if you wish your sample to have a slightly darker coloration you can add a few drops of lugols iodine to the sterile seawater before you pipette the amount.
- 5.3.4 The volume can be measured in different ways, using an accurate pipette is one way to do it. However, you can use a gravimetric method also by weighing the amount using a balance. If you use a gravimetric method, remember that the density of Seawater at 33-35ppt is roughly 1.025g, so that 47ml = 48.175g in weight.
- 5.3.5 The seawater used should be of a salinity of 33-35ppt
- 5.3.6 Once the sterilin tubes containing 47ml seawater are ready you can start working with the ampoules.
- 5.3.7 First adapt the ampoule and test tube to room temperature, before aliquoting.
- 5.3.8 Make sure the ampoule contents are at the bottom of the ampoule. If some contents are trapped on the top, flick the ampoule using your fingers to dislodge any liquid.

- 5.3.9 Break the ampoule by the neck pre-marked break line using gloves and a wad of paper to avoid cuts and grazes. Avoid losing any sample content. If you think some content is lost, you have an extra sample to work with and if this fails, ask for another set.
- 5.3.10 Use one dropper per sample, do not mix or use the same dropper. Using the dropper, aspirate the contents from the ampoule into the tube.
- 5.3.11 Once all the sample has been aliquoted into the tube, using the same dropper, take a 3ml sample from the tube itself and rinse the ampoule with it once, collect the liquid again back into the tube.
- 5.3.12 Close the lid of the tube, invert the sample 50 times minimum and pour into a sedimentation chamber of your choice.
- 5.3.13 Once the sample has been taken out of the ampoule into the tube, the sample should be settled and analysed. **Do not keep the sample in the tube for several days as this will invalidate your analysis.**

5.4 Chamber preparation:

- 5.4.1 All sedimentation chambers should be cleaned before you start
- 5.4.2 Place a new disposable cover slip base plate inside a cleaned metal plate.
- 5.4.3 Screw the plastic cylinder into the metal plate until tight. Extra care should be taken when setting up chambers. Disposable cover slip base plates are fragile and break easily causing cuts and grazes.
- 5.4.4 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 Sample homogenisation and filling:

- 5.5.1 To set up a sample for analysis, firmly invert the sample at least 50 times before pouring the sample to ensure that the contents are homogenised properly. Avoid hard shaking of the samples
- 5.5.2 Place the chamber in a flat horizontal surface protected from vibration and strong sunlight and gently pour the sample into the counting chamber to the top. Cover the chamber with the glass plate to complete the vacuum, making sure that there are no air bubbles or pockets between the sample and the cover glass.
- 5.5.3 Label the sedimentation chamber with the sample number from the ampoule.

5.6 Sedimentation time:

- 5.6.1 Settling time is dependent on the height of the chamber. 10ml chambers should be allowed to settle for a minimum of 8 hours, 25ml chambers for a minimum of 12 hours and 50ml chamber for a minimum of 24 hours.
- 5.6.2 Set the chamber on the inverted microscope and start the analysis.

6. Counting Procedure and strategy

- a. Scan the entire chamber at low magnification first to get an initial overview of the density, distribution and composition of phytoplankton in the samples.
- b. Assess the random distribution pattern of the organisms in the sample before starting the analysis. Larger organisms tend to sediment towards the edges and smaller ones towards the centre if the temperature of the chamber is higher than the sample and vice-versa if the temperature of the chamber is lower than the sample. A visual inspection is enough to assess these patterns.
- c. If the sample is not randomly distributed, then the sample will have to be returned to its original container and settled again after a period of acclimatization. This is particularly important if other counting strategies are to be used in some organisms other than the whole chamber count, in which case, the sample count wouldn't be affected.

- d. Make a preliminary list of species and densities to help you choose the best counting strategy for the sample.
- e. Choose the correct organism/s from the dropdown species list in the Excel worksheet Form2.
- f. Start at the lower magnification to count the larger species if present, depending on size even x 4 or x 10 objectives could be used. Then, go over the sample again at higher magnifications to count the rest of the species.
- g. The smaller species should be counted at a higher objective magnification (x 20) or x 40 if necessary.
- h. Each analyst should carry out a whole chamber cell count (WC) 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 half chamber (HC), field of view (FV) or transect (Tr) counting strategy.
- j. If half of the chamber is to be counted, analyse every second transect.
- k. If a transect counting strategy is used for one or several organism, count at least three transects and average your results. Be consistent as to which cells lying on which borders are to be counted or omitted.
- I. Fields of view should be avoided if possible but if you need to use this counting strategy, count at least ten different randomly selected fields and average your results.

7. Samples

- Analysts must analyse three samples in total to complete this part of the exercise. The samples are replicates. A fourth sample is additional and should be used as a replacement in case of one sample leaking or breaking. All the samples are made up in sterile filtered Seawater and spiked with culture material consisting of several species. Participants are asked to carry out a whole sedimentation chamber cell counts (where possible; see section 6.) on each organism and sample.
- The Master mix, have been made up with different aliquots of cell cultures at different concentrations and estimates have been carried out in 1ml lugol's preserved samples and

counted in Sedgewick-Rafter chambers for each species. This is done to check the condition and the densities of the cultures prior to inoculating into the Master mix.

- Once the master mix have been made up in a 2L brown schott glass bottle with the target species at the required concentrations, this mixture has been homogenised using an automated tumbler mixer (Inversina 2L) that uses the Paul-Schatz movement for 4 minutes at 60 rpm approximately and divided in 8 x 250ml batches. These in turn have been homogenised again at the same speed and time. 3mls of the Master mix have been inoculated using an automated multi-pipette eppendorf into a batch of 10ml brown glass ampoules, containing 100µl of lugols iodine.
- The ampoules have been purged using nitrogen gas and sealed using a torch. The ampoules have been checked for leaks by submerging on a water bath and then stored at 2-5°C in the dark. The ampoules have been assigned a random number.

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

8. Counting guide IPI2019

Spend some time becoming familiar with the samples and how the cells appear on the base plate before commencing any counts. This guide should give you some hints as to how to count the organisms.

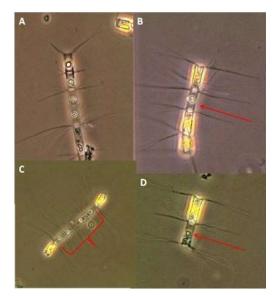


Fig.1 Diatoms. A-D cells are not in good condition, the chloroplasts are plasmolised and hardly visible inside the frustule. Image A do not count any cells. Image B count 3 cells but don't

count the second cell in the chain. In image C count the terminal cells only and in image D only count one cell.

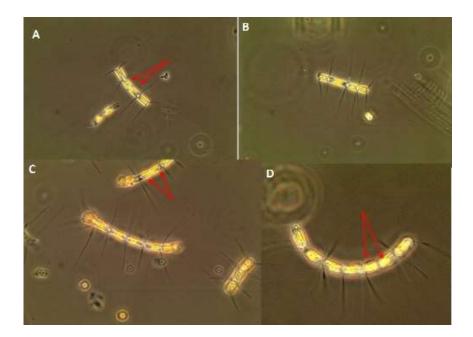


Fig.2 A-D. In image A (red arrows), the middle cells have no setae, but the foramen between the cells is well developed, so we consider 2 cells here. The only difficulty could be with image C and D (red arrows) where 2 cells have divided but not setae are visible yet between the cell pair. In this case, as cells are well differentiated, count these as 2 cells rather than just 1.

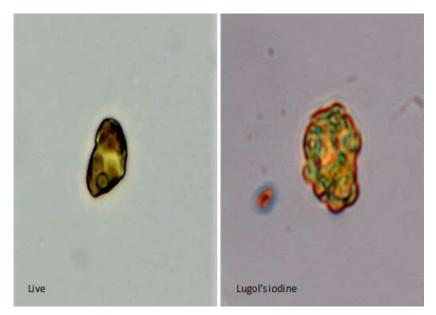


Fig. 3 Cells after preservation may look completely different.



Fig.4 Be aware that some organisms may not settle on the same focal plane. Use Z-focus in your samples. Images taken @ x20 magnification

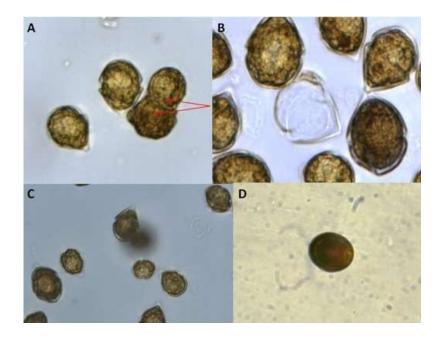


Fig.5 Image A shows two cells fusing or dividing, count only one cell. B: Dinoflagellates empty theca should not be counted. C: Cells may also vary in size, some cells will appear smaller than others, this is normal in culture conditions. Count all cells big or small. D: Sometimes Plasmolysis may occur and the cells appear naked and rounded. Do not count plasmolised cells as we don't know what they are.

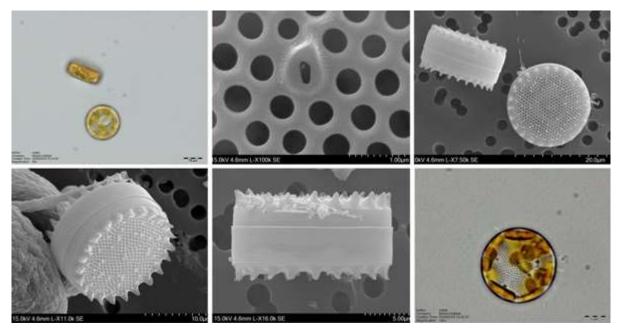


Fig.6 Samples contain a centric diatom. Due to their taxonomic difficulty, here we offer SEM images of this organism to aid in their identification.

These rules are applicable to this intercomparison exercise only.

9. Online HABs taxonomic assessment in Oceanteacher

- A taxonomic assessment is developed annually in the web platform 'Ocean teacher' <u>https://classroom.oceanteacher.org/</u> and should be completed as part of the IPI exercise. All participants need internet access to complete this section.
- Once you register for the exercise in <u>www.iphyi.org</u>, we will automatically enroll you in the Oceanteacher platform using the e-mail provided. Once everybody is enrolled, an e-mail will be sent to all of you with instructions on how to access the test. In order to access the exercise you need to go to the webpage <u>http://classroom.oceanteacher.org/</u> and login. It is very important that the e-mail you provide is unique to you and not generic within your organisation. Once you are all registered, we will grant you access to the test. The test will open on **September 30th 2019** and close on the **31st October 2019**.
- At the top right hand corner of the page; <u>http://classroom.oceanteacher.org/</u> press login and use your username and password provided to access the course, if you forgot your password press the forgotten password link. Once you are logged in, in the main page go to my courses and in the drop down menu choose the IPI 2019 course and start your test. Please note that you can login and out as many times as you want from the exercise as long as you

don't submit the exercise, the questions you answered are saved every time, so do not press submit until you are sure you are finished.

- Analysts have only one attempt to the exercise and once the exercise is submitted you won't be able to access it again. 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 in each test (true/false, numerical, matching, multiple choice short answer, etc.). Please note that if you are asked for a number as the answer do not use text, use a numerical value. Also, in questions where you are asked to write the answer, please make sure that the grammar is correct. Incorrect grammar will give an incorrect answer. Please review your work carefully before submitting.

ANNEX IV: Workshop agenda



Agenda 'International Phytoplankton Intercomparison' (IPI) workshop

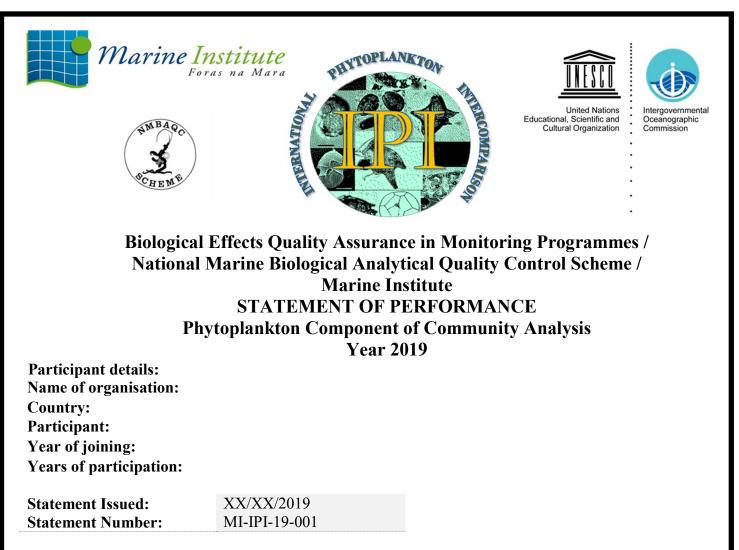
Danhostel, Hillerød, Denmark. 23–27 February 2020

	Morning, 9.00-12.00	Afternoon, 13.30-17.00
Sunday, 23 Feb		Arrival to the venue, arr. time 16.00 Danhostel, Lejrskolevej 4, 3400 Hilleroed, Sandwich is served in the evening
Monday, 24 Feb	Results and discussion of the inter- calibration and taxonomic quiz, <u>Rafael,</u> <u>Dave</u>	Presentations by the participants Microscope demonstration: Nanoplanktonic flagellates, ichthyotoxic flagellates, Jacob, Rafael, Dave
Tuesday, 25 Feb	Application of molecular techniques in identification of species of the Alexandrium tamarense-complex, <u>Dave</u> , Rafael Microscopy of cultures and samples, <u>Jacob</u> , Rafael, Dave	Microscope demonstration: Benthic dinoflagellates, <u>Jacob</u> , Rafael, Dave
Wednesday, 26 Feb	Flagellate-cysts relationship, <u>Dave, Jacob</u>	Microscopy of own samples, mixed samples from different areas
Thursday, 27 Feb	Breakfast, check-out at 10.00	

Company Name	Company Name	Company Name
Marine Institute	Fondazione Centro Ricerche Marine	lstituto Zooprofilattico Sperimentale della Sardegna
Marine Institute	CENTRO RICERCHE MARINE	4
Microalgal Services microalgal services	Dalcon Environmental	Marine Biological Association (ex SAHFOS)
phytoplasition monitoring and identification	<u>dal</u> con	SMHOS
Hydrorlab R-LAB	Institute of Marine Research, Flødevigen	Laboratorio de Control de Calidad de los Recursos Pesqueros
	INSTITUTE OF MARINE RESEARCH	
Agri Food and Biosciences Institute (AFBI)		Rijkswaterstaat 越
ARPA FVG	ARPAM Ancona	IRTA
SAMS Research Services Ltd (SRSL)	IFREMER	Agenzia Regionale Protezione Ambientale Campania
SAMS SRSL	- Ífremer	ZAMIZANIZ
SANIPES	ARPA Puglia	ARPAL La Spezia
SANIPES Second Report	ARPA PUGLIA	
Wageningen Marine Research	Instituto de Fomento Pesquero	SMHI / Swedish Meteorological and Hydrological Institute
		SMHI
Istituto Zooprofilattico Sperimentale delle Venezie	ARPA Campania	National Institute of Science and Technology of the Sea
Exercise deve Vecen		C. Servers
Institut za oceanografiju i ribarstvo (IOR) (Institute of Oceanography and Fisheries)	PLANCTON ANDINO SPA	IPMA (Portuguese Institute for Sea and Atmosphere)
Bureau Waardenburg by	Banco Español de Algas	Marine Scotland Marine Laboratory
Bureau Waardenburg bv Ecology & landscape	BANCO ESPANOL DE ALGAS ne-resolutionagyon	Scottish Government Riegholdtas no h-Alba gov.scot
Institut za Biologiju Mora	Aristotle University of Thessaloniki	Ministry of Ocean Economy, Marine Resources, Fisheries and Shipping
INSTITUT ZA BIOLOGIJU MORA		
Cefas	ARPA Lazio	Albion Fisheries Research Centre
Cefas	Λ	ALBION FISHERIES RESEARCH CENTRE
Institut National de Recherche Halieutique	APEM Limited	Laboratorio de Control de Calidad de los Recursos Pesqueros
Lamar Asociados Ltda	PIRSA	AquaEcology GmbH & Co. Aqua
m. P. I	SULTAALLA ALTAALLA	AquaEcology GmbH & Co. Aqua E
Northern Ireland Environment Agency (NIEA)	Sydney Water Sydney WATER	
Universidad Nacional Autonoma de Nicaragua	Universidad Catolica del Norte, Chile	Centro de Estudios Ambientales de Cienfuegos
Macronal Mac		

ANNEX V: Participating Laboratories

ANNEX VI: Statement of performance certificate



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:

Debbie Walsh

Debbie Walsh Laboratory technician

Palaet Gallorde blas

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 IPI 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

Azadinium spinosum homogeneity test

IP12019

Survey of homogeneity test results



Date: 21/02/2020

Sample:	Homogeneity		
Measurand:	Azadinium		
Mean:		14992 cells/Litre	
Analytical standa	rd deviation:	1917	
Heterogeneity sta	andard deviation s(samples):	1499	
Standard deviation	on for proficiency assessment:	3878 (Manual)	

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Homogeneity and stability test 19 w ere randomly selected, and the measurand Azadinium spinosum w as analyzed 2 times. The mean across all 10 proficiency test items is 14992 cells/Litre. The standard deviation w ithin proficiency test items s(analytical) (-analytical precision) is 1917 cells/Litre, and the standard deviation betw een proficiency test items s(sample) is 1499 cells/Litre.

F test

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

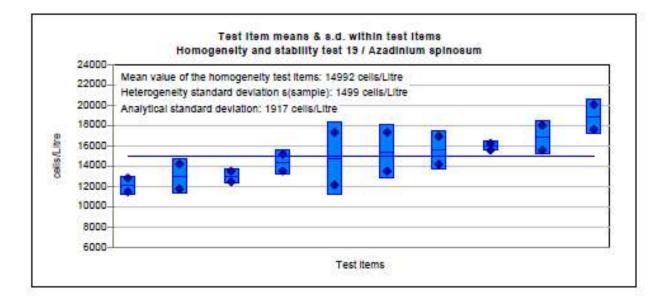
ISO 13528:2015 - Test for adequate homogeneity

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

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 3878 cells/Litre (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity

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



Sample: Homogeneity Measurand: Azadinium

Mean of homogeneity:	14992 cells/Litre
Mean of stability:	14840 cells/Litre
Uncertainty of mean for homogeneity measurement	t: 639 cells/Litre
Uncertainty of mean for stability measurement:	1482 cells/Litre
Standard deviation for proficiency assessment:	3878 (Manual)

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Homogeneity and stability test 19 have been selected randomly and the measurand Azadinium spinosum has been analyzed 2 times.

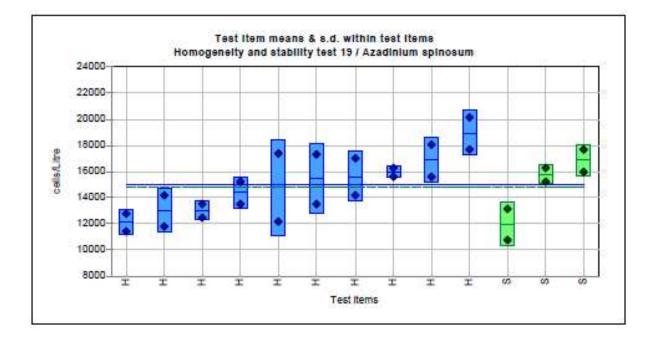
The mean value across all proficiency test items of the homogeneity analysis equals 14992 cells/Litre, the mean value across all proficiency test items of the stability analysis equals 14840 cells/Litre.

Therefore, the mean value of the stability analysis ites 1.0 % below the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Therefore, given the standard deviation for proficiency assessment of 3876 cells/Litre, the proficiency test items may be considered as adequately stable.

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

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





Date: 17/02/2020

Survey of homogeneity test results



Sample:	Homogeneity		Date: 2	1/02/2020
Measurand:	Akashiwo			
Mean:		480 cells/Litre		
Analytical standa	rd deviation:	111		
Heterogeneity st	andard deviation s(samples):	110		
Standard deviati	on for proficiency assessment:	13D (Manual)		

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Homogeneity and stability test 19 were randomly selected, and the measurand Akashiw o sanguinea was analyzed 2 times. The mean across all 10 proficiency test items is 480 cells/Litre. The standard deviation within proficiency test items s(analytical) (-analytical precision) is 111 cells/Litre, and the standard deviation between proficiency test items s(sample) is 110 cells/Litre.

F test

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

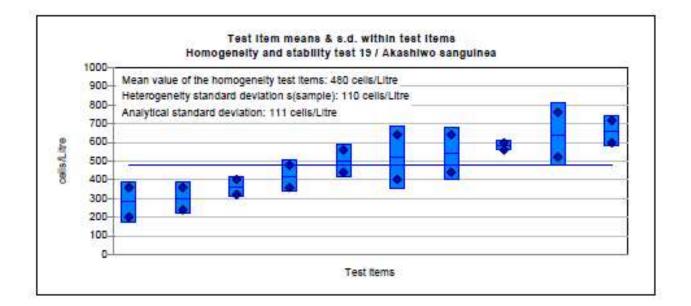
ISO 13528:2015 - Test for adequate homogeneity

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

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 130 cells/Litre (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity

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



Sample:

Measurand:

Survey of stability test results

Homogeneity

Akashiwo



Date: 17/02/2020

Mean of homogeneity:	480 cells/Litre
Mean of stability:	400 cells/Litre
Uncertainty of mean for homogeneity measurement	43 cells/Litre
Uncertainty of mean for stability measurement:	42 cells/Litre
Standard deviation for proficiency assessment:	130 (Manual)

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Homogeneity and stability test 19 have been selected randomly and the measurand Akashiw o sangulnea has been analyzed 2 times.

The mean value across all proficiency test items of the homogeneity analysis equals 480 cells/Litre, the mean value across all proficiency test items of the stability analysis equals 400 cells/Litre.

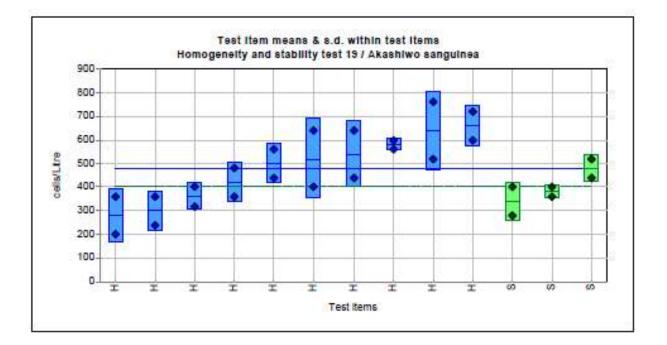
Therefore, the mean value of the stability analysis lies 16.7 % below the mean value of the homogeneity analysis.

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

Although for the given standard deviation for proficiency assessment of 130 cells/Litre, the proficiency test items may not be considered as adequately stable, the expanded acceptance criterion by adding the uncertainty of the difference to the standard deviation for proficiency assessment is fulfilled. Hence, stability of the proficiency test items is given only according to the expanded criterion of ISO 13528/2015.

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

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



IP12019

Survey of homogeneity test results



Date: 21/02/2020

 Sample:
 Homogeneity

 Measurand:
 Chaetoceros

 Mean:
 7566 cells/Litre

 Analytical standard deviation:
 1322

 Heterogeneity standard deviation s(samples):
 702

 Standard deviation for proficiency assessment:
 3662 (Manual)

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Homogeneity and stability test 19 w ere randomly selected, and the measurand Chaetoceros curvisetus w as analyzed 2 times. The mean across all 10 proficiency test items is 7566 cells/Litre. The standard deviation w ithin proficiency test items s(analytical) (-analytical precision) is 1322 cells/Litre, and the standard deviation betw een proficiency test items s(sample) is 702 cells/Litre.

F test

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

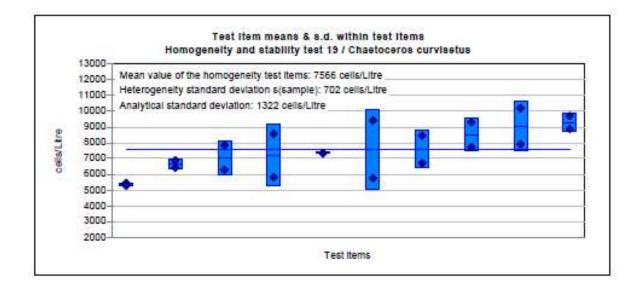
ISO 13528:2015 - Test for adequate homogeneity

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

The heterogeneity standard deviation is less than 30 % of the standard deviation for proficiency assessment 3662 cells/Litre (Mahual), therefore the proficiency test items can be considered adequately homogeneous according to ISO 13528:2015.

ISO 13528:2015 - Test for significant heterogeneity

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



Sample:	Homogeneity
Measurand:	Chaetoceros

 Mean of homogeneity:
 7566 cells/Litre

 Mean of stability:
 7293 cells/Litre

 Uncertainty of mean for homogeneity measurement:
 370 cells/Litre

 Uncertainty of mean for stability measurement:
 618 cells/Litre

 Standard deviation for proficiency assessment:
 3662 (Manual)

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Homogeneity and stability test 19 have been selected randomly and the measurand Chaetoceros curvisetus has been analyzed 2 times.

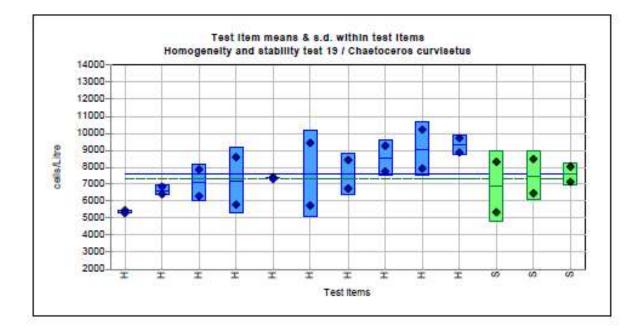
The mean value across all proficiency test items of the homogeneity analysis equals 7566 cells/Litre, the mean value across all proficiency test items of the stability analysis equals 7293 cells/Litre.

Therefore, the mean value of the stability analysis lies 3.6 % below the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Therefore, given the standard deviation for proficiency assessment of 3662 cells/Litre, the proficiency test items may be considered as adequately stable.

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

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





Date: 17/02/2020

IP12019

Survey of homogeneity test results



Sample:	Homogeneity		Date:	21/02/2020
Measurand:	Chaetoceros			
Mean:		13472 cells/Litre		
Analytical stands	ard deviation:	1340		
Heterogeneity st	andard deviation s(samples):	1153		
Standard deviat	ion for proficiency assessment.	2879 (Manual)		

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Homogeneity and stability test 19 were randomly selected, and the measurand Chaetoceros danicus was analyzed 2 times. The mean across all 10 proficiency test items is 13472 cells/Litre. The standard deviation within proficiency test items s(analytical) (-analytical precision) is 1340 cells/Litre, and the standard deviation between proficiency test items s(sample) is 1153 cells/Litre.

F test

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

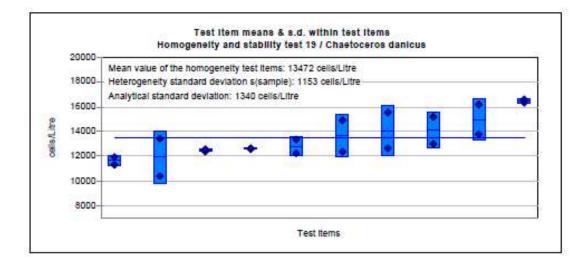
ISO 13528:2015 - Test for adequate homogeneity

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

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 2879 celis/Litre (Manuai), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528 2015 - Test for significant heterogeneity

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



Sample:	Homogeneity
Measurand:	Chaetoceros

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Date: 17/02/2020

Mean of homogeneity:	13472 cells/Litre
Mean of stability:	14173 cells/Litre
Uncertainty of mean for homogeneity measurement:	472 cells/Litre
Uncertainty of mean for stability measurement:	819 cells/Litre
Standard deviation for proficiency assessment:	2879 (Manual)

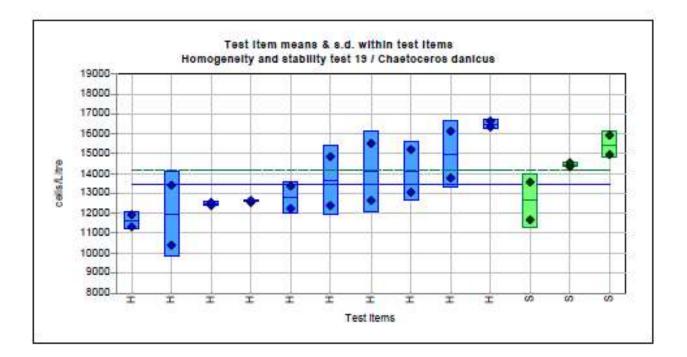
Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Homogeneity and stability test 19 have been selected randomly and the measurand Chaetoceros danicus has been analyzed 2 times. The mean value across all proficiency test items of the homogeneity analysis equals 13472 cells/Litre, the mean value across all proficiency test items of the stability analysis equals 14173 cells/Litre. Therefore, the mean value of the stability analysis lies 5.2 % above the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Therefore, given the standard deviation for proficiency assessment of 2879 cells/Litre, the proficiency test items may be considered as adequately stable.

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

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



Survey of homogeneity test results



Sample:	Homogeneity		Date:	21/02/2020
Measurand:	Corethron			
Mean:		2132 cells/Litre		
Analytical standard deviation:		300		
Heterogeneity standard deviation s(samples):		0		
Standard deviation for proficiency assessment:		551 (Manual)		

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Homogeneity and stability test 19 w ere randomly selected, and the measurand Corethron Hystris w as analyzed 2 times. The mean across all 10 proficiency test items is 2132 cells/Litre. The standard deviation within proficiency test items s(analytical) (-analytical precision) is 300 cells/Litre, and the standard deviation betw een proficiency test items s(sample) is 0 cells/Litre.

F test

The heterogeneity standard deviation s(sample) is 0 cells/Litre, and hence no statistically significant difference to 0 can be detected by the F test.

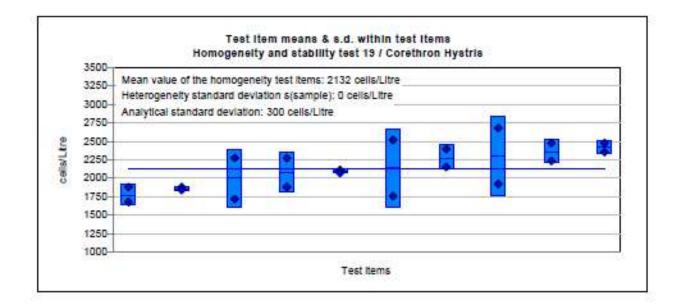
ISO 13528:2015 - Test for adequate homogeneity

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

The heterogeneity standard deviation is less than 30 % of the standard deviation for proficiency assessment 551 cells/Litre (Manual), therefore the proficiency test items can be considered adequately homogeneous according to ISO 13528:2015.

ISO 13528:2015 - Test for significant heterogeneity

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



Sample: Homogeneity Measurand: Corethron

Measurand: Corethron Mean of homogeneity:

Mean of stability: 2240 cells/Litre Uncertainty of mean for homogeneity measurement: 67 cells/Litre Uncertainty of mean for stability measurement: 162 cells/Litre Standard deviation for proficiency assessment: 551 (Manual)

Results of Stability Test

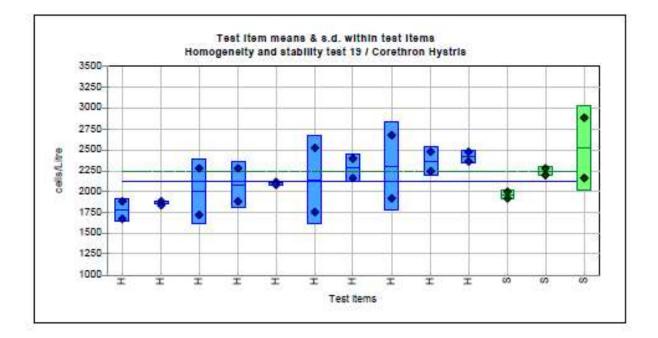
For the test for stability, 3 of the proficiency test items of sample Homogeneity and stability test 19 have been selected randomly and the measurand Corethron Hystris has been analyzed 2 times. The mean value across all proficiency test items of the homogeneity analysis equals 2132 cells/Litre, the mean value across all proficiency test items of the stability analysis equals 2240 cells/Litre. Therefore, the mean value of the stability analysis lies 5.1 % above the mean value of the homogeneity analysis.

2132 celis/Litre

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Therefore, given the standard deviation for proficiency assessment of 551 cells/Litre, the proficiency test items may be considered as adequately stable.

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

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





Date: 17/02/2020

Survey of homogeneity test results



Date: 21/02/2020

 Sample:
 Homogeneity

 Measurand:
 Gonyaulax

 Mean:
 6744 cells/Litre

 Analytical standard deviation:
 791

 Heterogeneity standard deviation s(samples):
 836

Standard deviation for proficiency assessment:

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Homogeneity and stability test 19 w ere randomly selected, and the measurand Gonyaulax spinifera w as analyzed 2 times. The mean across all 10 proficiency test items is 6744 cells/Litre. The standard deviation within proficiency test items s(analytical) (-analytical precision) is 791 cells/Litre, and the standard deviation betw een proficiency test items s(sample) is 836 cells/Litre.

1790 (Manual)

F test

According to the F test, the heterogeneity standard deviation is significantly different from 0 (significance level 5 %), therefore the proficiency test items should be considered heterogeneous according to this criterion.

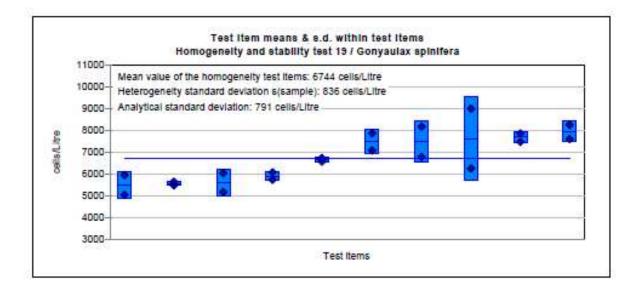
ISO 13528:2015 - Test for adequate homogeneity

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

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 1790 cells/Litre (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity

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





17/02/2020

Date:

Sample: Homogeneity Measurand: Gonyaulax

Mean of homogeneity:	6744 cells/Litre
Mean of stability:	7053 cells/Litre
Uncertainty of mean for homogeneity measurement	: 318 cells/Litre
Uncertainty of mean for stability measurement:	374 cells/Litre
Standard deviation for proficiency assessment:	1790 (Manual)

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Homogeneity and stability test 19 have been selected randomly and the measurand Gonyaulax spinifera has been analyzed 2 times.

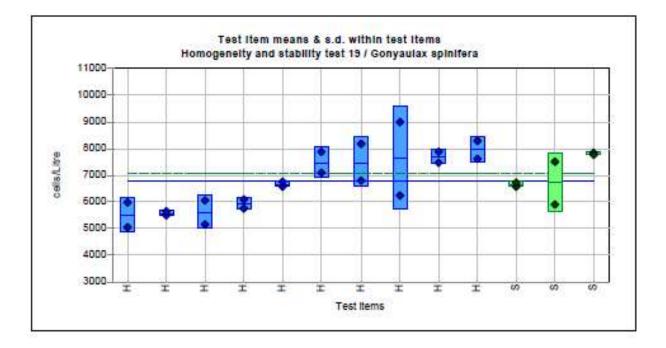
The mean value across all proficiency test items of the homogeneity analysis equals 6744 cells/Litre, the mean value across all proficiency test items of the stability analysis equais 7053 cells/Litre.

Therefore, the mean value of the stability analysis lies 4.6 % above the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Therefore, given the standard deviation for proficiency assessment of 1790 cells/Litre, the proficiency test items may be considered as adequately stable.

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

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



IPI2019

Survey of homogeneity test results



Sample: Measurand:	Homogeneity Heterosigma		Date:	15/05/2020
Mean:		34946		
Analytical standar	rd deviation:	4014		
Heterogeneity sta	ndard deviation s(samples):	3899		
Standard deviatio	n for proficiency assessment:	7502 (Manual)		

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Homogeneity and stability test 19 were randomly selected, and the measurand Heterosigma akashiwo was analyzed 2 times. The mean across all 10 proficiency test items is 34946. The standard deviation within proficiency test items s(analytical) (=analytical precision) is 4014, and the standard deviation between proficiency test items s(sample) is 3899.

F test

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

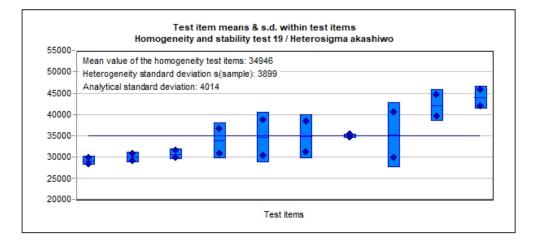
ISO 13528:2015 - Test for adequate homogeneity

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

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 7502 (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity

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





15/05/2020

PROLab Page 1

ANNEX VII: Heterosigma akashiwo stability test

IPI2019

Survey of stability test results



 Sample:
 Homogeneity
 Date:
 15/05/2020

 Measurand:
 Heterosigma
 Date:
 15/05/2020

 Mean of homogeneity:
 34946

 Mean of stability:
 31107

 Uncertainty of mean for homogeneity measurement:
 1525

 Uncertainty of mean for stability measurement:
 1120

 Standard deviation for proficiency assessment:
 7502 (Manual)

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Homogeneity and stability test 19 have been selected randomly and the measurand Heterosigma akashiwo has been analyzed 2 times. The mean value across all proficiency test items of the homogeneity analysis equals 34946, the mean value across all

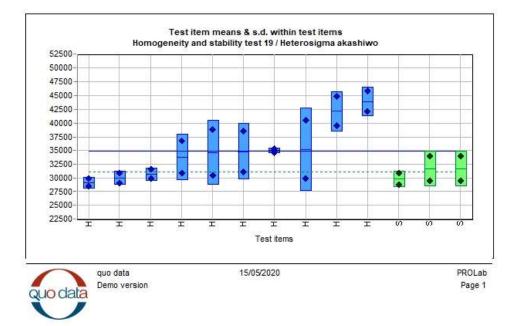
proficiency test items of the stability analysis equals 31107.

Therefore, the mean value of the stability analysis lies 11.0 % below the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Although for the given standard deviation for proficiency assessment of 7502, the proficiency test items may not be considered as adequately stable, the expanded acceptance criterion by adding the uncertainty of the difference to the standard deviation for proficiency assessment is fulfilled. Hence, stability of the proficiency test items is given only according to the expanded criterion of ISO 13528:2015.

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

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



Survey of homogeneity test results



 Sample:
 Homogeneity

 Measurand:
 Prorocentrum

 Mean:
 4894 cells/Litre

 Analytical standard deviation:
 488

 Heterogeneity standard deviation s(samples):
 366

Standard deviation for proficiency assessment:

Date: 21/02/2020

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Homogeneity and stability test 19 w ere randomly selected, and the measurand Prorocentrum micans was analyzed 2 times. The mean across all 10 proficiency test items is 4894 cells/Litre. The standard deviation within proficiency test items s(analytical) (-analytical precision) is 488 cells/Litre, and the standard deviation betw een proficiency fest items s(sample) is 366 cells/Litre.

1079 (Manual)

F test

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

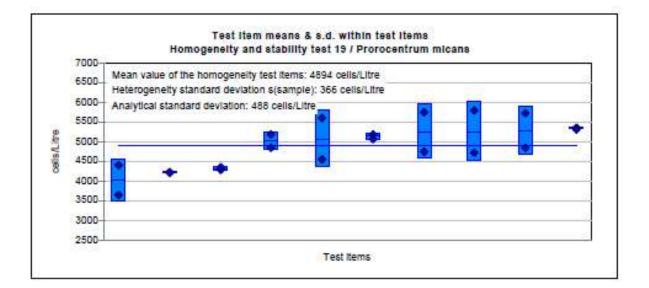
ISO 13528:2015 - Test for adequate homogeneity

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

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 1079 cells/Litre (Manuai), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity

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



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Sample:	Homogeneity
Measurand:	Prorocentrum

Date: 17/02/2020

Mean of homogeneity:	4894 cells/Litre
Mean of stability:	5347 cells/Litre
Uncertainty of mean for homogeneity measurement:	159 cells/Litre
Uncertainty of mean for stability measurement:	291 cells/Litre
Standard deviation for proficiency assessment:	1079 (Manual)

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Homogeneity and stability test 19 have been selected randomly and the measurand Protocentrum micans has been analyzed 2 times.

The mean value across all proficiency test items of the homogeneity analysis equals 4894 cells/Litre, the mean value across all proficiency test items of the stability analysis equals 5347 cells/Litre.

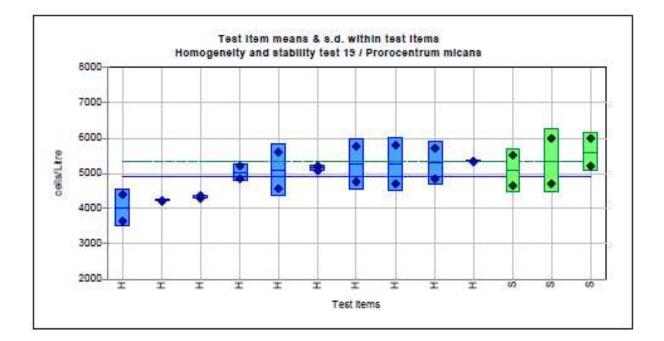
Therefore, the mean value of the stability analysis lies 9.2 % above the mean value of the homogeneity analysis.

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

Although for the given standard deviation for proficiency assessment of 1079 cells/Litre, the proficiency test items may not be considered as adequately stable, the expanded acceptance criterion by adding the uncertainty of the difference to the standard deviation for proficiency assessment is fulfilied. Hence, stability of the proficiency test items is given only according to the expanded criterion of ISO 13528:2015.

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

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



IP12019

Survey of homogeneity test results



	Date: 21/02/2020
185541 cells/Litre	
21035	
23173	
32944 (Manual)	
	21035 23173

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Homogeneity and stability test 19 w ere randomly selected, and the measurand Pseudo-nitzschia seriata group w as analyzed 2 times. The mean across all 10 proficiency test items is 185541 cells/Litre. The standard deviation within proficiency test items s(analytical) (-analytical precision) is 21035 cells/Litre, and the standard deviation betw een proficiency test items s(sample) is 23173 cells/Litre.

F test

According to the F test, the heterogeneity standard deviation is significantly different from 0 (significance level 5 %), therefore the proficiency test items should be considered heterogeneous according to this criterion.

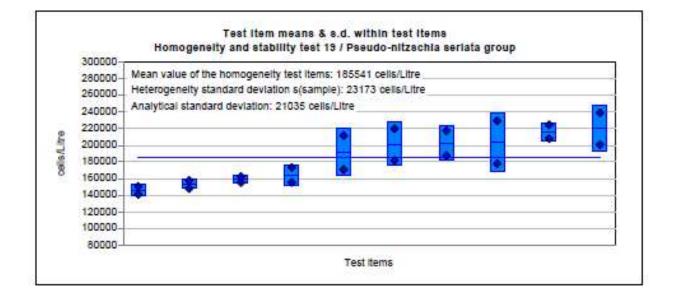
ISO 13528:2015 - Test for adequate homogeneity

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

The heterogeneity standard deviation is greater than 30 % of the standard deviation for proficiency assessment 32944 cells/Litre (Manual), therefore the proficiency test items cannot be considered as adequately homogeneous, i.e. they have to be considered heterogeneous.

ISO 13528:2015 - Test for significant heterogeneity

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



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Date: 17/02/2020

IP12019

Survey of stability test results

Sample: Homogeneity Measurand: Pseudo-

 Mean of homogeneity:
 185541 celis/Litre

 Mean of stability:
 175351 celis/Litre

 Uncertainty of mean for homogeneity measurement:
 8707 celis/Litre

 Uncertainty of mean for stability measurement:
 15637 celis/Litre

 Standard deviation for proficiency assessment:
 32944 (Manual)

Results of Stability Test

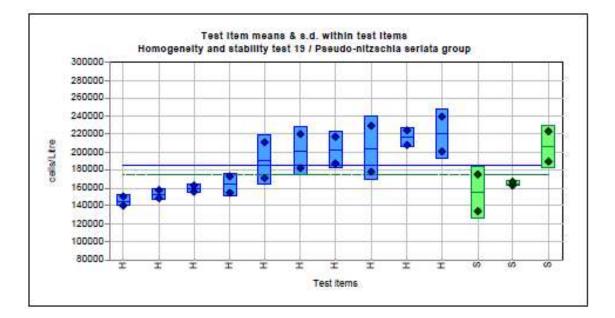
For the test for stability, 3 of the proficiency test items of sample Homogeneity and stability test 19 have been selected randomly and the measurand Pseudo-nitzschia seriata group has been analyzed 2 times. The mean value across all proficiency test items of the homogeneity analysis equals 185541 cells/Litre, the mean value across all proficiency test items of the stability analysis equals 175351 cells/Litre. Therefore, the mean value of the stability analysis lies 5.5 % below the mean value of the homogeneity analysis.

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

Although for the given standard deviation for proficiency assessment of 32944 cells/Litre, the proficiency test items may not be considered as adequately stable, the expanded acceptance criterion by adding the uncertainty of the difference to the standard deviation for proficiency assessment is fulfilled. Hence, stability of the proficiency test items is given only according to the expanded criterion of ISO 13528:2015.

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

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



IP12019

Survey of homogeneity test results



Sample:	Homogeneity		Date:	21/02/2020
Measurand:	Thalassiosira			
Mean:		13542 cells/Litre		
Analytical standard deviation:		767		
Heterogeneity standard deviation s(samples):		668		
Standard deviation for proficiency assessment:		2978 (Manual)		

Results of homogeneity analysis (with statistical background)

For the homogeneity test, 10 of the prepared proficiency test items of sample Homogeneity and stability test 19 were randomly selected, and the measurand Thalassiosira tenera was analyzed 2 times. The mean across all 10 proficiency test items is 13542 celis/Litre. The standard deviation within proficiency test items s(analytical) (-analytical precision) is 767 celis/Litre, and the standard deviation between proficiency test items s(sample) is 668 celis/Litre.

F test

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

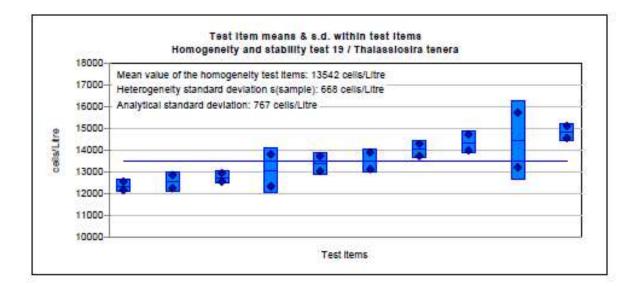
ISO 13528:2015 - Test for adequate homogeneity

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

The heterogeneity standard deviation is less than 30 % of the standard deviation for proficiency assessment 2978 cells/Litre (Manual), therefore the proficiency test items can be considered adequately homogeneous according to ISO 13528:2015.

ISO 13528:2015 - Test for significant heterogeneity

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



Sample: Homogeneity Measurand: Thalassiosira

Mean of homogeneity:	13542 cells/Litre
Mean of stability:	13867 cells/Litre
Uncertainty of mean for homogeneity measurement	272 cells/Litre
Uncertainty of mean for stability measurement:	362 cells/Litre
Standard deviation for proficiency assessment:	2978 (Manual)

Results of Stability Test

For the test for stability, 3 of the proficiency test items of sample Homogeneity and stability test 19 have been selected randomly and the measurand Thalassiosira tenera has been analyzed 2 times.

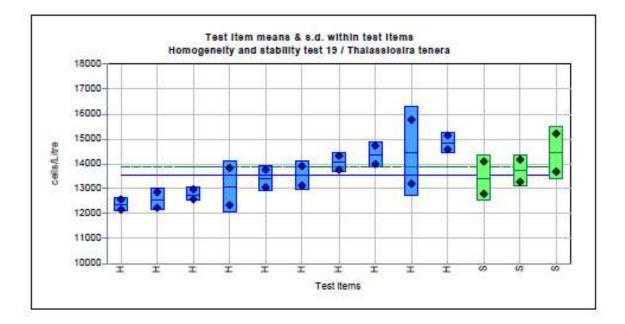
The mean value across all proficiency test items of the homogeneity analysis equals 13542 cells/Litre, the mean value across all proficiency test items of the stability analysis equals 13867 cells/Litre.

Therefore, the mean value of the stability analysis lies 2.4 % above the mean value of the homogeneity analysis.

According to ISO 13528:2015, the absolute difference between the mean values of the homogeneity analysis and the stability analysis should not exceed 30 % of the standard deviation for proficiency assessment. Therefore, given the standard deviation for proficiency assessment of 2978 cells/Litre, the proficiency test items may be considered as adequately stable.

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

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



Date: 17/02/2020

ANNEX VIII: Analysts' results 1-54 Akashiwo sanguinea + Prorocentrum micans

Analyst code	ASANG 1	ASANG	G 2	ASANG 3	Identification flag	Analyst code	PMIC 1	PMIC 2	PMIC 3	Identification flag
1	80	1	40	120		1	1240	3080	2240	
3	0	I.	0	80		3	5920	4560	2960	
4	200	1	160	0		4	1360	5720	2120	
5	40	1	80	0		5	3360	2160	1080	
8	75	1	111	37		8	3731	3037	1791	
9	240	2	200	280		9	4840	5280	5200	
10	120	2	280	80		10	4280	3880	4520	
11	n.d.	n.d.		n.d.	not detected	11	3700	2900	7100	
12	40	1	80	80		12	1400	2080	2480	
13	80	1	160	120		13	1680	3880	1840	
14	40	1	40	440		14	2840	720	440	
15	80	1	120	160		15	2520	3560	1560	
16	40	1	40	40		16	2080	1800	1920	
18	80	1	120	40		18	2840	2240	3600	
19	100		0	50		19	2450	2550	900	
20	43		0	43		20	2217	2217	1913	
21	160	4	140	240		21	2480	2320	2680	
24	160	l .	80	160		24	1200	920	1680	
25	160	. 3	320	280		25	1880	3320	2760	
26	0	1	160	0		26	1760	3640	3040	
27	754		0	0		27	10053	7540	7540	
28	250	2	250	250		28	2250	1875	2625	
29	40	1	160	240		29	1360	1520	1360	
30	120	1	120	120		30	2080	1760	3160	
31	n.d.	n.d.		n.d.	not detected	31	840	1760	2520	
32	80	1	160	280		32	3040	3040	2760	
33	80	1	80	120		33	4560	3480	4160	
34	80	1	40	40		34	2320	1400	3720	
35	120	1	160	120		35	2320	2120	4000	
36	40	1	140	40		36	2080	1980	1480	
37	120	2	280	0		37	1520	3040	1480	
38	140	1	120	120		38	1640	2380	1920	
39	200	2	200	200		39	3280	2600	3720	
40	120		80	240		40	2280	2480	2280	
42	n.d.	n.d.		n.d.	not detected	42	5200	4200	4800	
43	nd	nd		nd	not detected	43	11667	2000	8333	
44	200	1	120	160		44	3840	2880	2960	
46	240	2	200	160		46	2360	2880	3040	
47	40		40	160		47	2120	1720	1200	
48	50		0	50		48	2450	1500	2400	
49	0	7	700	0	Gymno/Gyro	49	700	700	700	
50	120		80	120		50	2840	2520	1200	
51	80		40	160		51	4280	3600	4520	
52	150		0	50		52	3600	2900	3050	
53	40		120			53	840	2000	2880	
54	120	1	120	120		54	680	920	1400	

Analyst code	ASANG 1	ASANG 2	ASANG 3	Identification flag	Analyst code	PMIC 1	PMIC 2	PMIC 3	Identification flag
56	240	280	280		56	4360	5040	4440	
57	40	120	80		57	1680	1720	1960	
58	120	320	480		58	2840	3520	4080	
59	43	43	174		59	2131	1348	2565	
60	224	115	261		60	1866	1769	2239	
61	40	0	0		61	2400	2000	2880	
62	160	40	120		62	3280	4000	4200	
63	200	240	200		63	3840	3560	4320	
64	160	120	120		64	3280	3320	4160	
65	160	200	160		65	2320	4000	2320	
66	100	400	300		66	1600	3600	3100	
67	160	200	160		67	3880	4160	3920	
70	400	360	440		70	3400	3000	3120	
71	40	80	160		71	3880	2800	2400	
72	80	160	320		72	3840	2080	4120	
73	160	60	80		73	2080	1400	2240	
74	0	120	200		74	2000	2880	3760	
76	120	40	160		76	3840	3520	2440	
77	120	100	40		77	3340	3300	2680	
78	80	80	40		78	1800	3800	3200	
79	240	240	240		79	3800	3680	2280	
80	0	180	120		80	4260	4500	3720	
	-	n.d.	n.d.	not detected	82	2800	1300	2600	
85	280	400	400	not detected	85	3160	4160	4160	
86	40	400 0	400		86	1880	2040	2400	
87	120	120	40 80		87	3760	2800	3120	
87	80	0	40		87	960	1960	3120	
89		nd	nd	not detected	89	4200	3900	2000	
90	160	40	40	not detected	90	3320	4840	4040	
90	200	600	40 0		91	6400	2200	5000	
92	0	102	102		92	1990	2653	1786	
93	40	280	200		93	3920	3600	3520	
	40 0	160	80			2800	2000	2400	
94 95	40	100	120		94	2800	1760	2400	
	200	0				2840	2080	1760	
96	200	200	100		96	1300	4400	4500	
97	80	40	100		97 98	2320	3280	3240	
98 99	360	40	560		99	3960	1760	2640	
	60	440				1160	1560	1280	
100	185	224			100 101	1704	2984	2714	
101 102	185	113	297		101	1462	2984	1636	
	192	200							
103	40	200 160	160 120		103	2520 2640	6000 2160	4240 2960	
105		200	40		105		3160	2960	
106	320				106	4000	2880		
107	80 754	40	80		107	2000	2880	1440	
108	754	0			108	10053	10053	7540	
109	80	240	160		109	3920	3520	2800	
110	40	40	200		110	1160	1240	3440	
112	0	0			112	2400	1080	760	
113	0	40	40		113	2240	2120	1920	
114	99	0			114	990	1386	1881	
115	20	0	0		115	840	880	540	

ANNEX VIII: Analysts' results 1-54 Gonyaulax spinifera + Azadinium spinosum

Analyst code	GSPIN 1	GSPIN 2	GSPIN 3	Identification flag	Analyst code	ASPIN 1	ASPIN 2	ASPIN 3	Identification flag
1	4040	5600	4280		1	6600	14800	12120	
3	9920	7440	6400	l.polyedrum	3	960	800	80	Azadinium/heterocpasa
4	5080	7000	5760		4	5600	11960	3640	Heterocapsa sp.
5	6080	3200	2800		5	6520	6480	1120	sp.
8	6082	4926	4291		8	7537	7407	6903	
9	7360	8000	5920	l.polyedrum	9	17640	19400	14640	Azadinium/heterocpasa
10	6120	6280	7280		10	9200	7880	9640	sp.
11	5900	4000	7600	sp.	11	4900	4600	9200	Azadinium/heterocpasa
12	3520	4440	3400		12	2280	2840	3520	sp.
13	5280	6640	5280		13	7600	11200	9000	
14	3560	3840	6360	sp.	14	14800	4520	9760	Azadinium/heterocpasa
15	6200	7400	4040		15	8200	11200	9000	
16	4120	3640	4840		16	4480	3800	5000	Azadinium/heterocpasa
18	4320	6800	5760	G.polygramma	18	5400	5680	8000	Azadinium/heterocpasa
19	5050	5200		Scrippsiella spinifera	19	4950	3750	2000	
20	5957	5913	4565		20	10348	11609	6218	sp.
21	4960	6000	8360		21	6120	8640	15560	
24	3920	4960	3760		24	2760	4360	4480	Azadinium/heterocpasa
25	5120	6000	5440		25	6480	8200	8160	sp.
26	4120	6880	4760	l.polyedrum	26	8560	14200	11040	Azadinium/heterocpasa
27	12566	12566	5026		27	16085	16085	28148	
28	7250	5500	5875		28	11125	8375	9250	
29	5600	4360	4120		29	5720	4680	8000	Azadinium/heterocpasa
30	5200	5040	6080		30	7520	9240	10880	
31	2400	3080	7000		31	2600	4920	11200	Azadinium/heterocpasa
32	6960	6200	5840	sp.	32	10400	8120	7040	sp.
33	7920	6120	7040		33	8480	6720	8800	
34	5680	3880	7040		34	5320	5080	11520	sp.
35	6120	5880	5520		35	8440	10320	12680	
36	8920	9238	8601		36	15291	11468	10194	
37	4680	7480	5480		37	4320	9840	6880	Azadinium/heterocpasa
38	5020	6600	4540		38	6500	7600	5540	
39	6000	7720	6440	sp.	39	11080	14160	14000	sp.
40	4360	4560	4040		40	12000	11160	13560	Azadinium/heterocpasa
42	7700	7900	8500	sp.	42	6800	5400	4400	Azadinium/heterocpasa
43	11667	3666	41666	Alexandrium/scripps	43	6667	666	3333	Azadinium/H.illdefina
44	7560	5560	6440		44	5160	7480	6240	
46	5640	6280	5720	l.polyedrum	46	13040	17520	15200	
47	3800	3600	1800		47	3960	5440	7560	Azadinium/heterocpasa
48	4600	4100	4300	Scrippsiella spinifera	48	2500	3850	5450	
49	5600	3500	4200		49	6300	4900	14000	Heterocapsa sp.
50	4120	5440	3360	sp.	50	4960	7240		Azadinium/heterocpasa
51	6400	7120	7720		51	8720	9760	8160	sp.
52	7550	6800	6600		52	17000	14500	12200	
53	2000	3000	5520		53	2360	7400	9040	Azadinium/heterocpasa
54	1200	1960	2160	l.polyedrum	54	1240	2400	3320	Heterocapsa sp.

ANNEX VIII: Analysts' results 55-115 Gonyaulax spinifera + Azadinium spinosum

Analyst code	GSPIN 1	GSPIN 2	GSPIN 3	Identification flag	Analyst code	ASPIN 1	ASPIN 2	ASPIN 3	Identification flag
56	6880	6800	6400		56	20400	20640	19520	sp.
57	3440	4400	4720		57	1520	2840	3200	Azadinium/heterocpasa
58	3440	6720	7320		58	5200	10400	13600	Azadinium/heterocpasa
59	6044	5261	6870		59	5957	9957	13131	
60	1866	4577	4104		60	3918	8456	9562	sp.
61	4560	4520	5760		61	2920	2840	8120	sp.
62	6560	5120	6320	sp.	62	9520	12200	14160	
63	6120	6520	6600		63	14000	20320	15600	
64	7680	6880	7320		64	9840	6360	13840	Azadinium/heterocpasa
65	4360	7280	6400	sp.	65	10400	12480	10600	sp.
66	3800	5200	6900		66	12000	10500	10800	Azadinium/heterocpasa
67	7160	6960	7440	sp.	67	3280	8400	11000	Azadinium/heterocpasa
70	6800	5360	6360	Scrippsiella trochoidea	70	4960	7720	6880	Azadinium/heterocpasa
71	5640	6000	5600		71	7600	6680	7560	sp.
72	7240	5520	7360		72	15720	8240	16200	Azadinium/heterocpasa
73	5680	5720	6320	sp.	73	5200	5668	5216	Amphidoma languida
74	5600	7840	6240	sp.	74	6360	11160	4920	Azadinium/heterocpasa
76	7760	7160	5920		76	13440	19720	12360	sp.
77	6340	5880	6020		77	10320	5780	7400	sp.
78	6360	7000	7600		78	3480	6440	3200	
79	9520	8800	4160		79	17920	15280	8320	
80	9780	8400	9120		80	20100	22200	16380	
82	5300	2200	3100		82	3400	1900	2000	
85	8000	8560	6880		85	17760	21560	11160	
86	2040	2040	4280		86	2960	7480	6800	Azadinium/heterocpasa
87	6120	4880	6240		87	13511	15343	14198	
88	4840	4080	4640		88	1200	1720	3760	
89	7300	6300	5500	sp.	89	4500	4500	2700	Azadinium/heterocpasa
90	5240	6760	6800		90	5080	5120	7280	Azadinium/heterocpasa
91	6800	7200	7200		91	18000	7600	29400	Azadinium/heterocpasa
92	7296	6122	5153		92	16735	12041	14325	
93	6040	6840	7200	sp.	93	8080	8880	10760	sp.
94	4800	4000	4800	l.polyedrum	94	4600	1600	4400	Azadinium/heterocpasa
95	4560	4960	5080		95	7400	9440	11160	
96	5160	4200	6600		96	7480	5080	5720	
97	5900	8700	9000		97	14700	17200	28400	
98	2840	5120	7200		98	4520	7400	9640	
99	5840	4200	5560		99	10360	7000	5960	Azadinium/heterocpasa
100	3820	5160	4720	sp.	100	6510	6324	5580	A.languida
101	3778	6493	4164		101	6656	6095	6275	Azadinium/heterocpasa
102	6231	5865	5613	sp.	102	6112	9389	11605	
103	6360	7600	7520		103	9640	10760	17560	H.rotundata
105	6000	6120	6200		105	10480	11800	11160	
106	6480	6480	5840		106	4840	4040	3120	
107	4360	5360	4360	sp.	107	7160	10000	6960	
108	15079	17592	10053		108	24127	16085	8042	
109	5720	5920	6000		109	9240	11280	9600	
110	3800	3720	5480		110	2760	4160	15400	
112	6000	2240	3480		112	6360	2560	2520	Azadinium/heterocpasa
113	1080	3640	5120		113	400	640	2600	Azadinium/heterocpasa
114	2574	3564	3762		114	3267	3267	4158	
115	1240	1440	1000	Scripsiella sp.	115	n.d.	n.d.	n.d.	not detected

ANNEX VIII: Analysts' results 1-54 Heterosigma akashiwo + Chaetoceros danicus

Analyst code	HAKA 1	HAKA 2	НАКА З	Identification flag	Analyst code	CDAN 1	CDAN 2	CDAN 3	Identification flag
1	8720	19920	17400		1	19720	20400	20760	
3	2480	1680	1280		3	10960	2640	1840	
4	4480	5560	3400		4	21520	17560	16760	
5	23800	11640	6000		5	16960	20160	9240	
8	6157	9963	9813		8	17463	15704	13321	
9	14040	13080	10280		9	13400	20360	18760	
10	12280	12480	12040		10	13000	13520	13400	
11	5200	2900	11600		11	18900	19100	23400	
12	7000	8600	8920		12	13400	14040	18640	
13	13000	14960	13920		13	15600	17200	15640	
14	21440	6400	17160		14	18160	20480	16000	
15	11440	14720	12640		15	18200	20000	15080	
16	5280	4400	6080		16	20440	12280	16560	
18	2240	1600	4800		18	14840	15680	17040	
19	7750	6000	1700		19	17650	18600	13000	C.danicus/similis
20	11044	11696	8957		20	20001	18044	10827	
21	19320	12000	22360		21	22520	18440	21480	
24	4360	3720	3840		24	14000	20200	16840	
25	11040	12400	11440		25	5400	10760	13560	
26	6520	24040	11440	F.japonica	26	16560	16640	12960	
27	n.d	n.d	n.d	not detected	27	22619	27645	22619	
28	13250	9500	9750		28	19125	17625	19125	
29	8720	6000	9440		29	14400	15080	17200	
30	10640	15280	13840		30	17600	14440	17520	
31	2000	5560	16480		31	13760	20720	16080	
32	12360	7800	11040		32	18360	17360	16320	Phaeoceros
33	14080	13200	14880		33	19440	12720	16080	
34	6120	7080	14480		34	18400	23120	19360	
35	11000	7880	18840		35	22800	20120	19000	
36	15610	14973	8283		36	21344	24848	21026	
37	8200	23640	13760		37	16720	18880	15920	
38	11060	11020	7360		38	15820	18300	15460	
39	11400	18280	18320		39	19200	22360	20200	
40	16840	9840	16800		40		13960	16720	
42	4600	4100	5500		42		24000	19700	
43	18333	6333	25000		43		0	15000	
44	12480.0	17120.0	16640.0		44		11200	11080	
46	14720	21920	18880		46		18720	20640	
47	11840	7480	8400		47		13760	11720	
48	3400	6000	6650		48		17550		C.danicus/similis
49	2100	700	700		49		11900	17500	Phaeoceros
50	7840	2640	2320		50		15480	12000	
51	11440	11880	10160		51		14800	12520	
52	26200	13650	15400		52		22350	19800	
53	2960	7040	13520		53		12800	18880	
54	n.d	n.d	n.d	not detected	54	13600	16000	14200	

ANNEX VIII: Analysts' results 55-115 Heterosigma akashiwo + Chaetoceros danicus

Analyst code	HAKA 1	HAKA 2	НАКА З	Identification flag	Analyst code	CDAN 1	CDAN 2	CDAN 3	Identification flag
56	35640	39600	35480		56	18040	17600	17760	
57	2480	5640	4680		57	9640	9440	9720	
58	4680	18000	20800		58	12760	18720	19640	
59	7435	6131	11044		59	17783	19957	17479	
60	10336	17184	18061		60	15229	15808	13694	
61	7280	3440	11640		61	16280	17960	19600	
62	12160	10680	13400		62	18200	21280	19880	
63	14040	23840	26600		63	14920	17200	16800	
64	9480	5720	14960		64	19320	22528	19120	
65	13600	21680	13520	sp.	65	13760	22520	18280	
66	15500	16800	13600		66	17200	18100	22300	
67	5640	12600	38440		67	19200	12600	20560	
70	8120	7000	6680		70	20400	19360	20480	
71	12440	11600	11720		71	9680	16800	10400	
72	22560	7440	14640		72	18560	15760	15880	
73	3440	4520	4960		73	18080	14392	16776	
74	8520	12040	14560		74	16040	17080	14360	
76	23400	28320	12480		76	18120	23840	20440	
77	10520	11180	7900		77	20520	16720	13440	
78	4360	7800	8120		78	8760	14880	10640	
79	30880	14880	13440		79	21120	17120	13920	
80	19080	27900	16800		80	23160	25020	22020	
82	1200	800	100		82	29091	25455	25455	
85	27000	40360	27160		85	18160	18280	18520	
86	3800	8120	7200		86	8240	15240	19160	
87	21984	16030	20381		87	21297	21526	20152	
88	2240	2600	3040		88	13440	15760	12640	
89	2500	4200	2500		89	21400	19400	18400	
90	3720	3800	5760		90	18640	22920	21440	
91	27000	7200	36600		91	20200	19800	20000	
92	17551	7959	15000		92	19082	20918	15561	
93	19720	21000	18280		93	20160	9800	16560	
94	5200	2000	4000		94	17600	12400	14600	
95	13280	14720	14640		95	21040	21120	19600	
96	6080	4240	4960		96	16880	16360	17480	
97	12100	18600	33100		97	10100	15800	21000	Atheya sp.
98	10280	11360	6600		98	15240	15840	18840	
99	29240	15240	7760		99	18440	16960	17080	
100	5160	4440	4320		100			15624	
101	5244	19912	10729		101			13309	
102	5239	19417	10339		102			11450	
103	8360	14480	15120		103			28640	Phaeoceros
105	14080	17880	15400		105			18520	
106	6800	9640	5520		106			11280	
107	9880	11960	10160		107			16240	
108			n.d	not detected	108			20106	
109	16080	14040	13760		109		15840	16080	
110	2600	4160	22440		110			17800	
112	8360	3440	4640		112			15120	
113	1840	2880	4080		113			18320	
114	5940	8910	5247		114			14256	
115	3840	1160	1180		115	3020	3640	3380	

ANNEX VIII: Analysts' results 1-54 Corethron hystris + Chaetoceros curvisetus

Analyst code	CHYS 1	CHYS 2	CHYS 3	Identification flag	Analyst code	CCURV 1	CCURV 2	CCURV 3	Identification flag
1	2200	2400	2800		1	8920	15320	10240	
3	1840	1040	1120	criophilum	3	n.d.	n.d.	n.d.	not detected
4	2440	2360	1920	criophilum	4	11320	8640	6000	Hyalochates
5	1760	2000	1480		5	7280	4200	3200	Hyalochates
8	2500	2444	2425	criophilum	8	12425	10926	9888	Hyalochates
9	1320	1400	2320		9	6000	4520	9320	Hyalochates
10	1960	1440	1880		10	5520	7320	6760	Hyalochates
11	2200	3200	2700	criophilum	11	5000	5900	7700	
12	1720	1920	2040		12	2040	3080	3720	Hyalochates
13	2280	2000	2160		13	9880	15040	6000	
14	1080	1760	640		14	9400	4640	5600	
15	2360	2560	1280		15	9840	12080	10480	
16	2880	2360	2600		16	8800	6360	11640	Hyalochates
18	960	1600	2000		18	3440	9360	10000	
19	2100	2000	3150	criophilum	19	9700	8450	4400	C.brevis/curvisetus/debilis
20	1957	2044	1783		20	9435	10522	9305	Hyalochates
21	2880	1800	3320		21	6040	12360	13160	Hyalochates
24	2040	2640	2120	criophilum	24	4120	8800	7280	
25	1160	3320	2200		25	3480	4240	4080	Phaeoceros
26	2320	2720	2920		26	6920	10520	11280	socialis
27	2513	5026	2513		27	8294	17592	17592	C.coronatus/debilis
28	2750	2500	1750		28	13625	12000	11625	Hyalochates
29	1560	2520	2000		29	7680	5600	9240	
30	760	2320	2400		30	10360	9800	15240	
31	2800	3120	2560		31	2960	7360	15800	Hyalochates
32	2000	1920	2440		32	9920	5760	8240	Hyalochates
33	3400	2480	2280		33	5800	2800	4560	Phaeoceros
34	1720	1760	2080		34	3200	5680	7440	Hyalochates
35	2880	2400	3400	criophilum	35	5720	10600	10000	Hyalochates
36	1720	2040	2320		36	15928	17203	11787	Hyalochates
37	2040	1680	2440		37	6960	8280	8920	debilis
38	1040	1580	1500		38	2180	4840	5260	
39	2120	2840	2240		39	7640	11160	8640	Hyalochates
40	2640	2680	2320		40	12840	12000	13240	
42	1800	3000	2500	criophilum	42	6100	9600	7700	
43	15000	1000	28333	criophilum/hystris	43	45000	1667	106667	didymus
44	1280	1320	1680	criophilum	44	5800	4720	6720	Hyalochates
46	2360	1920	2160		46	10000	10320	9120	Phaeoceros
47	960	680	1480		47	4720	6360	5480	Hyalochates
48	2000	2900	2900	criophilum	48	7750	7600	11500	C.curvisetus/brevis
49	1400	2800	700		49	4200	1400	8400	Hyalochates
50	1240	2120	1960		50	6600	10120	7560	socialis
51	1760	2000	2080		51	5440	4480	6320	Hyalochates
52	1950	2500	2400		52		9950	13550	
53	1080	1960	2280		53		6600		Hyalochates
54	1120	1640	1120		54	2680	3440	3560	

Analyst code	CHYS 1	CHYS 2	CHYS 3	Identification flag	Analyst code	CCURV 1	CCURV 2	CCURV 3	Identification flag
56	2120	2200	2440		56	8560	5760	7200	
57	1160	1120	1760		57	1760	3000	3120	Hyalochates
58	1680	1880	2080		58	4160	4520	7600	
59	2304	2783	1739		59	8087	12522	13696	Hyalochates
60	1567	923	1194		60	5821	11183	13280	Hyalochates
61	2720	2160	2360		61	6640	4320	9240	Hyalochates
62	2160	2440	2720		62	4320	4280	7000	
63	920	1600	1760	criophilum	63	11920	11080	8640	Hyalochates
64	2880	1960	2520		64	8520	6040	14280	
65	2200	2280	2360		65	8600	8080	8160	
66	2700	1800	2900	criophilum	66	7000	8400	16000	
67	1480	2000	1360		67	11000	7160	10960	Hyalochates
70	2440	2440	2280	criophilum	70	nd	nd	nd	not detected
71	2040	2960	1760		71	3280	3760	4400	Hyalochates
72	2640	1560	1720		72	10480	8000	7240	
73	2080	1400	2240		73	9600	2492	6800	Hyalochates
74	1200	1640	1440		74	4240	3320	8440	Hyalochates
76	1840	2720	2760		76	9600	11960	6880	Hyalochates
77	1080	1600	1980		77	6660	6740	5860	
78	1520	1520	1440		78	2040	3400	2120	Hyalochates
79	2720	2080	1920		79	16320	10000	4000	debilis
80	2160	2700	2880		80	19980	20220	19980	
82	1200	800	500	criophilum	82	21818	18182	10909	brevis
85	2480	2840	2360		85	18120	17800	12800	
86	720	1400	1480		86	1840	5760	5320	
87	1040	920	1360		87	13282	7557	13053	Hyalochates
88	2320	2400	1760		88	3360	4320	4400	Hyalochates
89	2400	2900	3100	criophilum	89	11500	6800	8300	
90	3040	2400	2600		90	7200	7680	13400	Hyalochates
91	2200	1000	2400		91	15800	12400	18800	
92	7296	6122	5153		92	17959	5153	12806	
93	3760	3280	3400		93	5240	5600	4360	Hyalochates
94	3000	1800	2200		94	4400	1400	4400	Hyalochates
95	2040	2160	2360		95	12040	11920	11520	Hyalochates
96	2400	2680	1960		96	11120	12360	10640	Hyalochates
97	3200	2700	2300		97	22600	21300	20900	danicus
98	1760	2440	2880		98	6280	5240	14880	Hyalochates
99	800	1480	1200		99	16320	14880	20560	Hyalochates
100	1440	2640		criophilum	100	6000	4640	5880	Hyalochates
101	1148	1231	1041	criophilum	101	6453	2641	11537	Hyalochates
102	2000	1203	1227		102	5894	11096	16036	Hyalochates
103	2480	2960	3280		103	7880	12680		Hyalochates
105	2560	2800		criophilum	105	7000	9240	10000	
106	1840	2240	1480		106	5400	3600	3600	Phaeoceros
107	1160	2560		criophilum	107	6520	9320	5560	diadema
108	2513	5026	2513		108	13320	15079	10807	C.coronatus/debilis
109	2800	1920	2240	criophilum	109	5440	7400		Hyalochates
110	3200	2680	2200		110	7160	6520	12880	Hyalochates
112	2840	2160	1800		112	7840	4840	3480	Hyalochates
113	2520	2480	2160		113	3200	4080	6600	Hyalochates
114	1782	1782	1089		114	3960	3465	5643	
115	520	8000	300		115	560	560	840	Hyalochates

ANNEX VIII: Analysts' results 1-54 P.seriata complex + Thalassiosira tenera

Analyst code	PSER 1	PSER 2	PSER 3	Identification flag	Analyst code	TTEN 1	TTEN 2	TTEN 3	Identification flag
1	47480	73720	94280		1	15080	15720	13240	
3	41280	12800	19680		3	7440	6240	6000	sp.
4	80560	109480	59840	P. delicatissima group	4	13920	8240	11680	sp.
5	70080	33840	36000		5	12800	10680	6480	T. pacifica
8	74552	71519	63806		8	9590	9370	11194	sp.
9	43160	78240	65720		9	15200	15640	16240	sp.
10	75440	88120	86800		10	8800	7880	10120	sp.
11	108300	100700	146500		11	10300	5000	8300	sp.
12	59200	68000	94040	P. seriata	12	11360	11760	10120	
13	42000	60000	36800	P. fraudulenta	13	12600	11600	9600	T.rotula/gravida
14	146880	63120	118320	P.caliantha	14	14040	4960	5920	sp.
15	52000	49680	51600	P. fraudulenta	15	12600	14200	10400	T. rotula/gravida
16	81880	69200	88040		16	11720	11560	10480	sp.
18	78200	55320	59680		18	5040	5000	8720	T. aestivalis
19	76200	66550	27050		19	9650	8150	8050	
20	45219	47132	42176		20	13783	14957	6218	sp.
21	30640	44400	65720		21	11920	10360	15680	T. eccentrica
24	40760	43920	67320		24	9080	11720	6520	sp.
25	26720	36360	35160		25	5600	11000	7360	sp.
26	41400	63120	63120	P. fraudulenta	26	13320	15400	9480	sp.
27	120634	113094	113094	P.multiseries	27	12566	10053	10053	Coscinodiscus sp.
28	32375	37875	35625	P. delicatissima group	28	13000	16875	12250	sp.
29	40400	30400	43440		29	9160	12160	12000	sp.
30	42000	50960	56160	P. fraudulenta	30	11400	10920	13760	T. rotula/gravida
31	11960	30720	78320		31	8640	9840	12920	T.pacifica
32	41320	29520	31680		32	13680	12520	12560	sp.
33	63000	63720	92280		33	14250	13020	9640	sp.
34	60240	75065	133398		34	12000	12080	15440	sp.
35	50680	62160	59600		35	13920	12280	14760	sp.
36	71997	46511	67218	P. fraudulenta	36	13380	21981	16247	sp.
37	31360	76480	80720		37	13480	13600	12440	T. eccentrica
38	40160	37540	27120		38	8580	11080	11480	T. eccentrica
39	40800	79120	81040	P. seriata	39	14200	14880	8640	sp.
40	99400	69200	47720		40	12400	13000	11240	sp.
42	124800	116300	126400		42	8900	11300	14100	sp.
43	68334	28666	331667	P.pungens/seriata	43	35000	5000	65000	Actynoptychus/cyclus
44	79400	85680	89920		44	11520	7200	7120	sp.
46	48640	64720	86080		46	14640	18080	14880	sp.
47	43400	38480	62400		47	6120	10360	10720	sp.
48	42600	48550	98850		48	9400	9500	11500	sp.
49	21000	30800	76300	P. seriata	49	7000	4900	10500	sp.
50	57640	79400	47680		50	3120	5280	7920	sp.
51	73640	79600	76760		51	12320	12200	8760	sp.
52	65900	94100	49500	P. fraudulenta	52	15500	15250	16550	sp.
53	17640	34560	115920		53		7240		-
54	19440	43400	36680		54	9320	10080	9680	sp.

ANNEX VIII: Analysts' results 55-115 P.seriata complex+ Thalassiosira tenera

Analyst code	PSER 1	PSER 2	PSER 3	Identification flag	Analyst code	TTEN 1	TTEN 2	TTEN 3	Identification flag
56	176715	187803	181566	identification hag	56	13440	13640	14120	•
57	18720	43320	36440		57	7920	5680	5480	•
58	23600	68000	108000		58	5800	13200	12400	
59	60394	46567	54393		59			12392	-
60	43507	60000	102000		60	6530	9272	8234	-
61	103080	65880	113440		61	3160	9000	15800	-
62	55920	76000	109720		62				
63	100720	105120	81240		63	11600	10800	12080	•
64	39008	52736	63240		64	16640	14280	16320	sp.
65	40320	80080	47840		65	11200	14400		-
66	59200	59100	95400	P. fraudulenta	66	16800	17200	9100	sp.
67	11880	112560	112320		67	6960	7360	11760	sp.
70	64600	60560	58480		70	13440	14680	15400	T. anguste-lineata
71	80000	76320	72200		71		12600		
72	72880	32360	70880		72		13720	12400	
73	47600	19492	29240		73	5664	10992	10200	
74	36360	39760	47600	P. delicatissima group	74	6800	9080	9200	-
76	72280	87440	42240		76	14360	16480		T. pacifica
77	37240	71600	63560		77	11620	9360	9560	T. pacifica
78	66880	95800	86000		78	7000	9360	7440	
79	57750	47600	46200	P. fraudulenta	79	18160	15120	11040	sp.
80	87648	79200	90425		80	19740	17160	16680	sp.
82	134545	138182	144242	P. australis	82	14545	7273	7273	sp.
85	29760	49120	39520	P. fraudulenta	85	9640	13320	8400	T. eccentrica
86	24480	57880	54920		86	2840	5800	13640	sp.
87	129156	54273	70761		87	11450	17862	17404	sp.
88	86320	81560	118800		88	5440	8400	8720	sp.
89	110200	113600	92700		89	11300	9600	7600	sp.
90	66000	52080	100000		90	12040	14960	13120	sp.
91	66400	71000	122600	P. fraudulenta	91	9600	14400	16600	sp.
92	117449	96224	108265		92	11224	10306	13214	sp.
93	105240	64840	65240	P. seriata	93	9520	10880	14280	sp.
94	34400	12000	41200		94	9600	4800	10800	sp.
95	67040	56480	46400		95	11560	14040	13080	T. pacifica
96	94080	85200	84600		96	13200	8240	11200	sp.
97	27100	49700	112700	P. fraudulenta	97	5300	15400	16300	
98	71120	71840	106080		98	9440	11160	15120	sp.
99	30000	10120	11040		99		5480	7520	
100	31620	33108	32736		100		10520	11120	sp.
101	52841	77211	56479		101		14630	9717	-
102	28816	68494	38191		102		18651		•
103	46240	66000	64400		103		14320		
105	41600	56160	67920		105		12960		
106	72680	81120	72000		106			8640	•
107	43560	68240	42680		107				T. rotula/gravida
108	118120	105554		P.multiseries	108		12566		Coscinodiscus sp.
109	55680	47680	44080		109		10640	9600	
110	44040	17920	62000		110		13360		
112	100400	53120	48640		112		8560	5200	
113	85440	60360	83880		113			12000	
114	86526	45342	72666		114		10395		
115	20240	13580	15960		115	1840	1260	1020	sp.

to algorithm A annex C ISO13528 Akashiwo sanguinea iteration

Homogeneity and stab	ility test IP	12019					
Akashiwo sanguinea							
					sample		_
	Date	Sample	M1	M2	a ve ra ge		*2
	14/10/2019	water19	720	600	660	120	14400
	14/10/2019	water19	200	360	280	160	2560
	14/10/2019	water19	400	320	360	80	640
	14/10/2019	water19	560	440	500	120	1440
	14/10/2019	water19	600	560	580	40	160
	14/10/2019	water19	440	640	540	200	4000
	14/10/2019	water19	640	400	520	240	5760
	14/10/2019	water19	360	480	420	120	1440
	14/10/2019	water19	520	760	640	240	5760
	14/10/2019	water19	360	240	300	120	1440
				Average:	480	Sum	24640
				SD	135	P=	1
			SD within s	amples:	111		
			SD between	n samples:	110		
		Sample	Test	Test	sample	test portion	
	Date	number	portion 1	portion 2	average	range	*2
	20/112019	water19	400	360	380	40	160
	20/112019	water19	520	440	480	80	640
	20/112019	water19	280	400	340	120	1440
	-,			Average:	400	Sum	2240
				SD	72		
			SD within s		61		
			SD between		58		

Analysts iteration for Akashiwo sanguinea

Average X	132		119	119	119
SD S	88		62	62	62
robust average X*	107	newX*	119	119	119
robust stdev S*	69	new S*	71	70	70
δ= 1.5S*	104		106	106	105
Χ*-δ	3		13	14	14
Χ*+δ	210		225	225	225
no of analysts P	92		92	92	92
Between Samples SD	110				
new stdev for ASANG	130				

to algorithm A annex C ISO13528 Prorocentrum micans iteration

Homogeneity and stabili	ty test IPI2	019						
Prorocentrum micans	CELLS / L							
						sample		
		Date	Sample	M1	M2	average		*2
		14/10/2019	water19	5200	5080	5140	120	1440
		14/10/2019	water19	4760	5760	5260	1000	100000
		14/10/2019	water19	5720	4840	5280	880	77440
		14/10/2019	water19	5360	5320	5340	40	160
		14/10/2019	water19	4720	5800	5260	1080	116640
		14/10/2019	water19	5200	4840	5020	360	12960
		14/10/2019	water19	4400	3640	4020	760	57760
		14/10/2019	water19	5600	4560	5080	1040	108160
		14/10/2019	water19	4200	4240	4220	40	160
		14/10/2019	water19	4280	4360	4320	80	640
					Average:	4894	Sum	475360
					SD	503	P=	1
				SD within s	amples:	488		
				SD between	n samples:	366		
							Between	
							test	
			Sample	Test	Test	sample	portion	
		Date	number	portion 1	portion 2	average	range	*2
		20/112019	water19	5200	6000	5600	800	64000
	CELLS / L	20/112019	water19	4640	5520	5080	880	77440
		20/112019	water19	4720	6000	5360	1280	163840
					Average:	5347	Sum	305280
					SD	260	P=	
				SD within s	amples:	713		
				SD betweer	n samples:	432		

Analysts iteration for Prorocentrum micans

Average X	2893		2756	2756
SD S	1353		895	895
robust average X*	2763	newX*	2756	2756
robust stdev S*	984	new S*	1015	1015
δ= 1.5S*	1476		1522	1522
Χ*- δ	1288		1234	1234
Χ*+δ	4239		4278	4278
no of analysts P	98		98	98
Between Samples SD	366			
new stdev for PMICANS	1079			

Annex IX: Robust mean and Standard deviation calculation according to algorithm A annex C ISO13528 *Gonyaulax spinifera* iteration

Homogeneity and stab	ility test II	PI2019						
Gonyaulax spinifera	CELLS / L							
		Date	Sample	M1	M2	sample average		*2
		14/10/2019	water19	7880	7480	7680	400	16000
		14/10/2019	water19	6240	9000	7620	2760	761760
		14/10/2019	water19	6560	6760	6660	200	4000
		14/10/2019	water19	7080	7880	7480	800	64000
		14/10/2019	water19	8280	7600	7940	680	46240
		14/10/2019	water19	6800	8160	7480	1360	184960
		14/10/2019	water19	5480	5640	5560	160	2560
		14/10/2019	water19	5760	6080	5920	320	10240
		14/10/2019	water19	6040	5160	5600	880	77440
		14/10/2019	water19	5960	5040	5500	920	84640
					Average:	6744	Sum	1251840
					SD	1006	P=	1
				SD within s	amples:	791		
				SD betweer	n samples:	836		
							Between test	
			Sample	Test	Test	sample	portion	
		Date	number	portion 1	portion 2	average	range	*2
		20/112019	water19	6560	6720		160	2560
	CELLS / L	20/112019	water19	7520			1600	256000
		20/112019	water19	7840	7760	7800	80	640
					Average:	7053	Sum	259200
					SD	648	P=	
				SD within s	amples:	657		
				SD betweer	n samples:	451		

Analysts iteration for Gonyaulax spinifera

Average X	5841		5689	5689
SD S	2177		1324	1324
robust average X*		new X*	5837	5837
robust stdev S*		new S*	1583	1583
δ= 1.5S*	2375		2375	2375
Χ*-δ	3462		3462	3462
Χ*+δ	8211		8211	8211
no of analysts P	98		98	98
Between Samples SD	836			
new stdev for GSPIN	1790			

to algorithm A annex C ISO13528 Azadinium spinosum iteration

Azadinium spinosum	CELLS / L							
	01110 / 1					sample		
		Date	Sample	M1	M2	a ve ra ge		*2
		14/10/2019	water19	17360	12160	14760	5200	2704000
		14/10/2019	water19	20120	17680	18900	2440	595360
		14/10/2019	water19	16280	15600	15940	680	46240
		14/10/2019	water19	14200	17000	15600	2800	784000
		14/10/2019	water19	15600	18040	16820	2440	595360
		14/10/2019	water19	17320	13520	15420	3800	1444000
		14/10/2019	water19	13520	15240	14380	1720	295840
		14/10/2019	water19	11760	14200	12980	2440	595360
		14/10/2019	water19	12800	11440	12120	1360	184960
		14/10/2019	water19	12480	13520	13000	1040	108160
					Ave rage :	14992	Sum	7353280
					SD	2021	P=	1
				SD within s	amples:	1917		
				SD betwee	n samples:	1499		
							Between	
			Sample	Test	Test	complo	test	
		Date	number	Test portion 1	portion 2	sample average	portion range	*2
		20/112019	water19	16280			1040	108160
	CELLS / L	20/112019	water19	17680			1720	295840
		20/112019	water19	10720			2440	595360
				10,20	Average:	14840	-	999360
					SD	2567		555500
				SD within s		1291		
				SD betwee	•	2399		

Analysts iteration for Gonyaulax spinifera

Average X	8698		8156	8156
SD S	4521		3404	3404
robust average X*	7773	newX*	7773	7773
robust stdev S*	3577	new S*	3577	3577
δ= 1.5S*	5365		5365	5365
Χ*- δ	2408		2408	2408
Χ*+δ	13139		13139	13139
no of analysts P	97		97	97
Between Samples SD	1499			
new stdev for ASPIN	3878			

				0				
Homogeneity and stabilit	y test IPI20	019						
Heterosigma akashiwo	CELLS / L							
		Date	Sample	M1	M2	sample average		*2
		14/10/2019	water19	28440	29880	29160	1440	207360
		14/10/2019	water19	45800	42000	43900	3800	1444000
		14/10/2019	water19	39520	44760	42140	5240	2745760
		14/10/2019	water19	30880	36760	33820	5880	3457440
		14/10/2019	water19	40560	29840	35200	10720	11491840
		14/10/2019	water19	38840	30520	34680	8320	6922240
		14/10/2019	water19	34680	35360	35020	680	46240
		14/10/2019	water19	31200	38480	34840	7280	5299840
		14/10/2019	water19	29840	31560	30700	1720	295840
		14/10/2019	water19	30880	29120	30000	1760	309760
					Ave rage :	34946	Sum	32220320
					SD	4823	P=	1
				SD within s	amples:	4014		
				SD between	n samples:	3899		
							Between test	
		Date	Sample number	Test portion 1	Test portion 2	sample average	portion range	*2
		20/112019	water19	28800	30880	29840	2080	432640
	CELLS / L	20/112019	water19	34000	29480	31740	4520	2043040
		20/112019	water19	29480	34000	31740	4520	2043040
		20/112019	water19	25400	Average:	31740		4518720
					SD	1097		4318720
				SD within s	-	2744	r -	
					•	1601		
				SD between	i sampies:	1001		

to algorithm A annex C ISO13528 Heterosigma akashiwo iteration

Analysts iteration for Heterosigma akashiwo

Average X	11435		11145	11357	11357
SD S	6418		5479	5652	5652
robust average X*	11627	new X*	11145	11357	11357
robust stdev S*	6169	new S*	6213	6410	6410
δ= 1.5S*	9254		9320	9614	9614
Χ*- δ	2373		1825	1743	1743
Χ*+δ	20881		20465	20972	20972
no of analysts P	95		95	95	95
Between Samples SD	3899				
new stdev for HAKA	7502				

Homogeneity and stability	y test IPI2019							
Chaetoceros danicus	CELLS / L							
		Date	Sample	M1	M2	sample average		*2
		14/10/2019	water19	11320	11960	11640	640	409600
		14/10/2019	water19	16320	16640	16480	320	102400
		14/10/2019	water19	13360	12240	12800	1120	1254400
		14/10/2019	water19	16160	13760	14960	2400	576000
		14/10/2019	water19	12400	12560	12480	160	25600
		14/10/2019	water19	15520	12640	14080	2880	8294400
		14/10/2019	water19	12560	12640	12600	80	6400
		14/10/2019	water19	10400	13440	11920	3040	9241600
		14/10/2019	water19	15200	13040	14120	2160	4665600
		14/10/2019	water19	14880	12400	13640	2480	6150400
					Average:	13472	Sum	35910400
					SD	1492	P=	10
				SD within s	amples:	1340		
				SD between	n samples:	1153		
			Sample	Test	Test	sample	Between test portion	
		Date	number	portion 1	portion 2	a ve ra ge	range	*2
		20/112019	water19	14960	15920	15440	960	921600
	CELLS / L	20/112019	water19	14320	14560	14440	240	5760
		20/112019	water19	13600	11680	12640	1920	368640
					Average:	14173	Sum	466560
					SD	1419	P=	
				SD within s	amples:	882		
				SD betweer	n samples:	1275		

to algorithm A annex C ISO13528 Chaetoceros danicus iteration

Analysts iteration for Chaetoceros danicus

Average X	16812		16963	16963
SD S	3803		2563	2563
robust average X*	16840	newX*	16840	16840
robust stdev S*	2639	new S*	2639	2639
δ= 1.5S*	3958		3958	3958
Χ*- δ	12882		12882	12882
Χ*+δ	20798		20798	20798
no of analysts P	98		98	98
Between Samples SD	1153			
new stdev for CDAN	2879			

			1					
Homogeneity and st	ability tes	t IPI2019						
Corethron Hystris	CELLS / L							
						sample		
		Date	Sample	M1	M2	a ve ra ge		*2
		14/10/2019	water19	2480	2240	2360	240	5760
		14/10/2019	water19	1720	2280	2000	560	31360
		14/10/2019	water19	2520	1760	2140	760	5776
		14/10/2019	water19	2120	2080	2100	40	160
		14/10/2019	water19	2360	2480	2420	120	1440
		14/10/2019	water19	1880	2280	2080	400	1600
		14/10/2019	water19	2400	2160	2280	240	576
		14/10/2019	water19	2680	1920	2300	760	5776
		14/10/2019	water19	1880	1840	1860	40	16
		14/10/2019	water19	1880	1680	1780	200	400
					Average:	2132	Sum	18016
					SD	212	P=	
				SD within s	amples:	300		
				SD betweer	•	12		
							Between	
							test	
			Sample	Test	Test	sample	portion	
		Date	number	portion 1	portion 2	a ve ra ge	range	*2
		20/112019	water19	2880	2160	2520	720	5184
	CELLS / L	20/112019	water19	2320	2400	2360	80	64
		20/112019	water19	2600	2680	2640	80	64
					Ave rage:	2507	Sum	53120
					SD	140	P=	
				SD within s	amples:	298		
				SD betweer	n samples:	157		

to algorithm A annex C ISO13528 Corethron hystris iteration

Analysts iteration for Corethron hystris

Average X	2280		2149	2145	2145	2145	2144
SD S	1447		495	488	486	486	486
robust average X*	2233	new <i>X*</i>	2149	2145	2145	2145	2144
robust stdev S*	554	new S*	561	553	552	551	551
δ= 1.5S*	830		841	830	827	827	827
Χ*- δ	1403		1308	1316	1317	1318	1318
Χ*+δ	3064		2990	2975	2972	2971	2971
no of analysts P	98		98	98	98	98	98
Between Samples SD	12						
new stdev for CHYS	551						

Homogeneity and stability t	test IPI2019							
Chaetoceros curvisetus	CELLS / L							
		Date	Sample	M1	M2	sample average		*2
		14/10/2019	water19	5760	9400	7580	3640	13249600
		14/10/2019	water19	6720	8440	7580	1720	2958400
		14/10/2019	water19	5440	5280	5360	160	25600
		14/10/2019	water19	9720	8880	9300	840	705600
		14/10/2019	water19	7840	6280	7060	1560	2433600
		14/10/2019	water19	7400	7320	7360	80	6400
		14/10/2019	water19	8600	5800	7200	2800	7840000
		14/10/2019	water19	6400	6880	6640	480	230400
		14/10/2019	water19	9280	7760	8520	1520	2310400
		14/10/2019	water19	10200	7920	9060	2280	5198400
					Average:	7566	Sum	34958400
					SD	1169	P=	10
				SD within s	amples:	1322		
				SD betweer	n samples:	702		
							Between test	
			Sample	Test	Test	sample	portion	
		Date	number	portion 1	portion 2	average	range	*2
		20/112019	water19	5360	8320	6840	2960	8761600
	CELLS / L	20/112019	water19	8480	6440	7460	2040	4161600
		20/112019	water19	8040	7120	7580	920	846400
					Average:	7293	Sum	13769600
					SD	397	P=	3
				SD within s	amples:	1515		
				SD betweer	n samples:	995		

to algorithm A annex C ISO13528 Chaetoceros curvisetus iteration

Analysts iteration for Chaetoceros curvisetus

Average X	8917		8320	8286	8273	8268	8266	8265	8264	8264	8264
SD S	5803		3305	3226	3192	3179	3173	3171	3170	3170	3170
robust average X*	8427	new X*	8320	8286	8273	8268	8266	8265	8264	8264	8264
robust stdev S*	3954	new S*	3748	3658	3620	3605	3598	3596	3595	3594	3594
δ=1.5S*	5932		5621	5487	5430	5407	5398	5394	5392	5391	5391
Χ*-δ	2495		2699	2799	2843	2861	2868	2871	2872	2873	2873
Χ*+δ	14358		13941	13772	13703	13675	13663	13658	13656	13656	13656
no of analysts P	96		96	96	96	96	96	96	96	96	96
Between Samples SD	702										
new stdev for CCURV	3662										

Homogeneity and stability test IPI2019								
Pseudo-nitzschia seriata complex	CELLS / L							
						sample		
		Date	Sample	M1	M2	a ve ra ge		*2
		14/10/2019	water19	140882	150598	145740	9716	94400656
		14/10/2019	water19	172806	154762	163784	18044	325585936
		14/10/2019	water19	181828	219998	200913	38170	1.457E+09
		14/10/2019	water19	187380	217222	202301	29842	890544964
		14/10/2019	water19	224162	208200	216181	15962	254785444
		14/10/2019	water19	229020	178358	203689	50662	2.567E+09
		14/10/2019	water19	155456	162396	158926	6940	48163600
		14/10/2019	water19	170724	210976	190850	40252	1.62E+09
		14/10/2019	water19	157538	148516	153027	9022	81396484
		14/10/2019	water19	200566	239430	219998	38864	1.51E+09
					Average:	185541	Sum	8.849E+09
					SD	27535	P=	10
				SD within s	amples:	21035		
				SD betweer	n samples:	23173		
							Between	
							test	
		Date	Sample number	Test portion 1	Test portion 2	sample	portion	*2
		20/112019	water19	134636		a ve rage 155109	range 40946	2 1.677E+09
	65116 (J			162396				
	CELLS / L	20/112019	water19				4858	23600164
		20/112019	water19	222774			33312	1.11E+09
					Average:	175351		2.81E+09
					SD	27085	P=	3
				SD within s		21640		
				SD between	n samples:	22348		

to algorithm A annex C ISO13528 Pseudo-nitzschia seriata complex iteration

Analysts results for Pseudo-nitzschia seriata complex

Average X	66517		64060	64102	64108	64108	64108
SD S	27533		20734	20660	20651	20650	20650
robust average X*	63010	new X*	64060	64102	64108	64108	64108
robust stdev S*	23728	new S*	23512	23428	23418	23417	23417
δ= 1.5S*	35592		35268	35142	35127	35125	35125
Χ*-δ	27418		28792	28960	28980	28983	28983
Χ*+δ	98602		99328	99245	99235	99234	99233
no of analysts P	98		98	98	98	98	98
Between Samples SD	23173						
new stdev for PSERGRUP	32944						

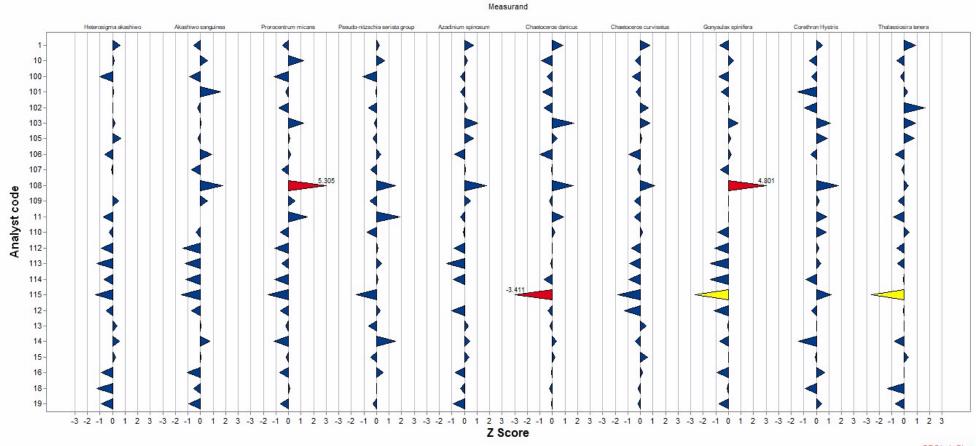
Homogeneity and stab	ility test II	PI2019						
Thalassiosira tenera	CELLS / L							
		Date	Sample	M1	M2	sample average		*2
		14/10/2019	water19	12560	12960	12760	400	16000
		14/10/2019	water19	14720	14000	14360	720	51840
		14/10/2019	water19	12880	12240	12560	640	40960
		14/10/2019	water19	14560	15120	14840	560	31360
		14/10/2019	water19	12360	13840	13100	1480	219040
		14/10/2019	water19	13200	15760	14480	2560	655360
		14/10/2019	water19	13120	13920	13520	800	64000
		14/10/2019	water19	13760	13040	13400	720	51840
		14/10/2019	water19	12160	12560	12360	400	16000
		14/10/2019	water19	13760	14320	14040	560	31360
					Average:	13542	Sum	1177760
					SD	861	P=	1
				SD within s	amples:	767		
				SD betweer	n samples:	668		
							Between test	
			Sample	Test	Test	sample	portion	
		Date	number	portion 1	portion 2	average	range	*2
		20/112019	water19	13280		13720	880	7744(
	CELLS / L	20/112019	water19	15200			1520	231040
		20/112019	water19	14080	12800	13440	1280	163840
					Average:	13867	Sum	472320
					SD	516	P=	
				SD within s	amples:	887		
				SD betweer	n samples:	357		

to algorithm A annex C ISO13528 Thalassiosira tenera iteration

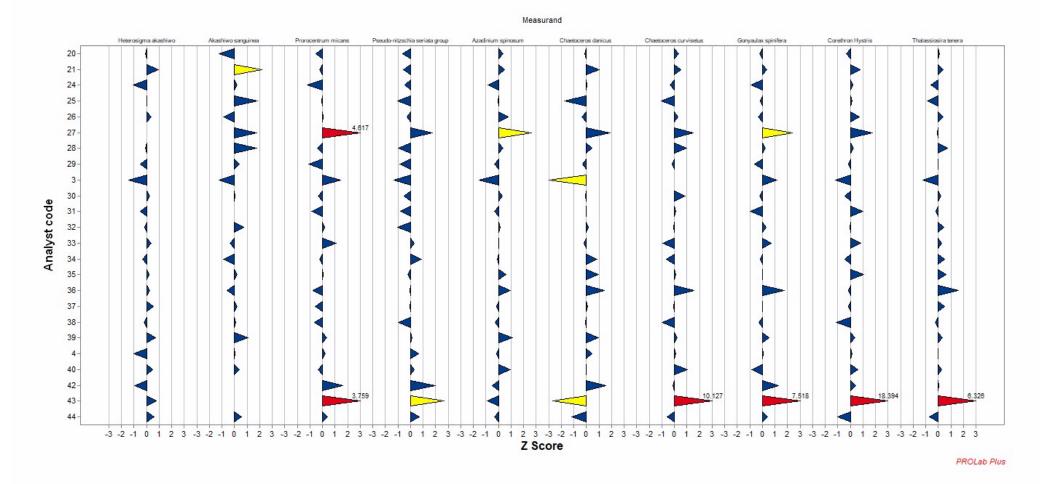
Analysts results for *Thalassiosira tenera*

Average X	11461		11288	11290	11289	11288	11288	11288	11288
SD S	3732		2591	2571	2564	2561	2560	2559	2559
robust average X*	11260	new X*	11288	11290	11289	11288	11288	11288	11288
robust stdev S*	3006	new S*	2939	2916	2907	2904	2903	2902	2902
δ= 1.5S*	4508		4408	4374	4361	4356	4354	4353	4353
Χ*-δ	6752		6880	6916	6928	6932	6934	6935	6935
Χ*+δ	15768		15696	15664	15650	15644	15642	15641	15641
no of analysts P	98		98	98	98	98	98	98	98
Between Samples SD	668								
new stdev for TTENERA	2978								

ANNEX X: Summary of Z-scores IPI2019 for all measurands pg1

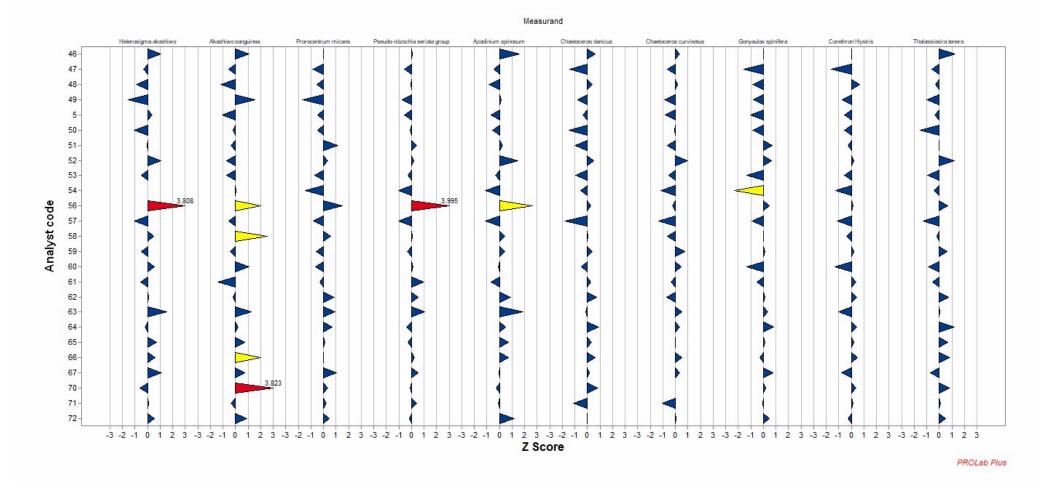


ANNEX X: Summary of Z-scores IPI2019 for all measurands pg2

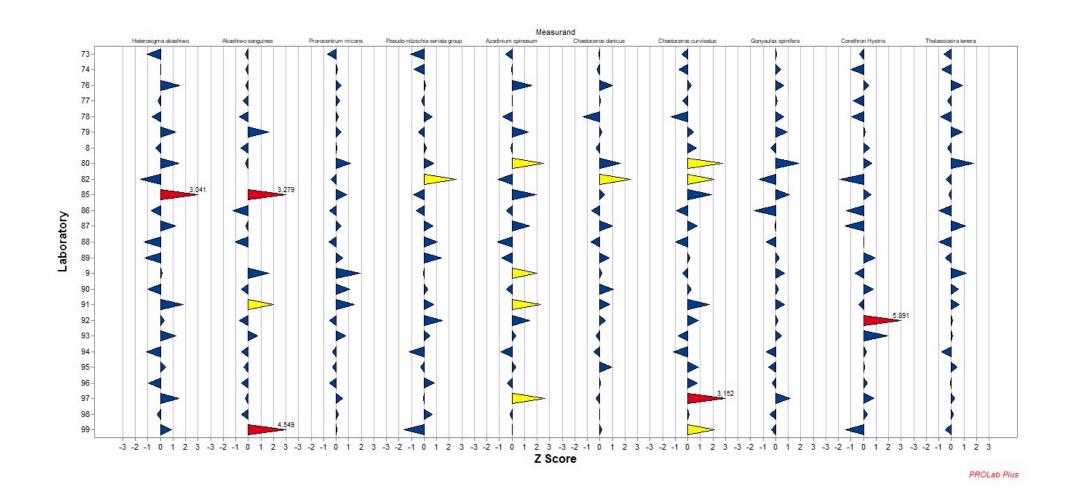


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ANNEX X: Summary of Z-scores IPI2019 for all measurands pg3



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Analyst code	Total	Within tolerance	%	Successful	Analyst code	Total	Within tolerance	%	Successful	Analyst code	Total	Within tolerance	%	Successful
65	10	10	100	Yes	16	10	10	100	Yes	67	10	10	100	Yes
39	10	10	100	Yes	61	10	10	100	Yes	114	10	10	100	Yes
93	10	10	100	Yes	96	10	10	100	Yes	90	10	10	100	Yes
8	10	10	100	Yes	112	10	10	100	Yes	36	10	10	100	Yes
102	10	10	100	Yes	88	10	10	100	Yes	38	10	10	100	Yes
101	10	10	100	Yes	62	10	10	100	Yes	77	10	10	100	Yes
60	10	10	100	Yes	14	10	10	100	Yes	98	10	10	100	Yes
95	10	10	100	Yes	100	10	10	100	Yes	50	10	10	100	Yes
40	10	10	100	Yes	24	10	10	100	Yes	49	10	10	100	Yes
105	10	10	100	Yes	73	10	10	100	Yes	58	10	9	90	Yes
29	10	10	100	Yes	10	10	10	100	Yes	42	10	9	90	Yes
64	10	10	100	Yes	4	10	10	100	Yes	11	10	9	90	Yes
72	10	10	100	Yes	71	10	10	100	Yes	89	10	9	90	Yes
47	10	10	100	Yes	25	10	10	100	Yes	66	10	9	90	Yes
44	10	10	100	Yes	12	10	10	100	Yes	31	10	9	90	Yes
86	10	10	100	Yes	51	10	10	100	Yes	92	10	9	90	Yes
19	10	10	100	Yes	106	10	10	100	Yes	21	10	9	90	Yes
48	10	10	100	Yes	78	10	10	100	Yes	9	10	9	90	Yes
32	10	10	100	Yes	33	10	10	100	Yes	54	10	8	80	Yes
103	10	10	100	Yes	76	10	10	100	Yes	3	10	8	80	Yes
110	10	10	100	Yes	5	10	10	100	Yes	70	10	8	80	Yes
18	10	10	100	Yes	87	10	10	100	Yes	97	10	8	80	Yes
20	10	10	100	Yes	1	10	10	100	Yes	91	10	8	80	Yes
59	10	10	100	Yes	30	10	10	100	Yes	80	10	8	80	Yes
63	10	10	100	Yes	15	10	10	100	Yes	85	10	8	80	Yes
26	10	10	100	Yes	13	10	10	100	Yes	99	10	8	80	Yes
94	10	10	100	Yes	35	10	10	100	Yes	108	10	7	70	No
46	10	10	100	Yes	109	10	10	100	Yes	56	10	6	60	No
52	10	10	100	Yes	107	10	10	100	Yes	115	10	6	60	No
79	10	10	100	Yes	34	10	10	100	Yes	82	10	6	60	No
74	10	10	100	Yes	57	10	10	100	Yes	27	10	6	60	No
28	10	10	100	Yes	37	10	10	100	Yes	43	10	2	20	No
113	10	10	100	Yes	53	10	10	100	Yes					

ANNEX XI: Performance statistics for the test IPI2019

ANNEX XII: S	Summary of labora	tory means + star	tistical parameters

Analyst code	Akashiwo sanguinea (cells/Litre)	Z-score	Prorocentrum micans (cells/Litre)	Z-score2	Ps. seriata complex (cells/Litre)	Z-score3	Azadinium spinosum (cells/Litre)	Z-score4	Chaetoceros danicus (cells/Litre)	Z-score5	Chaetoceros curvisetus (cells/Litre)	Z-score6	Gonyaulax spinifera (cells/Litre)	Z-score7	Corethron hystris (cells/Litre)	Z-score8	Thalassiosira tenera (cells/Litre)	Z-score9	Heterosigma akashiwo (cells/Litre)	Z-score 10
1	80	-0.5	2187	-0.4	71827	0.2	11173	0.7	20293	0.8	11493	0.7	4640	-0.6	2467	0.4	14680	0.9	15347	0.5
3	27	-1.2	4480	1.4	24587	-1.3	613	-1.5	5147	-2.9			7920	1.1	1333	-1.1	6560	-1.2	1813	-1.4
4	120	0	3067	0.2	83293	0.6	7067	-0.1	18613	0.4	8653	0	5947	0	2240	0.1	11280	0	4480	-1
5	40	-1	2200	-0.4	46640	-0.5	4707	-0.6	15453	-0.3	4893	-0.7	4027	-1	1747	-0.5	9987	-0.3	13813	0.3
8	74	-0.6	2853	0	69959	0.1	7282	-0.1	15496	-0.3	11080	0.6	5100	-0.4	2456	0.4	10051	-0.3	8644	-0.4
9		1.6	5107	1.9	62373	0	17227	2	17507	0.1	6613	-0.3	7093	0.7	1680	-0.6	15693	1.1	12467	0.16
10	160	0.5	4227	1.2	83453	0.6	8907	0.2	13307	-0.8	6533	-0.4	6560	0.4	1760	-0.5	8933	-0.6	12267	0.1
11	67	0.7	4567	1.4	118500	1.8	6233	-0.3	20467	0.9	6200	-0.4	5833	0	2700	0.8	7867	-0.9	6567	-0.7
12	67	-0.7	1987	-0.6	73747	0.3	2880	-1	15360	-0.3	2947	-1.2	3787	-1.1	1893	-0.3	11080	0	8173	-0.4
13	120	0	2467	-0.2	46267	-0.6	9267	0.3	16147	-0.1	10307	0.4	5733	0	2147	0	11267	-	13960	0.3
14 15	173 120	0.7 0	1333 2547	-1.1 -0.1	109440 51093	1.5 -0.4	9693 9467	0.4 0.3	18213 17760	0.3 0.2	6547 10800	-0.4 0.5	4587 5880	-0.7 0	1160 2067	-1.4 -0.1	8307 12400	-0.7 0.2	15000 12933	0.5
16	40	-1	1933	-0.1	79707	-0.4	4427	-0.7	16427	-0.1	8933	0.3	4200	-0.9	2613	-0.1	12400	0.2	5253	-0.9
10	80	-0.5	2893	0.1	64400	0.5	6360	-0.7	15853	-0.1	7600	-0.1	5627	-0.9	1520	-0.9	6253	-1.3	2880	-0.9
19	50	-0.9	1967	-0.6	56600	-0.2	3567	-0.8	16417	-0.1	7517	-0.1	4500	-0.7	2417	0.3	8617	-0.7	5150	-0.9
20	29	-1.2	2116	-0.5	44842	-0.6	9392	0.3	16291	-0.1	9754	0.3	5478	-0.2	1928	-0.3	11653	0	10566	-0.1
21	280	2.1	2493	-0.2	46920	-0.5	10107	0.4	20813	1	10520	0.5	6440	0.3	2667	0.7	12653	0.3	17893	0.9
24	133	0.1	1267	-1.2	50667	-0.4	3867	-0.8	17013	0	6733	-0.3	4213	-0.9	2267	0.1	9107	-0.5	3973	-1.1
25	253	1.8	2653	0	32747	-1	7613	0	9907	-1.7	3933	0	5520	-0.1	2227	0.1	7987	-0.8	11627	0
26	53	-0.8	2813	0	55880	-0.2	11267	0.7	15387	-0.3	9573	0.3	5253	-0.3	2653	0.7	12733	0.3	14000	0.3
27	251	1.8	8378	4.6	115607	1.7	20106	2.6	24294	1.8	14493	1.4	10053	2.4	3351	1.7	10891	-0.1		
28	250	1.7	2250	-0.4	35292	-0.9	9583	0.3	18625	0.4	12417	0.9	6208	0.2	2333	0.2	14042	0.7	10833	0
29	147	0.3	1413	-1.1	38080	-0.8	6133	-0.3	15560	-0.3	7507	-0.1	4693	-0.6	2027	-0.1	11107	0	8053	-0.4
30	120	0	2333	-0.3	49707	-0.4	9213	0.3	16520	0	11800	0.8	5440	-0.2	1827	-0.4	12027	0.1	13253	0.2
31			1707	-0.8	40333	-0.8	6240	-0.3	16853	0	8707	0.1	4160	-0.9	2827	0.9	10467	-0.2	8013	-0.4
32	173	0.7	2947	0.1	34173	-1	8520	0.1	17347	0.1	7973	0	6333	0.2	2120	0	12920	0.4	10400	-0.1
33	93	-0.3	4067	1	73000	0.3	8000	0	16080	-0.1	4387	-0.9	7027	0.6	2720	0.8	12303	0.2	14053	0.4
34	53	-0.8	2480	-0.2	89568	0.8	7307	-0.1	20293	0.8	5440	-0.6	5533	-0.1	1853	-0.4	13173	0.5	9227	-0.3
35	133	0.1	2813	0	57480	-0.2	10480	0.5	20640	0.9	8773	0.1	5840	0	2893	1	13653	0.6	12573	0.1
36 37	73 133	-0.6	1847 2013	-0.7 -0.6	61909 62853	0	12318 7013	0.9 -0.1	22406 17173	1.4	14973 8053	1.5 0	8920 5880	1.7 0	2027 2053	-0.1 -0.1	17203 13173	1.5	12955 15200	0.2
37	133	0.1 0.1	1980	-0.6	34940	-0.9	6547	-0.1	1/1/3	0	4093	-0.9	5387	-0.2	1373	-0.1	10380	0.5 -0.2	9813	-0.2
39	200	1.1	3200	0.3	66987	-0.9	13080	1.1	20587	0.9	9147	0.2	6720	-0.2	2400	0.3	12573	-0.2	16000	0.6
40	147	0.3	2347	-0.3	72107	0.2	12240	0.9	16827	0.5	12693	1	4320	-0.8	2547	0.5	12213	0.3	14493	0.0
42		0.0	4733	1.6	122500	1.9	5533	-0.4	23000	1.5	7800	-0.1	8033	1.2	2433	0.4	11433	0.2	4733	-0.9
43			7333	3.7	142889	2.6	3555	-0.9	6111	-2.7	51111	10	19000	7.5	14778	18	35000	6.3	16555	0.7
44	160	0.5	3227	0.3	85000	0.7	6293	-0.3	12053	-1.2	5747	-0.5	6520	0.3	1427	-1	8613	-0.7	15413	0.6
46	200	1.1	2760	0	66480	0	15253	1.5	19227	0.6	9813	0.3	5880	0	2147	0	15867	1.2	18507	1
47	80	-0.5	1680	-0.8	48093	-0.5	5653	-0.4	11293	-1.4	5520	-0.6	3067	-1.5	1040	-1.6	9067	-0.5	9240	-0.3
48	33	-1.1	2117	-0.5	63333	0	3933	-0.8	18267	0.3	8950	0.1	4333	-0.8	2600	0.6	10133	-0.3	5350	-0.8
49	233	1.5	700	-1.6	42700	-0.7	8400	0.1	13767	-0.7	4667	-0.8	4433	-0.8	1633	-0.7	7467	-1	1167	-1.5
50	107	-0.1	2187	-0.4	61573	0	5067	-0.5	11067	-1.4	8093	0	4307	-0.8	1773	-0.5	5440	-1.5	4267	-1
51	93	-0.3	4133	1.1	76667	0.4	8880	0.2	12800	-1	5413	-0.6	7080	0.7	1947	-0.2	11093	0	11160	0
52	67	-0.7	3183	0.3	69833	0.1	14567	1.4	18733	0.4	12500	1	6983	0.6	2283	0.2	15767	1.1	18417	1
53	67	-0.7	1907	-0.6	56040	-0.2	6267	-0.3	13160	-0.9	5933	-0.5	3507	-1.3	1773	-0.5	7733	-0.9	7840	-0.5
54	120	0	1000	-1.4	33173	-1	2320	-1.1	14600	-0.5	3227	-1.1	1773	-2.3	1293	-1.2	9693	-0.4		
56	267	2	4613	1.5	182028	3.9	20187	2.6	17800	0.2	7173	-0.2	6693	0.4	2253	0.1	13733	0.6	36907	3.8
57	80	-0.5	1787	-0.7	32827	-1	2520	-1.1	9600	-1.8	2627	-1.3	4187	-0.9	1347	-1.1	6360	-1.3	4267	-1

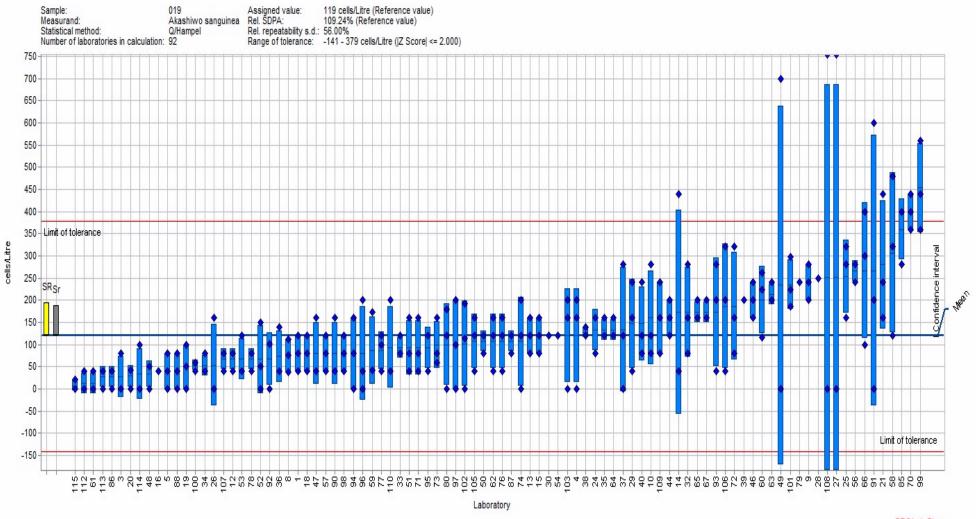
	Akashiwo		Prorocentrum		Ps. seriata		Azadinium		Chaetoceros		Chaetoceros		Gonyaulax		Corethron		Thalassiosira		Heterosigma	
Analyst code	sanguinea	Z-score	micans	Z-score2	complex	Z-score3	spinosum	Z-score4	danicus	Z-score5	curvisetus	Z-score6	spinifera	Z-score7	hystris	Z-score8	tenera	Z-score9	akashiwo	Z-score10
	(cells/Litre)		(cells/Litre)		(cells/Litre)		(cells/Litre)		(cells/Litre)		(cells/Litre)		(cells/Litre)		(cells/Litre)		(cells/Litre)		(cells/Litre)	
58	307	2.5		0.5	66533	0		0.4	17040	0		-0.6	5827	0		-0.3	10467	-0.2	14493	0.4
59	87	-0.4	2015	-0.6	53785	-0.3	9682	0.4	18406	0.3	11435	0.7	6058	0.1	2275	0.1	13638	0.6	8203	-0.4
60	200	1.1	1958	-0.6	68502	0.1	7312	0	14910	-0.4	10095	0.4	3516	-1.3		-1.3	8012	-0.8	15194	0.5
61	13	-1.4	2427	-0.2	94133	1	4627	-0.6	17947	0.2		-0.3	4947	-0.5	2413	0.3	9320	-0.5	7453	-0.5
62 63	107 213	-0.1 1.2	3827 3907	0.8 0.9	80547 95693	0.5	11960 16640	0.8 1.8	19787 16307	0.7 -0.1	5200 10547	-0.7 0.5	6000 6413	0 0.3		-1	14000 11493	0.7	12080 21493	0.1 1.5
64	133	0.1	3587	0.9	51661	-0.4	10040	0.4	20323	-0.1			7293	0.3		-1	11495	1.1	10053	-0.1
65	133	0.1	2880	0.0	56080	-0.4	11160	0.4	18187	0.3	8280	0.3	6013	0.8	2455	0.4	13747	0.6	16267	-0.1
66	267	2		0.1		0.2	11100	0.7	19200	0.5		0.5	5300	-0.3		0.4	14367	0.8	15300	0.5
67	173	0.7	3987	1	78920	0.5	7560	0	17453	0.1		0.3	7187	0.7	1613	-0.7	8693	-0.6	18893	1.1
70		3.8		0.3	61213	0.5		-0.2	20080	0.8		0.0	6173	0.1		0.3	14507	0.8	7267	-0.6
71	93	-0.3	3027	0.2	76173	0.4	7280	-0.1	12293	-1.1	3813	-1	5747	0	2253	0.1	11640	0	11920	0
72	187	0.9	3347	0.4	58707	-0.1	13387	1.2	16733	-	8573	0	6707	0.4	1973	-	13107	0.4	14880	0.5
73	100	-0.2	1907	-0.6	32111	-1	5361	-0.5	16416	-0.1	6297	-0.4	5907	0	1907	-0.3	8952	-0.6	4307	-1
74	107	-0.1	2880	0.1	41240	-0.7	7480	0	15827	-0.2	5333	-0.6	6560	0.4	1427	-1	8360	-0.7	11707	0
76	107	-0.1	3267	0.4	67320	0.1	15173	1.5	20800	1	9480	0.2	6947	0.6	2440	0.4	14760	0.9	21400	1.4
77	87	-0.4	3107	0.2	57467	-0.2	7833	0	16893	0	6420	-0.4	6080	0.1	1553	-0.8	10180	-0.2	9867	-0.2
78	67	-0.7	2933	0.1	82893	0.6	4373	-0.7	11427	-1.3	2520	-1.3	6987	0.6	1493	-0.9	7933	-0.8	6760	-0.6
79	240	1.6	3253	0.4	50517	-0.4	13840	1.2	17387	0.1	10107	0.4	7493	0.9	2240	0.1	14773	0.9	19733	1.2
80	100	-0.2	4160	1.1	85758	0.7	19560	2.5	23400	1.6		2.7	9100	1.8		0.6	17860		21260	1.4
82			2233	-0.4	138990	2.5	2433		26667	2.4		2	3533	-1.3		-1.9	9697	-0.4	700	-1.5
85	360	3.2	3827	0.8	39467	-0.8	16827	1.9	18320	0.3		1.8	7813	1.1	2560	0.6	10453	-0.2	31507	3
86	27	-1.2	2107	-0.5	45760	-0.6	5747	-0.4	14213	-0.6		-0.9	2787	-1.7		-1.3	7427	-1	6373	-0.7
87	107	-0.1	3227	0.3	84730	0.6	14351	1.4	20992	1	11297	0.7	5747	0	1107	-1.5	15572	1.1	19465	1.2
88	40	-1		-0.6	95560	1	2227	-1.1	13947	-0.7	4027	-1	4520	-0.7		0	7520		2627	-1.3
89	00	0.5	3367	0.5	105500	1.4	3900	-0.8	19733	0.7	8867	0.1	6367	0.3	2800	0.9	9500	-0.4	3067	-1.2
90 91	80 267	-0.5 2		1 1.4	72693 86667	0.2	5827 18333	-0.4 2.2	21000 20000	1 0.7	9427 15667	0.2	6267 7067	0.2	2680 1867	-0.4	13373 13533	0.5	4427 23600	-1
91	68	-0.6		-0.5		0.7	18333	2.2 1.4	18520	0.7		0.8	6190	0.7		-0.4	13533	0.5	13503	1.8 0.3
92	173	-0.8	3680	-0.5	78440	0.4	9240	0.3	18520	-0.3	5067	-0.7	6693	0.2	3480	1.9	11560	0	19667	1.2
94	80	-0.5		-0.2	29200	-1.1	3533	-0.9	13307	-0.3	3400	-0.7	4533	-0.7		0.2	8400		3733	-1.1
95	93	-0.3	2373	-0.3	56640	-0.2	9333	0.3	20587	0.9	11827	0.8	4867	-0.5	2333	0.2	12893	0.4	14213	0.4
96	80	-0.5		-0.5	87960	0.8	6093	-0.3	16907	0.5			5320	-0.2		0.2	10880	-0.1	5093	-0.9
97	100	-0.2	3400	0.5	63167	0	20100	2.6	15633	-0.3			7867	1.1	2733	0.8	12333	0.2	21267	1.4
98	80	-0.5	2947	0.1	83013	0.6	7187		16640	0		0.1	5053	-0.4		0.3	11907	0.1	9413	-0.2
99	453	4.5	2787	0	17053	-1.5	7773	0	17493	0.1		2.1	5200	-0.3	1160	-1.4	9467	-0.4	17413	0.9
100	53	-0.8	1333	-1.1	32488	-1	6138	-0.3	15252	-0.4	5507	-0.6	4567	-0.7	1867	-0.4	10000	-0.3	4640	-1
101	235	1.5	2467	-0.2	62177	0	6342	-0.3	13781	-0.7	6877	-0.3	4812	-0.5	1140	-1.4	12217	0.2	11962	0
102	102	-0.2	1810	-0.7	45167	-0.6	9035	0.2	14340	-0.6	11009	0.6	5903	0	1477	-0.9	17602	1.6	11665	0
103	120	0	4253	1.2	58880	-0.1	12653	1	23733	1.7	11507	0.7	7160	0.7	2907	1.1	14747	0.9	12653	0.1
105	107	-0.1	2920	0.1	55227	-0.3	11147	0.7	18400	0.3	8747	0.1	6107	0.1	2760	0.8	14320	0.8	15787	0.6
106	187	0.9	2968	0.1	75267	0.3	4000	-0.8	12960	-0.9	4200	-0.9	6267	0.2	1853	-0.4	8640	-0.7	7320	-0.6
107	67	-0.7	2107	-0.5		-0.4	8040	0	16520	0		-0.2	4693	-0.6		0	10440	-0.2	10667	-0.1
108	251	1.8	9215	5.3	108067	1.4	16085	1.7	23457	1.6		1.1	14241	4.8	3351	1.7	12566	0.3		
109	160	0.5	3413	0.5	49147	-0.5	10040	0.4	15760	-0.2		-0.4	5880	0		0.2	9533	-0.4	14627	0.4
110	93	-0.3	1947	-0.6	41320	-0.7	7440	0	17760	0.2	8853	0.1	4333	-0.8		0.8	12760	0.3	9733	-0.2
112	13	-1.4	1413	-1.1	67387	0.1	3813	-0.8	16720	0		-0.6	3907	-1.1		0.1	9013		5480	-0.8
113	27	-1.2	2093	-0.5	76560	0.4	1213	-1.4	16333	-0.1	4627	-0.8	3280	-1.4	2387	0.3	9333	-0.5	2933	-1.2
114	33	-1.1	1419	-1		0.1	3564	-0.9	14256	-0.6			3300	-1.4		-0.8	11022	0	6699	-0.6
115	7	-1.5	753	-1.6	16593	-1.6			3347	-3.4	653	-1.7	1227	-2.6	2940	1.1	1373	-2.6	2060	-1.3

ANNEX XII: Summary of laboratory means + statistical parameters

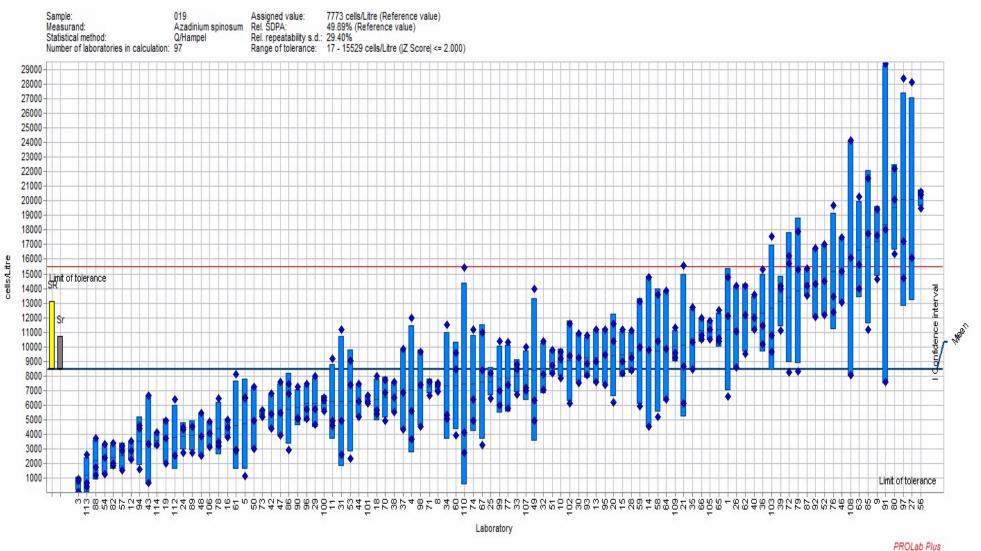
Akashiwo Prorocentrum Ps. seriata Azadinium Chaetoceros Chaetoceros Gonvaulax Corethron Thalassiosira Heterosigma Statistical parameters IPI2019 spinifera sanguinea complex spinosum danicus curvisetus hystris akashiwo micans tenera 97 95 Number of labs that submitted results 92 98 98 98 96 98 98 98 98 98 Number of participants according to design 98 98 98 98 98 98 98 98 2756 Assigned value 119 64108 7773 16840 8264 5837 2144 11288 11357 Mean 121 2726 64580 8441 16972 8263 5614 2104 11242 11049 64108 Reference values 119 2756 7773 16840 8264 5837 2144 11288 11357 74 SDPA 1218 29520 4677 3956 4231 1751 687 3748 6626 Reproducibility s.d. 74 1218 29520 4677 3956 4231 1751 3748 687 6626 67 469 719 17103 2286 2371 Repeatability s.d. 2503 2347 1018 2953 Rel. SDPA 61.77 % 44.18% 46.05 % 60.17 % 23.49 % 51.20% 29.99 % 32.04 % 33.20 % 58.34% Rel. reproducibility s.d. 61.77 % 51.20% 44.18% 46.05 % 60.17 % 23.49 % 29.99% 32.04 % 33.20 % 58.34% Rel. repeatability s.d. 56.00 % 26.09% 26.68 % 29.40% 28.40 % 17.45 % 21.88 % 21.01 % 26.00% 14.86 % 1790 Reference s.d. 130 1079 32944 3878 2879 3662 551 2978 7502 Limit of reproducibility, R (2.80 X sR) 13095 11078 11847 4902 10495 18553 206 3409 82655 1923 Limit of repeatability, r (2.80 X sr) 187 2013 47889 6400 7009 6571 2852 1314 6640 8269 Rel. limit of reproducibility 83.98 % 92.97 % 163.36% 172.95 % 123.70 % 128.93 % 168.47 % 65.78% 143.36 % 89.70 % Rel. limit of repeatability 156.81 % 73.04 % 74.70 % 82.33 % 41.62 % 79.52 % 48.85 % 61.27 % 58.82 % 72.81% 121.786 HORRAT 63.399 72.769 115.841 50.813 99.484 55.309 50.81 67.62 118925 Absolute classical Horwitz s.d. 56 1 17 242 40 78 43 32 14 55 0.97 % 0.52 % Relative classical Horwitz s.d. 0.61% 0.38 % 0.46 % 0.51% 0.54 % 0.63 % 0.49% 0.49% Lower limit of tolerance -28 321 5069 -1581 8927 -198 2336 770 3792 -1895 Upper limit of tolerance 266 5191 17127 9338 123147 24753 16726 3518 18784 24609 5 108 2627 385 58 633 Standard error 435 342 156 324 No. of laboratories after elimination of outliers type A-L except E (without laboratories that only gave states but no measured values) 92 98 98 97 98 96 98 98 98 95 No. of measurement values and states 98 98 98 98 98 98 98 98 98 98 No. of measurement values 276 294 294 291 294 288 294 294 294 285

ANNEX XII: Summary of laboratory means + statistical parameters

ANNEX XIII: Graphical summary of Akashiwo sanguinea results by analyst

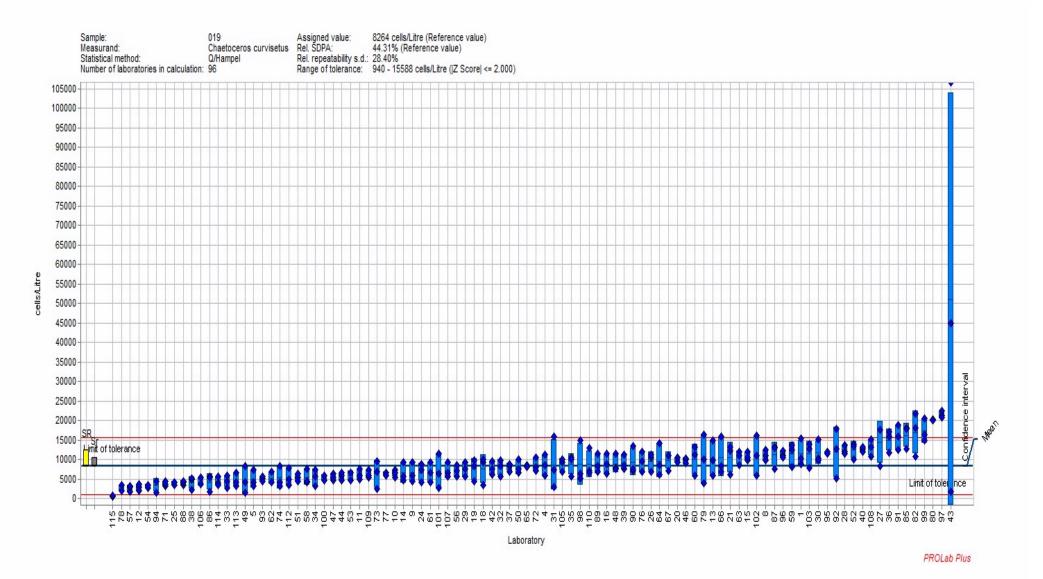


ANNEX XIII: Graphical summary of Azadinium spinosum results by analyst



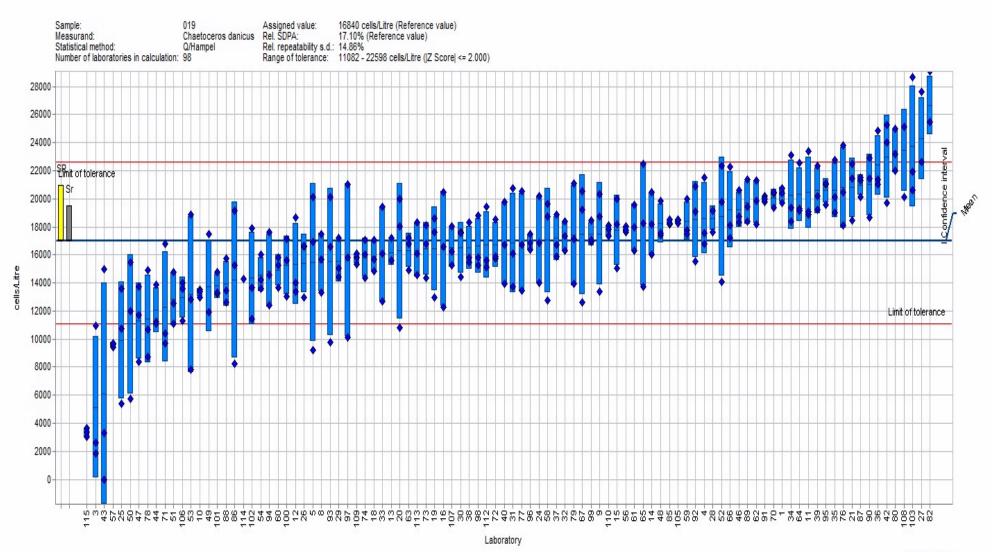
Photao Pi

ANNEX XIII: Graphical summary of *Chaetoceros curvisetus* results by analyst

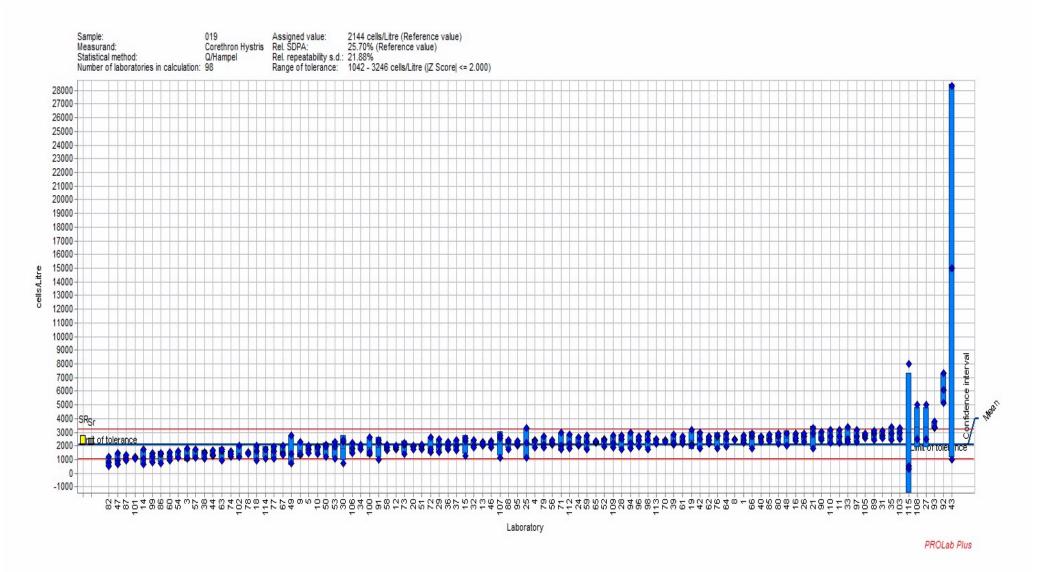


98

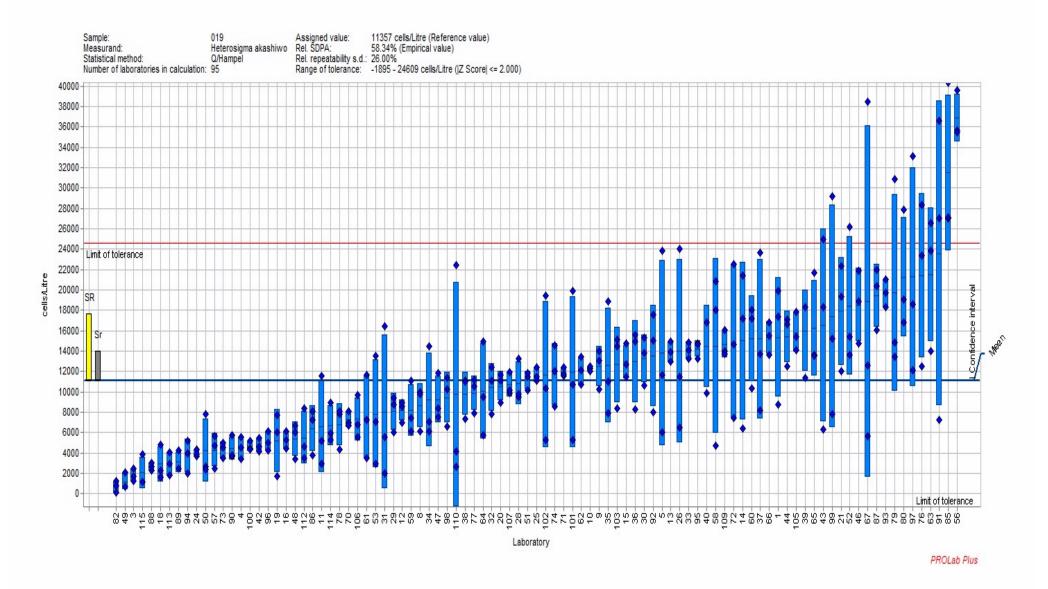
ANNEX XIII: Graphical summary of *Chaetoceros danicus* results by analyst



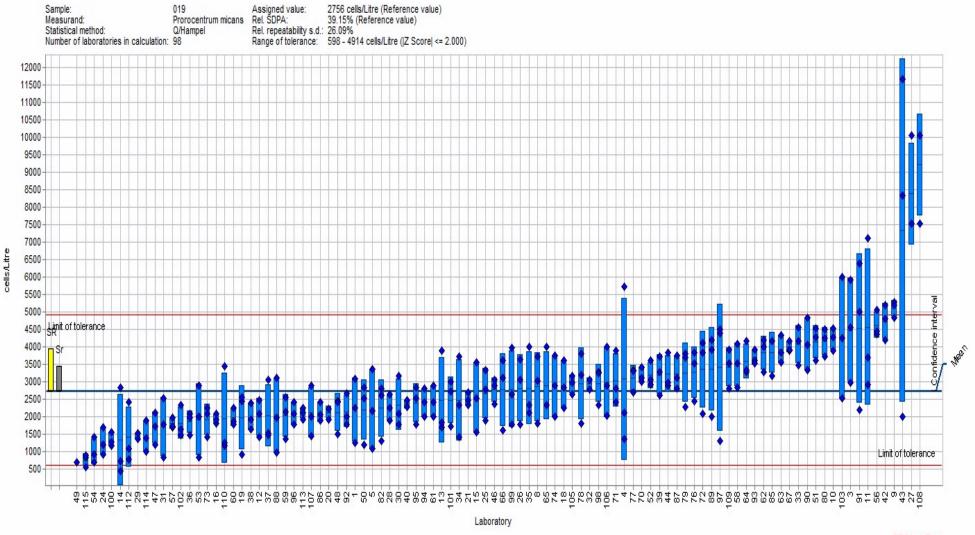
ANNEX XIII: Graphical summary of Corethron hystris results by analyst



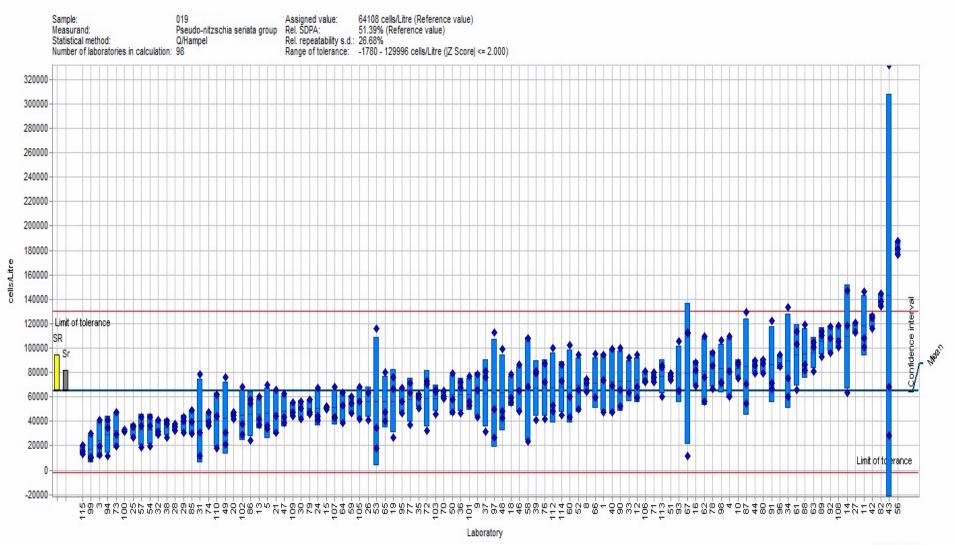
ANNEX XIII: Graphical summary of *Heterosigma akashiwo* results by analyst



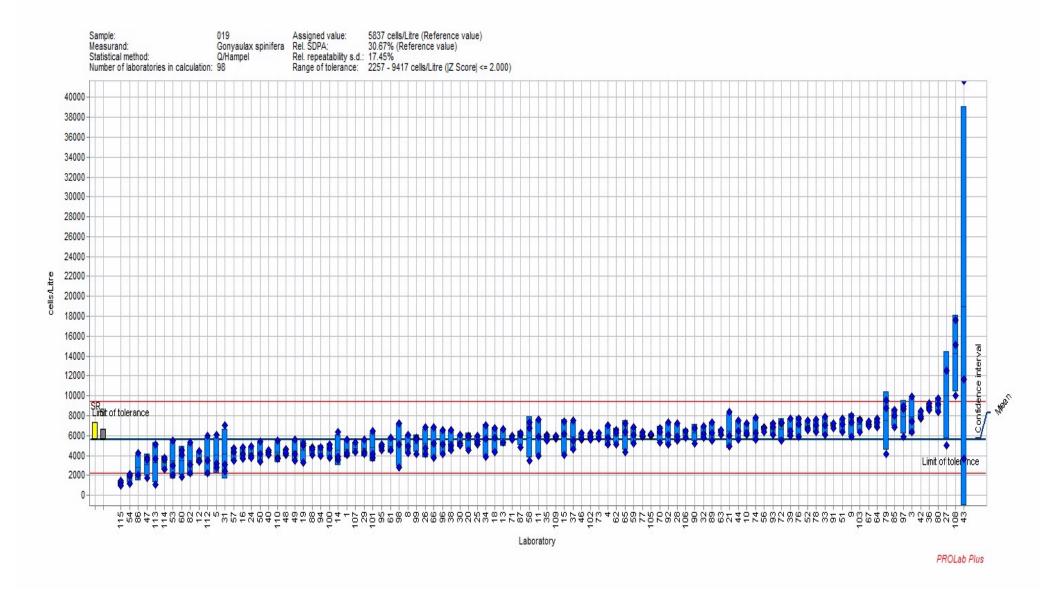
ANNEX XIII: Graphical summary of *Prorocentrum micans* results by analyst



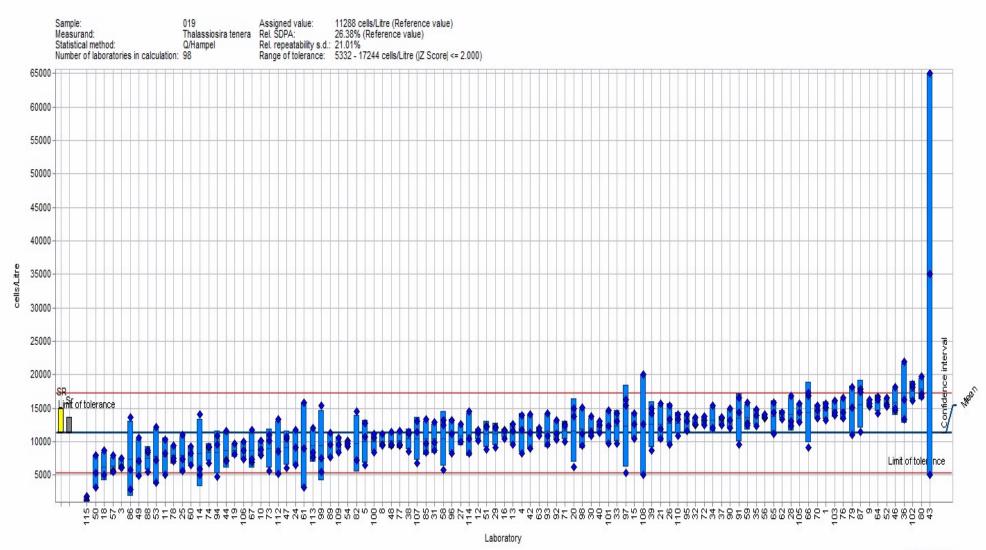
ANNEX XIII: Graphical summary of *Pseudo-nitzschia seriata group* results by analyst



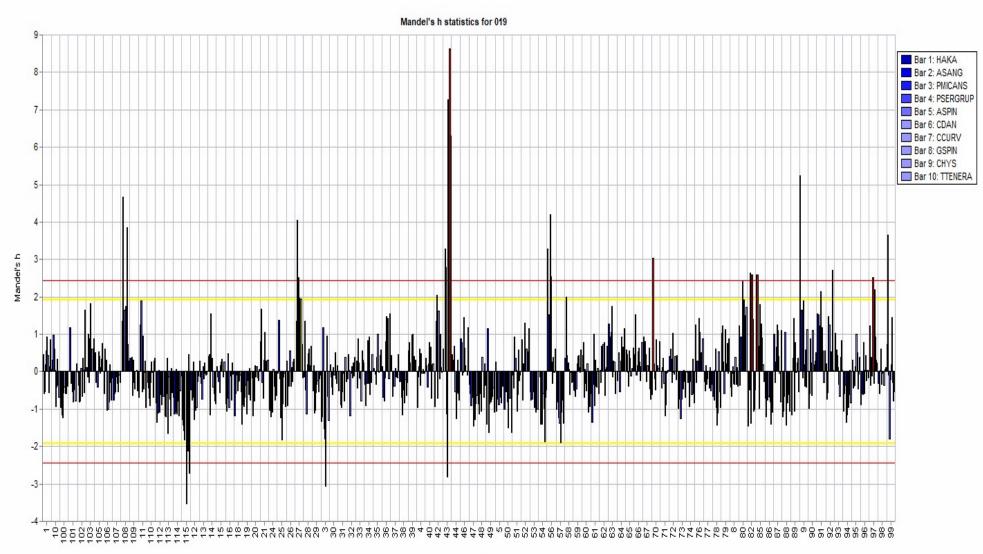
ANNEX XIII: Graphical summary of Gonyaulax spinifera results by analyst



ANNEX XIII: Graphical summary of Thalassiosira tenera results by analyst

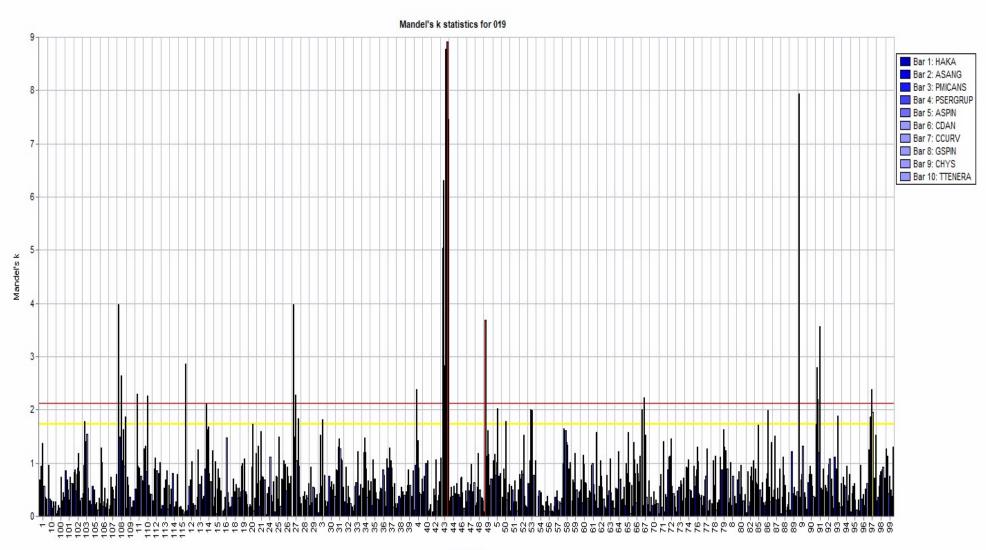


ANNEX XIV: Mandel's h statistics

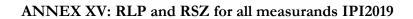


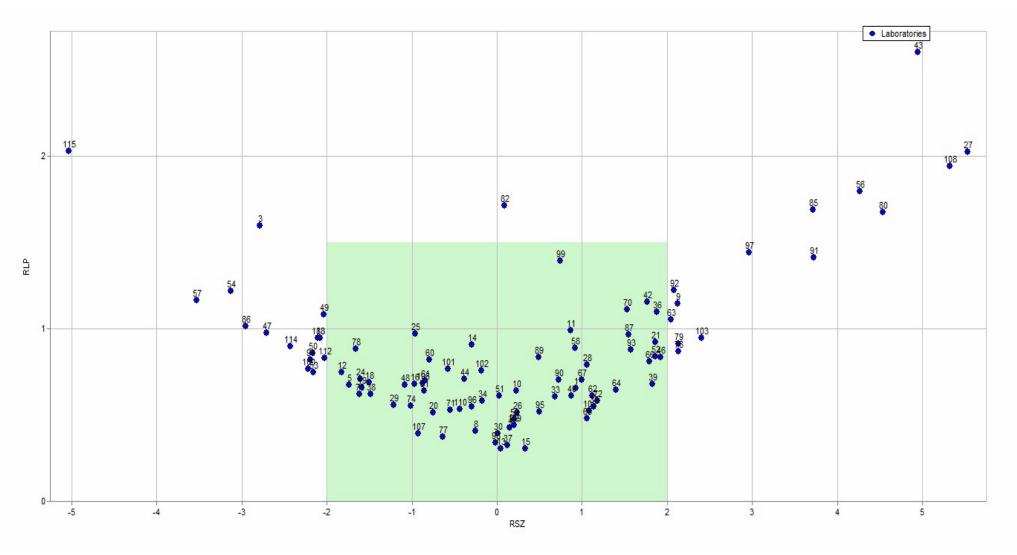
Laboratory

ANNEX XIV Mandel's k statistics

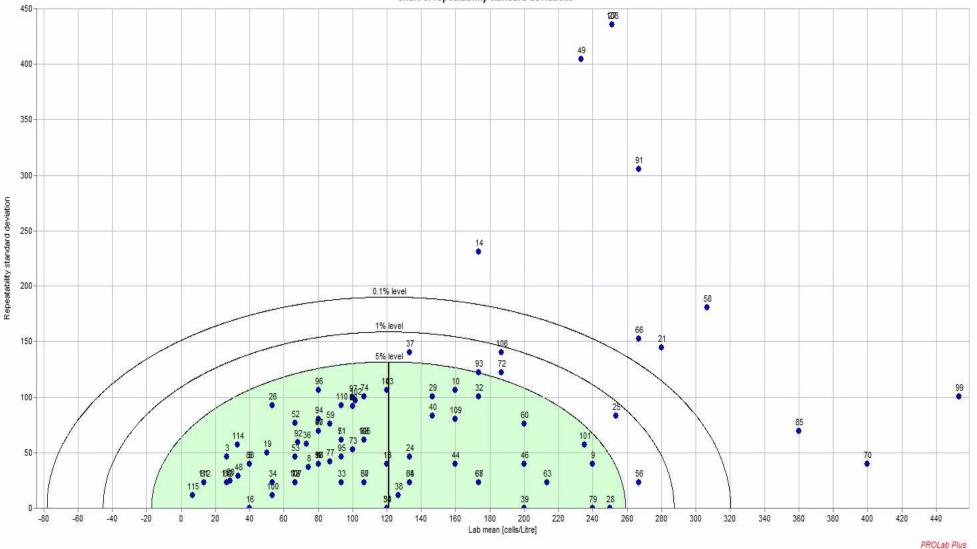


Laboratory

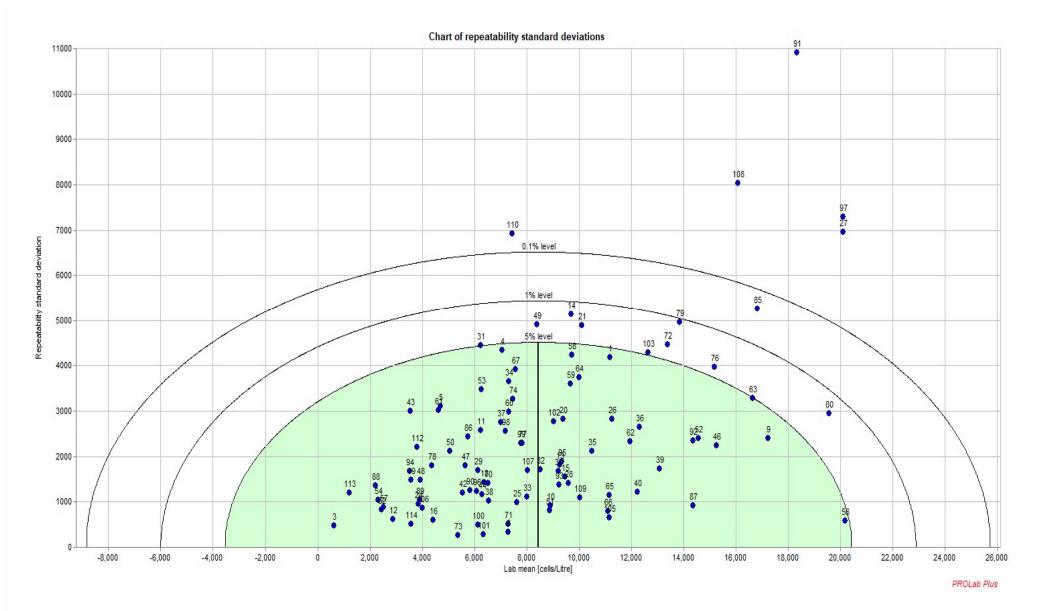




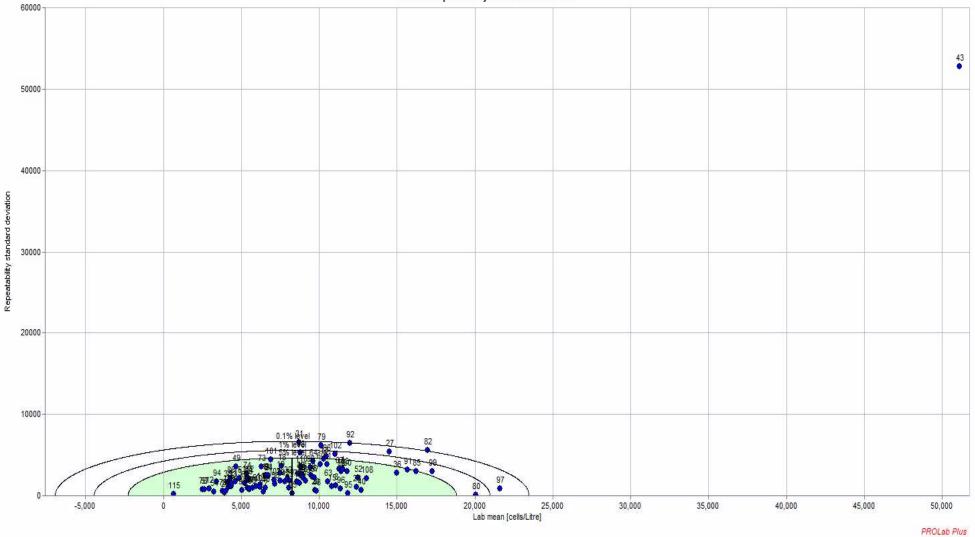
ANNEX XVI: Lischer plot Akashiwo sanguinea



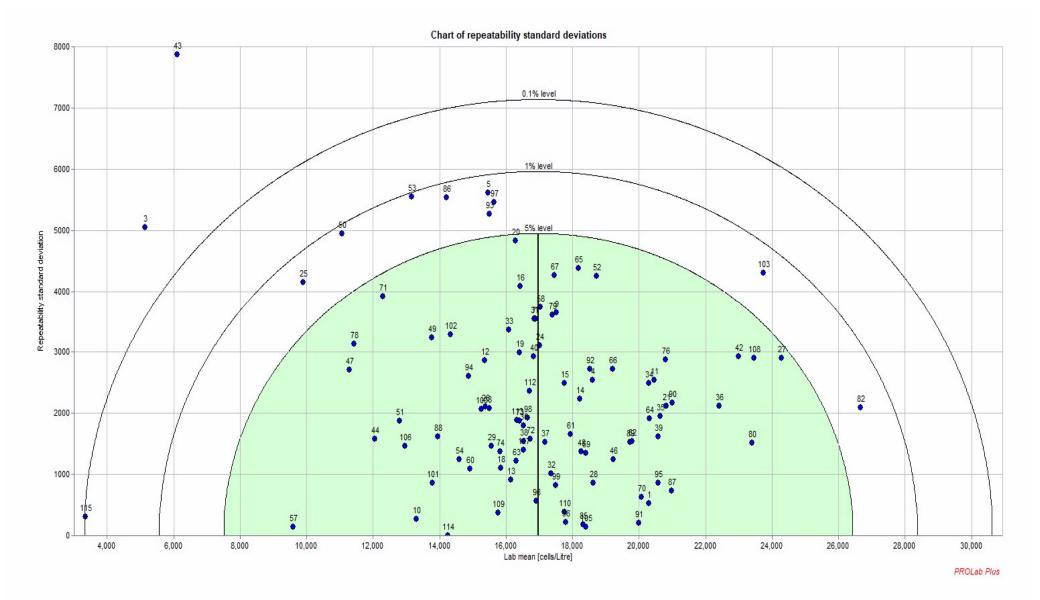
ANNEX XVI: Lischer plot Azadinium spinosum



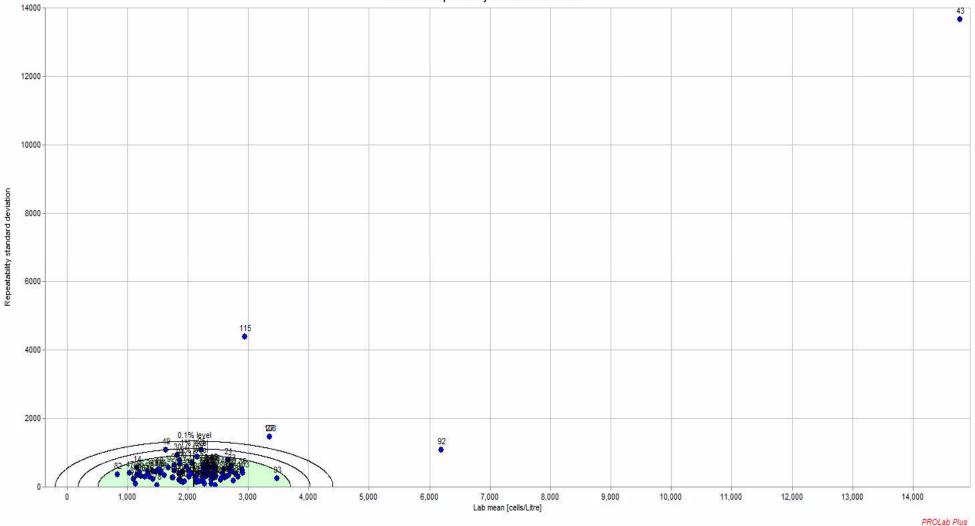
ANNEX XVI: Lischer plot *Chaetoceros curvisetus*



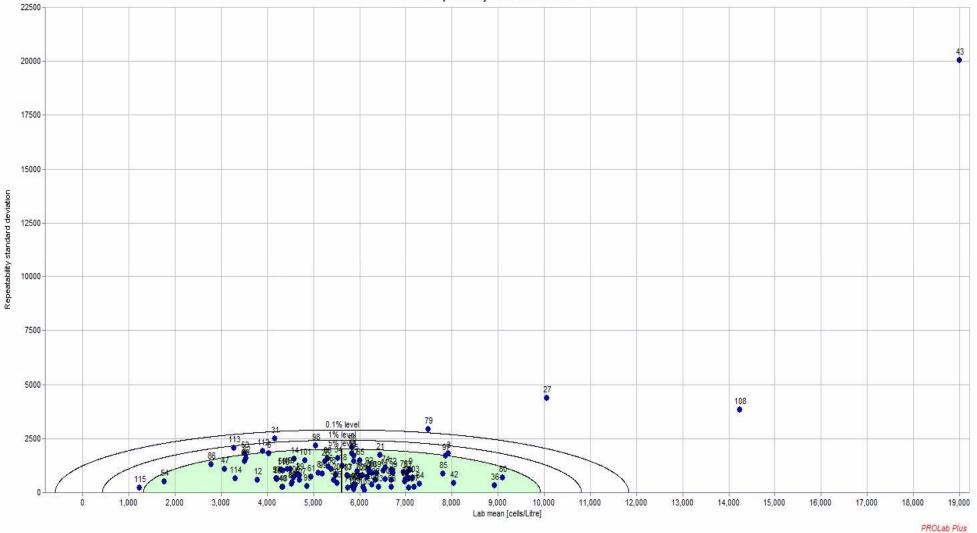
ANNEX XVI: Lischer plot Chaetoceros danicus



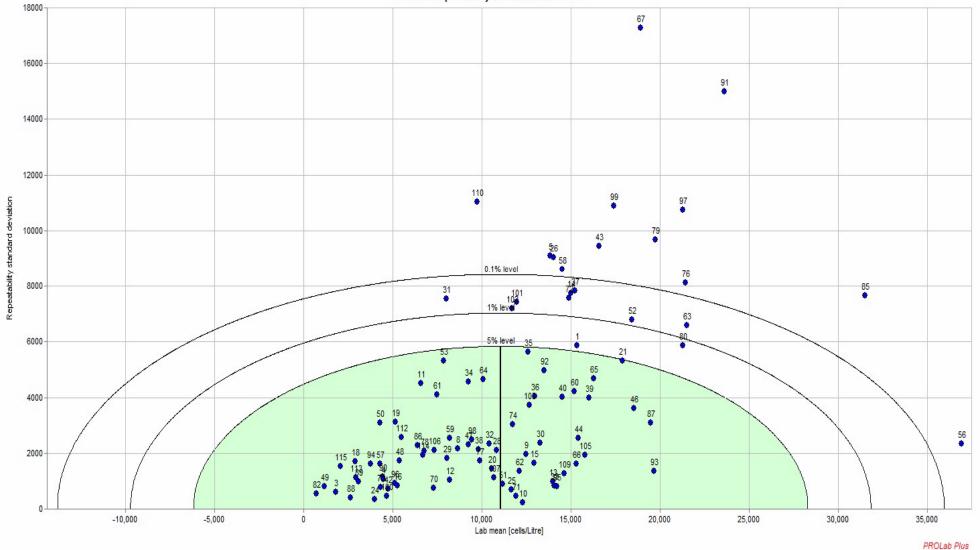
ANNEX XVI: Lischer plot Corethron hystris



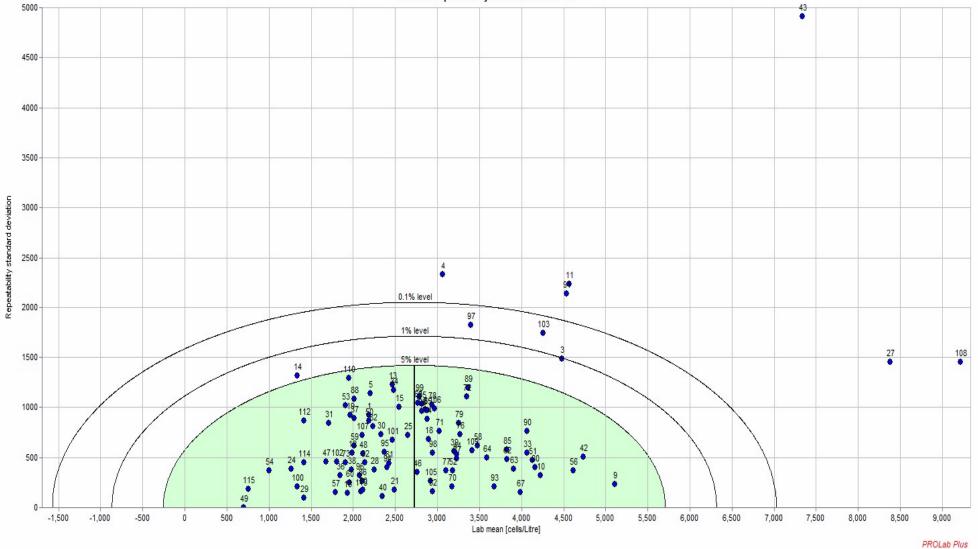
ANNEX XVI: Lischer plot Gonyaulax spinifera



ANNEX XVI: Lischer plot Heterosigma akashiwo

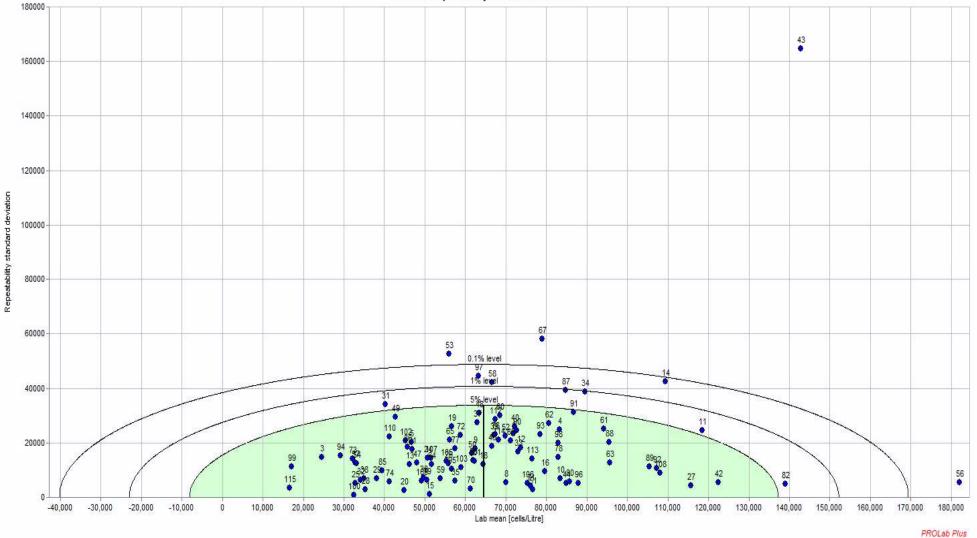


ANNEX XVI: Lischer plot Prorocentrum micans

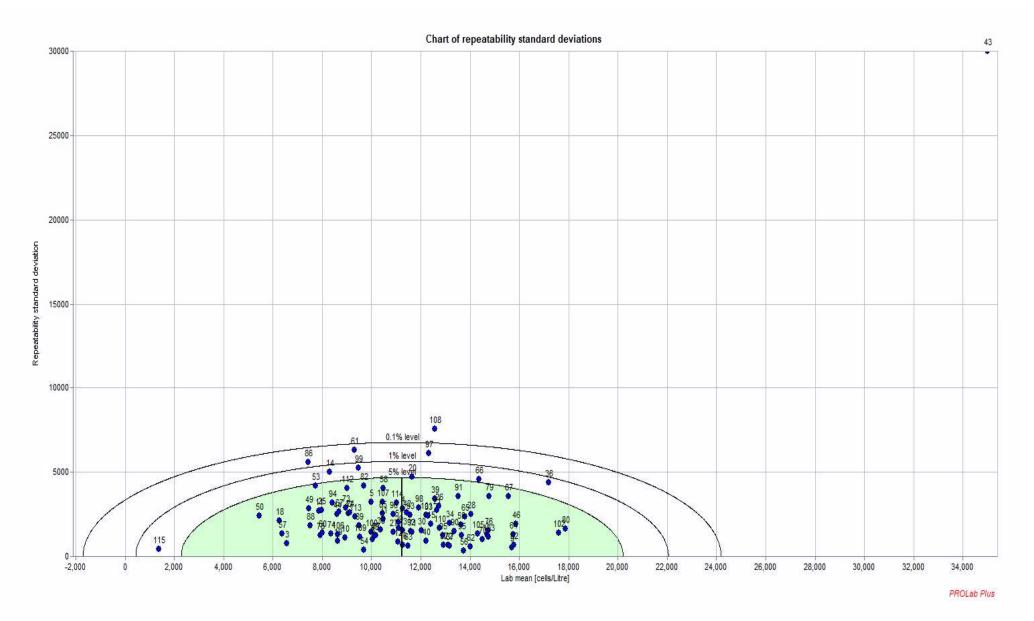


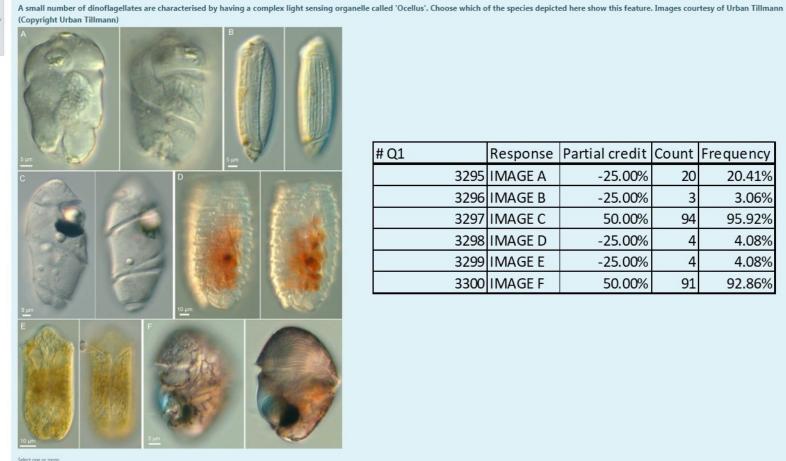
ANNEX XVI: Lischer plot Pseudo-nitzschia seriata complex





ANNEX XVI: Lischer plot *Thalassiosira tenera*





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- nage A b. Image B
- 🐑 c. Image C 🗸
- d. Image D
- e. Image E
- ∞ EImage F ✓

# Q1	Response	Partial credit	Count	Frequency
3295	IMAGE A	-25.00%	20	20.41%
3296	IMAGE B	-25.00%	3	3.06%
3297	IMAGE C	50.00%	94	95.92%
3298	IMAGE D	-25.00%	4	4.08%
3299	IMAGE E	-25.00%	4	4.08%
3300	IMAGE F	50.00%	91	92.86%

119

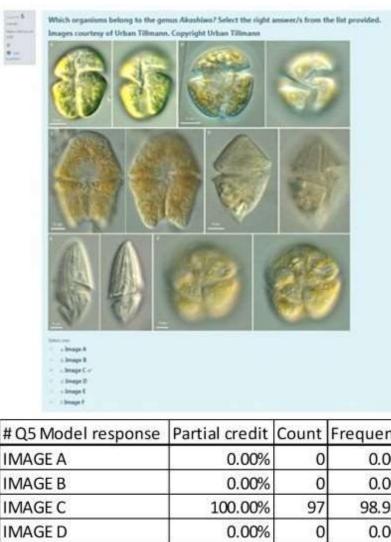


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	#Q3 Model response	Actual response	Partial credit	Count	Frequency
The following images depict live diatoms in net had samples. Please provide the right number of cells for each of the images.	2007 1. DETONULA : 8	8	16.67%	82	83.67%
	2007 1. DETONULA : 1		0.00%	1	1.02%
	2007 1. DETONULA : 4	4	0.00%	2	2.04%
	2007 1. DETONULA : 6	6	0.00%	2	2.04%
	2007 1. DETONULA : 7	7	0.00%	10	10.20%
	2008 2. CORETHRON : 1	1	15.67%	57	58.16%
	2008 2. CORETHRON : 2	2	0.00%	40	40.82%
	2009 3. GUINARDIA: 5	5	16.67%	89	90.82%
	2009 3. GUINARDIA: 3	1	0.00%	1	1.02%
4 🗢 5 6 🚳	2009 3. GUINARDIA: 4		0.00%	6	6.125
A SWIT A	2009 3. GUINARDIA: 6	-6	0.00%	1	1.079
SUBDRE OWN BBS	2010 4. EUCAMPIA: 8	8	15.67%	39	39.80%
A ABONT	2010 4. EUCAMPIA: 5	3	0.00%	1	1.025
	2010 4. EUCAMPIA: 4	4	0.00%	1	1.02%
	2010 4. EUCAMPIA: 6	é	0.00%	40	40.82%
	2010 4. EUCAMPIA: 7	-	0.00%	16	16.33%
and b Education of the second	2011 5. THALASSIOSIRA: 5	5	0.00%	1	1.07%
Detonais a + w	2011 S. THALASSIOSIRA: 15	15	15.67%	95	96.94%
Centron 1 8 a	2011 S. THALASSIOSIRA: 6		1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		1.02%
Advertis 1 4 4	2012 6. PARALIA: 12	12			95.923
Sucanata a a u	2012 6. PARALIA: 6	e	1 100423	1.10	1.029
The second secon	2012 6. PARALIA: 11	11			1.023
Literate 11 6 or	2012 6. PARALIA: 14	14			1.023

11 1.1

			# Q4 Model response	Actual response	Partial credit	Count	Frequency
			1998 Identify species 1: Prorocontrium micans	Protoonthim micans	11.11%	96	97,96%
1			1998 Identify species 1: Protocritrum soutellium	Prorocetrum soutellum	0.00%	1	1.02%
	100 Mar 1	and the second second	1999 Identify species 2: Promcentrum donghalense	Prorocentrum donghalense	11.11%	97	98,98%
		Contraction of the second seco	2000 identify species 3: Promontrum soutellum	Prorocitrum soutellium	11.11%	94	95.92%
		1 10 10 10 10 1	2000 Identify species 3: Promicentrum consistum	Protocentrum cordatum	0.00%	1	1.02%
		STAR STAR	2000 Identify species 3: Prorocentrum triangulatum	Protocentrum triangulatum	0.00%	I	1.02%
A MARCELLA	1	and the state of the	2000 Identity species 3: Prorocentrum lima	Promocentrum tima	0.00%	1	1.02%
	2	STORES !!	2001 (dentify species 4: Prorocentrum compressum	Protocentrum compressum	11,11%	RS	96,94%
10 µm	- C		2001 Identify species 4 Prorocentrum lima	Prorocentrum lima	0.00%	2	2.04%
	C 100	3	2002 Identify species 5: Prorocentrum compressum	Prorocentrum compressum	0.005	1	1.02%
ATTS AND			2002. Identify species 5: Mesoporus perforatua	Mesoponus perforatus	11.11%	95	97,56%
			2003 Identify species & Prorocentrum tilestenum	Prorocentrum triestenum	11.51%	90	91.84%
Very series		8 9	2003 Identify species & Prorocentrum gracile	Prorocentrum gracile	0.00%	4	4.08%
(hete)	100 M	Gen AL	2003 Identify species & Prorocentrum redfieldii	Prorocentrum redfieldii	0.00%	3	3.06%
	A READ	Side 2	2004 Identity species 7: Mesoporus pertoratus	Mesoporus perforatus	0.00%	.1	1.07%
		(ASE) [5-	2004 Identify species 7: Prorocentrum condutum	Protocentrum constatum	11.11%	88.	89.80%
Con B 13	1	SEX B	2004 Identify species 7. Prorocentrum cordiformis	Pronournitrum conditionms	0.00%	- 3	3.06N
REAL BALL		1381	2004 (dentify species 7, Prorecentrum triangulatum	Prorocentrum triangulatum	0.00%	5	5.30%
		Y VS	2005 Identify species 8 Prorocentrum triestenum	Prorocentrum triestenum	0.00%	2	2.04%
5 × 6 ×	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	-V -	2005 Identify species & Prorocentrum gracile	Protocentrum gracile	11.11%	81	82.65%
-	,		2005 Identify species & Prorocentrum arcuatum	Protocentrum accuatum	0.00%	14	14.29%
			2006 Identify species 9: Prorocentrum rostructum	Prorocentrum rostratum	11,115	97	98.98%
11							
The second s							
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#Q5 Model response	Partial credit	Count	Frequency
IMAGE A	0.00%	0	0.00%
IMAGE B	0.00%	0	0.00%
IMAGE C	100.00%	97	98.98%
IMAGE D	0.00%	0	0.00%
IMAGE E	0.00%	0	0.00%
IMAGE F	0.00%	0	0.00%

Connet 6 Connet Mark 110 out of 100 V 0 644 puettor

This video sequence shows a cell of *Dinophysis acuta*. Choose the biological process that best describes this sequence. Video sequence courtesy of Urban Tillmann (Copyright)



Select one:

* A Rotation of the nucleus (nuclear cyclosis) 🗸

b. Digestion of ingested Mesodinium rubrum

∠ Vermiforme stage of the parasite Amoebophrya

d Cytoplasmic streaming

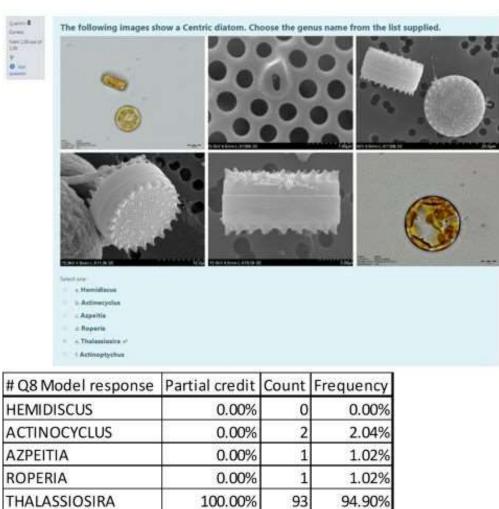
e Auxospore formation

#Q6 Model response	Partial credit	Count	Frequency
ROTATION OF THE NUCLEUS (NUCLEAR CYCLOSIS)	100.00%	91	92.86%
DIGESTION OF INGESTED _MESODINIUM RUBRUM_	0.00%	2	2.04%
VERMIFORME STAGE OF THE PARASITE AMOEBOPHRYA	0.00%	2	2.04%
CYTOPLASMIC STREAMING	0.00%	2	2.04%
AUXOSPORE FORMATION	0.00%	0	0.00%

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0	X)-out	
0	Annual and a state	COTT I	60 80 69	

#Q7	Response	Partial credit	Count	Frequenc
3284	IMAGE A	33.33%	84	85.71%
3285	IMAGE B	-10.00%	2	2.04%
3286	IMAGE C	33.33%	93	94.90%
3287	IMAGE D	-10.00%	0	0.00%
3288	IMAGE E	33.33%	92	93.88%
3289	IMAGE F	-10.00%	1	1.02%
3290	IMAGE G	-10.00%	2	2.04%
3291	IMAGE H	-10.00%	1	1.02%
3292	IMAGE I	-10.00%	1	1.02%
3293	IMAGE J	-10.00%	1	1.02%
3294	IMAGE K	-10.00%	1	1.02%

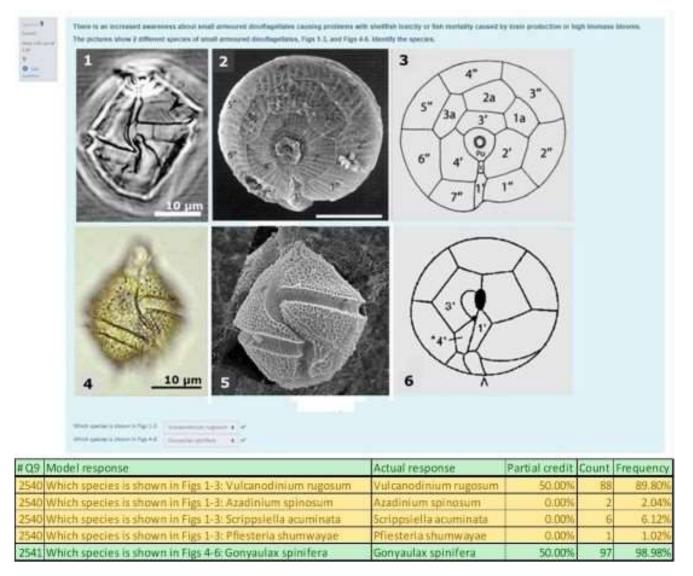


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The Willsaming Images depict live physical address in cert hand assesses. Please provide the right consider of cells for each of the images,	#Q10 Model response	Actual response	Partial credit	Count	Frequency
	2025 1. PROHOCENYRUM: S	.5	20.00%	99	94.90%
	2025 1. PROROCENTRUM; 4	4	0.02%	- 4	4.08%
	2026 2. POLYKRIKOS: 2	2	20.00%	83	84.69%
	2026 2. POLYKRIKOS: 1	1	0.00%	12	12.24%
	2026 2. POLYKRIKOS: 4	4	0.00%	1	1.02%
	2026 2. POLYKRIKOS: 16	16	0.00%	1	1.02%
2.4.3	2027 3. CHAETOCEROS: 17	17	20.00%	67	68.37%
	2027 3. CHAETOCEROS: 12	12	0.00%	2	2.04%
4	2027 3. CHAETOCEROS: 4	4	0.00%	1	1.02%
	2027 3 CHAETOCEROS: 8	8	0.00%	1	1.02%
	2027 3. CHAETOCEROS: 11	11	0.00%	- 2	2,04%
	2027 3. CHAETOCEROS: 13	13	0.00%	9	7.14%
	2027 3. CHAETOCEROS: 14	14	0.00%	1	1.02%
	2027 3. CHAETOCEROS: 15	15	0.00%	4	4.08%
	2027 1. CHAETOCEROS: 16	16	0.00%	12	12,24%
63 C	2028 4 DISSODINIUM 5	5	0.00%	1	1.02%
L Presentition a d	2028 4. DISSODINIUM: 1	1	20.00%	78	79.59%
I Nijirikas J k a	2028 4. DISSODINIUM: 4	4	0.00%	18	18.37%
3. Charmony 11 0 pt	2029 5. GUINARDIA: 5	5			1.02%
4 Description 4 or	2029 5 GUINARDIA: 2	2	0.00%	4	4.08%
1. Gutherdia a a	2029 5. GUINARDIA: 9	9	20.00%	80	81.63%
	2029 5. GUINARDIA: 4	4	0.00%	1	1.02%
	2029 5. GUINARDIA: 6				3.06%
	2029 S. GUINARDIA: 7	7	0.00%		4.08%
	HAR AND			1.00	20222

2029 S. GUANARDIA: #

2029 5. GUINARDIA: 10

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0.00%

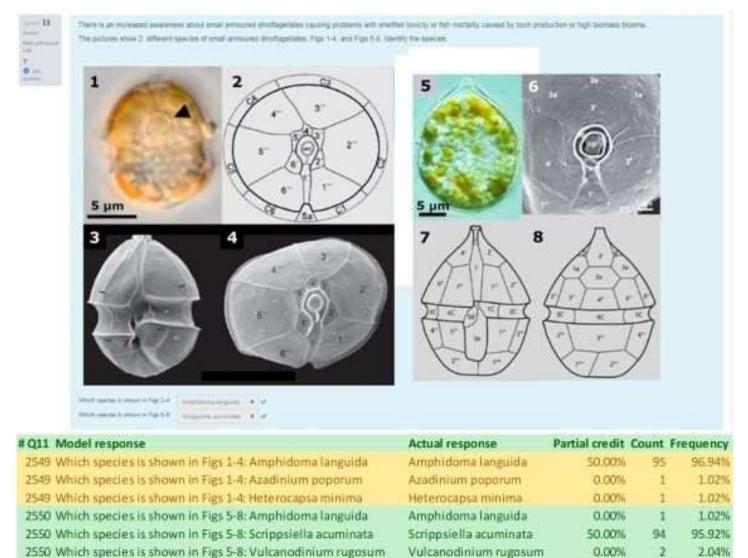
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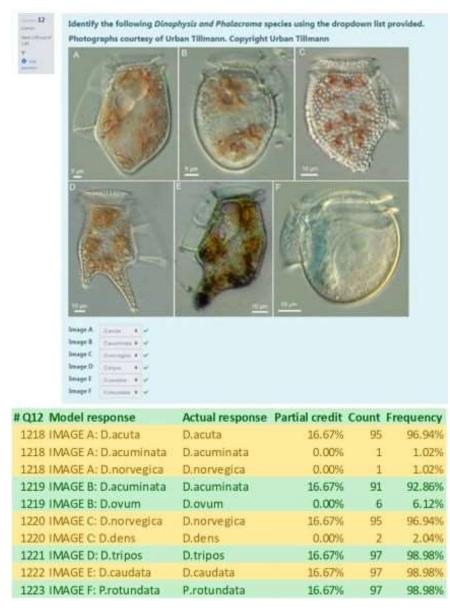
3.06%

1.02%

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ANNEX XVIII: Oceanteacher 2019 quiz results

Analyst code	Q. 1/0.83	Q. 2 /0.83	Q. 3 /0.83	Q. 4 /0.83	Q. 5 /0.83	Q. 6 /0.83	Q. 7 /0.83	Q. 8 /0.83	Q. 9 /0.83	Q. 10 /0.83	Q. 11/0.83	Q. 12 /0.83	Total score
1	100.00	100.00	83.13	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.59
3	100.00	39.76	67.47	100.00	100.00	100.00	67.47	100.00	100.00	60.24	100.00	100.00	
4		100.00	100.00	89.16	100.00		67.47	100.00	100.00	100.00	100.00		
5	75.90	100.00	67.47	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
8	100.00	80.72	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.39
9	25.30	80.72	0.00	100.00	100.00	100.00	0.00	100.00	100.00	80.72	100.00	100.00	
10	75.90	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	97.99
11	75.90	100.00	80.72	89.16	100.00	100.00	100.00	100.00	50.60	100.00	100.00	100.00	
12	100.00	39.76	83.13	100.00	100.00	0.00	100.00	100.00	100.00	80.72	100.00	100.00	83.63
13	0.00	80.72	83.13	100.00	100.00	100.00	33.73	100.00	100.00	100.00	100.00	100.00	83.13
14	0.00	60.24	83.13	89.16	100.00	100.00	100.00	100.00	100.00	39.76	50.60	100.00	76.91
15	100.00	80.72	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.39
16	100.00	100.00	83.13	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.59
18	50.60	60.24	83.13	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	91.16
19	100.00	39.76	100.00	78.31	100.00	100.00	100.00	100.00	100.00	80.72	100.00	100.00	91.57
20	100.00	60.24	83.13	100.00	100.00	100.00	100.00	100.00	100.00	80.72	100.00	100.00	93.67
21	100.00	100.00	83.13	100.00	100.00	100.00	100.00	100.00	100.00	80.72	100.00	100.00	96.99
24	100.00	80.72	100.00	100.00	100.00	100.00	100.00	100.00	100.00	60.24	100.00	100.00	95.08
25	75.90	39.76	80.72	78.31	100.00		100.00		100.00	100.00	50.60		
26	100.00	60.24	100.00	100.00	100.00		100.00		100.00	100.00	100.00		
27	25.30	100.00	83.13	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	92.37
28	100.00	100.00	83.13	100.00	100.00		100.00		100.00	80.72	100.00		
29	100.00	100.00	100.00	100.00	100.00		100.00		100.00	100.00	100.00		
30	100.00	80.72	100.00	100.00	100.00		100.00		100.00	100.00	100.00		
31	75.90	100.00	83.13	100.00	100.00		100.00		100.00	60.24	100.00		
32	75.90	60.24	100.00	100.00	100.00		100.00		100.00	100.00	100.00		
33	50.60	100.00	83.13	89.16	100.00		100.00		100.00	60.24	100.00		
34	75.90	60.24	100.00	100.00	100.00		100.00		100.00	100.00	100.00		
35	100.00	100.00	100.00		100.00		100.00		100.00	100.00	100.00		
36	100.00	100.00	83.13	100.00	100.00		100.00		100.00	80.72	100.00		
37	100.00	80.72	83.13	100.00	100.00		67.47		100.00	39.76			
38	100.00	100.00	100.00	100.00	100.00		100.00		100.00	80.72	100.00		
39 40	100.00	39.76 100.00	100.00	100.00 100.00	100.00		100.00		100.00	80.72 100.00	100.00		
40	100.00 100.00	60.24	100.00	55.42	100.00 100.00		100.00 13.25	100.00	100.00 100.00	80.72	100.00 100.00		
43	75.90	60.24	83.13	<u> </u>	100.00		13.25	100.00	100.00	100.00	100.00		
44 46	75.90	100.00	100.00	89.16	100.00		100.00	0.00	100.00	100.00	100.00		
46	100.00	60.24	67.47	100.00	100.00		100.00		100.00	80.72	100.00		
47	100.00	39.76	100.00	78.31	100.00		100.00	100.00	100.00	80.72	100.00		
48	100.00	60.24	100.00	67.47	100.00		100.00	100.00	100.00	80.72	100.00		
	100.00	100.00	83.13	100.00	100.00		100.00	100.00	100.00	100.00	100.00		
51	100.00	100.00	100.00	100.00	100.00		67.47	100.00	100.00	100.00	100.00		
52	75.90	100.00	100.00	100.00	100.00		100.00		100.00	100.00	100.00		
53	25.30	80.72	83.13	100.00	100.00		100.00	100.00	50.60	100.00	50.60		
54	100.00	60.24	67.47	89.16	100.00		100.00	100.00	100.00	100.00	100.00		
56	100.00	100.00	83.13	100.00	100.00		100.00	100.00	100.00	100.00	100.00		
57	75.90	60.24	50.60	100.00	100.00		100.00		50.60	80.72	100.00		
58		100.00	83.13	100.00	100.00		100.00	100.00	100.00	80.72	100.00		

ANNEX XVIII: Oceanteacher 2019 quiz results

Analyst code	Q. 1/0.83	Q. 2/0.83	Q. 3 /0.83	Q. 4 /0.83	Q. 5/0.83	Q. 6 /0.83	Q. 7 /0.83	Q. 8/0.83	Q. 9/0.83	Q. 10/0.83	Q. 11/0.83	Q. 12 /0.83	Total score
59	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
60	100.00	60.24	100.00	89.16	100.00	100.00	100.00	100.00	100.00	60.24	100.00	100.00	92.47
61	100.00	100.00	83.13	89.16	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	97.69
62	100.00	100.00	83.13	89.16	100.00	100.00	100.00	100.00	100.00	60.24	100.00	83.13	92.97
63	100.00	100.00	83.13	89.16	100.00	100.00	33.73	100.00	100.00	100.00	100.00	100.00	92.17
64	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
65	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
66	100.00	100.00	80.72	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.39
67	75.90	80.72	67.47	100.00	100.00	100.00	67.47	100.00	100.00	39.76	100.00	83.13	84.54
70	100.00	39.76	100.00	89.16	100.00	100.00	67.47	100.00	100.00	80.72	100.00	100.00	89.76
71	100.00	100.00	100.00	100.00	100.00	100.00	67.47	100.00	100.00	100.00	100.00	100.00	97.29
72	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
73	100.00	80.72	100.00	89.16	100.00	100.00	100.00	0.00	100.00	80.72	100.00	100.00	87.55
74	50.60	60.24	100.00	78.31	100.00	100.00	100.00	100.00	100.00	80.72	100.00	100.00	89.16
76	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
77	100.00	100.00	83.13	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.59
78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	83.13	98.59
79	100.00	80.72	100.00	100.00	100.00	100.00	100.00	100.00			100.00	100.00	96.79
80	100.00	100.00	83.13	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.59
82	100.00	100.00	83.13	89.16	100.00	100.00	100.00	100.00	50.60	60.24	100.00	83.13	88.86
85	100.00	100.00	83.13	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.59
86	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
87	75.90	100.00	83.13	89.16	100.00	100.00	100.00	100.00	100.00	80.72	100.00	100.00	94.08
88	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	80.72	100.00	100.00	98.39
89	100.00	100.00	83.13	89.16	100.00	100.00	100.00	100.00	50.60	100.00	100.00	100.00	93.57
90	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
91	100.00	100.00	83.13	100.00	100.00	100.00	100.00	100.00	100.00	80.72	100.00	100.00	96.99
92	100.00	100.00	83.13	100.00	100.00	100.00	100.00	100.00	100.00	80.72	100.00	100.00	96.99
93	75.90	80.72	83.13	78.31	100.00	100.00	100.00	100.00		100.00	100.00	100.00	89.06
94	100.00	100.00	83.13	100.00	100.00	100.00	67.47	100.00		100.00	100.00	100.00	95.88
95	100.00	80.72	100.00	100.00	100.00	100.00	100.00	100.00		100.00	100.00	100.00	98.39
96	100.00	100.00	67.47	89.16	100.00	100.00	100.00	100.00	100.00	39.76	100.00	100.00	91.37
97	100.00	100.00	100.00	89.16	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.10
98	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
99	75.90	100.00	100.00	89.16	100.00	0.00	67.47	100.00	50.60	80.72	100.00	67.47	77.61
100	75.90	100.00	83.13	100.00	100.00	100.00	33.73	0.00	100.00	80.72	100.00	100.00	81.12
101	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	50.60	100.00	100.00	100.00	95.88
102	100.00	100.00	83.13	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.59
103	50.60	80.72	83.13	89.16	100.00	100.00	100.00	100.00	100.00	39.76	100.00	100.00	86.95
105 106	100.00 100.00	100.00 100.00	67.47 100.00	100.00 100.00	100.00 100.00	100.00 100.00	100.00 100.00	100.00 100.00	100.00 100.00	60.24 100.00	100.00 100.00	100.00 100.00	93.98 100.00
106	100.00	80.72	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00		100.00	98.39
107	25.30	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	98.39 93.78
108	75.90	60.24	100.00	100.00	100.00	100.00	100.00	100.00		80.72	100.00	100.00	93.78 88.96
109	100.00	60.24 100.00	100.00	100.00	100.00	0.00	67.47	100.00		80.72	100.00	100.00	88.96
110	100.00	100.00	50.60	89.16	100.00	0.00	100.00	100.00				100.00	83.33
112	100.00	80.72	83.13	100.00	100.00	100.00	67.47	100.00	100.00	80.72	100.00	100.00	92.67
113	100.00	100.00	83.13	100.00	100.00	100.00	100.00	100.00	100.00	80.72	100.00	100.00	92.87
114			67.47			0.00		100.00	100.00	60.24	0.00		
115	25.30	39.76	67.47	89.16	100.00	0.00	67.47	100.00	100.00	60.24	0.00	67.47	59.74