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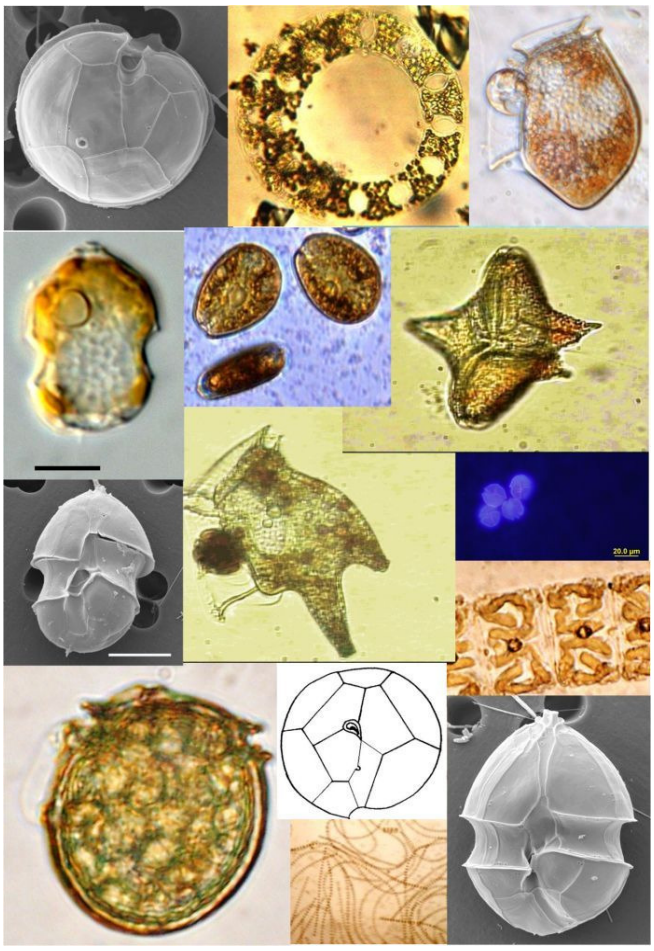
Marine Institute
Foras na Mara

BEQUALM

NMBAQC SCHEME

BEQUALM Phytoplankton proficiency test in the abundance and composition of marine microalgae 2013 report.

PHY-ICN-13-MI1 VR 1.0



Rafael Salas[1] & Jacob Larsen[2]

[1] Marine Institute, Rinville, Oranmore, Co.Galway, Ireland

[2] IOC Science and Communication center on harmful algae
Department of Biology, University of Copenhagen, Øster Farimagsgade 2D
1353 Copenhagen K. Denmark

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1. Summary of results

- 49 analysts from 34 laboratories took part in this intercomparison. 47 analysts and 32 laboratories returned results. This year, new laboratories have joined the scheme from France, Iceland, Italy, Singapore, Uruguay, USA and Australia

- Most participating laboratories are based in Europe (29): Ireland (3), Northern Ireland (1), Scotland (2), England (7), France (6), Netherlands (2), Sweden (1), Spain (3), Croatia (1), Iceland (1), Italy (1) and Greece (1). A small number come from different continents: USA (1), Australia (2), Singapore (1) and Uruguay (1).

- There were four species of interest in this intercomparison exercise. These were: *Chaetoceros diadema, Coscinodiscus granii, Gyrodinium instriatum* and *Heterosigma akashiwo.*

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

- The homogeneity and stability test show that samples don't meet the assessment criteria set out in the standard. The number of replicates needed for the samples to meet the criteria would be impractical. So, instead the between sample Standard deviation is taken into account for the final confidence limits. Outliers don't affect test result as we are using the robust analysis.

- The assigned values standard uncertainty was found to be negligible for all test items, so there is no bias in the method.

- The laboratory bias plot indicates that results are normally distributed around zero for all test items. The percentage difference plots show that only a few analysts are outside the warning (2SD) and action (3SD) limits. The % rank using probability plots gives an indication of the most extreme values.

- The Z-scores were calculated using the robust mean and standard deviation for each test item. There was one warning signal on the *C.diadema* count, two warning signals on the *H.akashiwo* count and two warning and two action signals in the *G.instriatum* count. A total of seven signals from 184 results. Also, four

analysts failed to identify one of the species in the samples, two analysts failed to identify *C.diadema* and two others *H.akashiwo.*

- The bar plot shows bias across all levels (test items) for three analysts which have tended to underestimate all counts. This could point out to methodology issues. The plots of repeatability standard deviation assume that there is no difference between laboratories means and standard deviations. The plots showed unusual results for two out of the four counts with extreme values found on the *C.diadema* count and on the *H.akashiwo* count. Some counts look implausible.

- Sample composition results show that the easiest items for identification were *C.granii* and *H.akashiwo,* with near perfect scores for all analysts, *G.instriatum* prove the most difficult item for identification, with ten incorrect answers and *C.diadema* proved difficult at species level but all correct to genus.

- The Ocean teacher online HAB quiz results suggests a high rate of proficiency. 45 analysts returned results and 27 analysts achieved 100% scores with another 12 analysts over 90% mark.

- Most questions average above 90%. The worst answered question was Q8 (planozygote) with a 73% on average.

- Problems arose from 'short answer' questions where grammar errors, punctuation or similar answers were given. In this case, where the answer was correct, notwithstanding these grammar issues, it was given as a valid answer and the scores should reflect this change.

- Issues regarding naming authority and use of synonyms in answers as in Preperidinium (Zygabikodinium). These answers were given as correct.

2. Introduction

The Phytoplankton Bequalm intercomparison study in 2013 was designed to test the ability of analysts to identify and enumerate correctly marine phytoplankton species in preserved water samples. As in previous years, samples have been designed using laboratory cultures. There were four species of interest in this intercomparison exercise. These were: *Chaetoceros diadema* (Ehrenberg) Gran, *Coscinodiscus granii* Gough, *Gyrodinium instriatum* Freudenthal & J.J.Lee and *Heterosigma akashiwo* (Y.Hada) Y.Hada ex Y.Hada & M.Chihara. Also, we asked participants to return cell counts on three replicate samples as part of a homogeneity test

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

This year, 49 analysts from 34 laboratories took part in this intercomparison. 47 analysts and 32 laboratories returned results. Laboratories from the USA, Singapore, Uruguay, France, Italy and Iceland took part in this exercise for the first time. Most laboratories are based in Europe (29): Ireland (3), Northern Ireland (1), Scotland (2), England (7), France (6), Netherlands (2), Sweden (1), Spain (3), Croatia (1), Iceland (1), Italy (1) and Greece (1). A small number of laboratories come from USA (1), Australia (2), Singapore (1) and Uruguay (1).

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-13-MI1. PHY standing for phytoplankton, ICN for intercomparison, 13 refers to the year 2013, 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 2013.

3. Materials and Methods

3.1 Sample preparation, homogenization and spiking

All samples were prepared following the following protocol. The seawater used in this experiment was natural field water collected at Ballyvaughan pier, Galway bay, Ireland, filtered through GF/C Whatmann filters (Whatmann™, Kent, UK), autoclaved (Systec V100, Wettenberg , Germany) and preserved using Lugol's iodine solution (Clin-tech, Dublin, Ireland). The sterilin tubes were made up to the required volume with sterile filtered seawater containing neutral lugol's iodine. This was carried out using a 25ml serological pipettes (Sardstedt, Nümbrech, Germany) and the volume weighted in a calibrated balance (ME414S Sartorius, AG Gottingen, Germany). The density of seawater was considered for this purpose to be 1.025g/ml. The final volume of each sample was 29 ml approximately before spiking the samples.

Two sample sets were prepared, the first one containing the four species and the second one containing one species for counting only to test the homogeneity and stability of the samples preparation. Both sets were prepared using the same technique.

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

Samples were capped and label, parafilm was used around the neck of the sterilin tube to avoid water loss, placed in envelopes and couriered via TNT couriers for a one day delivery across the world, in order for all the laboratories to have approximately the same arrival time.

3.2 Culture material, treatments and replicates.

The laboratory cultures used in this exercise were sourced from the Scandinavian Culture Collection of Algae and Protozoa (SCCAP) in Denmark. The algae *Chaetoceros diadema, Coscinodiscus granii, Gyrodinium instriatum* and *Heterosigma akashiwo* was used for this study. A fifth culture kept in the Marine Institute of *Scrippsiella sp.* was used for the homogeneity test.

There were two sample sets. The first set (set 1) comprised three samples spiked with one species (*Scrippsiella sp.*). The sterilin tubes were numbered in black pen and analysts were asked to return whole chamber counts. This data was used to test the homogeneity and stability of the samples. No identification of the organism was needed.

The second set (Set 2) consisted of four samples; three samples for analysis and one spare. Samples were numbered in red pen and four species were spiked in this set, which analysts were required to enumerate, identify and report the results in cells per litre.

A total of 200 samples were produced for the homogeneity test and 300 samples for the enumeration and identification study. Of the samples from the homogeneity test 150 were sent to the participants and 15 were sent to a reference laboratory. Of the 300 samples 200 samples were sent to the participants for analysis.

An expert laboratory carried out the homogeneity and stability test. The data generated by this laboratory was used to test the homogeneity and stability of the samples. A minimum of 10 samples (30ml volume) were necessary for the homogeneity test and a minimum of 3 samples for the stability test. These were randomly selected from the batch and sent to the expert laboratory to carry out the counts. Samples had to be divided in two portions of 10ml each.

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

3.3 Cell concentrations

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

Generally cell concentrations were low and ranging from approximately 3200 cells/Litre for *C.granii*, 7200 cells/L for *H.akashiwo*, 9200 cells/L for *C.diadema* and 10000 cells/L for *G.instriatum*. The highest concentration (10000) would correspond to a count of 250 cells in a 25ml sedimentation chamber.
The cell concentration for the homogeneity test was 8000 cells/L approximately.

3.4 Sample randomization

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

3.5 Forms and instructions

A set of instructions and forms required to complete the exercise were sent via e-mail to all the analysts including their unique identifiable laboratory and analyst code. Form 1 (Annex I) to confirm the receipt of materials; number and condition of samples and correct sample code. Form 2 (Annex II) and Form 3 (Annex III) in an Excel spreadsheet format to input species composition and calculate abundance for each species. Form 2 was used for the identification and enumeration part of the exercise and form 3 to input the homogeneity test counts. All analysts were asked to read and follow the instructions (Annex IV) before commencing the test.

3.6 Statistical analysis

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

We followed the standard ISO normative 13528 which describes the statistical methods to be used in proficiency testing by interlaboratory comparisons. Here, we use this standard to determine and assess the homogeneity and stability of the samples, the number of replicate measurements needed to meet the criteria, how to deal with outliers, how to determine assigned values for the test and calculate their standard uncertainty. How to compare these values with their standard uncertainty, how to calculate the performance statistics for the test, the graphical representation of these statistics and the combination of performance scores with a final discussion over this combination of scores over several rounds.

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

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

3.7 Bequalm online HAB quiz

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

First time participants had to register in the following web address: http://classroom.oceanteacher.org/ before allowed to access the quiz content, while analysts already registered from previous years, could go directly to the login page. Once registered, participants could login into the site and using a password, able to access the quiz. Eight weeks were given to analysts to register, complete and submit the online quiz. The course itself was found under the courses tab in the main menu page. Analysts could link to the Harmful Algal Bloom programme BEQUALM 2013 and quiz content from here.

The test itself consisted of 14 questions (see Annex VIII). There were different question types used in this quiz; matching, multiple choice and short answer questions. Matching questions had dropdown menus with the answers and analysts had to choose the right ones, multiple choice questions have different choices and analysts must tick the right ones and in short answer type questions analysts had to write what they thought was the correct answer.

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

4. Results

4.1 Homogeneity and stability study

The procedure for a homogeneity and stability test is recorded in annex b (pg 60) of ISO13528. The assessment criteria for suitability, is also explained in this annex.

CELLS / L							
	Date	Sample number	Test portion 1	Test portion 2	sample average	Between test portion range	*2
	28/05/2013	7	6854	7606	7230	752	565504
	28/05/2013	29	8357	7418	7888	939	881721
	30/05/2013	77	9390	10047	9719	657	431649
	30/05/2013	166	6761	6479	6620	282	79524
	02/06/2013	107	9390	9108	9249	282	79524
	02/06/2013	100	8451	8169	8310	282	79524
	03/06/2013	122	7743	8971	8357	1228	1507984
	03/06/2013	194	5540	7324	6432	1784	3182656
	04/06/2013	54	6949	7794	7372	845	714025
	04/06/2013	121	8545	7982	8264	563	316969
				Average:	7944	Sum	7839080
				SD	1061	P=	10
			SD within samples:		626		
			SD between samples:		965		
homogeneity criteria			965	318			

Figure 1: Homogeneity test results by expert laboratory

The results of ten samples for the homogeneity test by our expert laboratory are plotted in figure 1. The average was found to be 7944 ±1061 cells/L. The Standard deviation (SD) within samples (*Sw*) was 626 calculated using equation (A) where *Wt* is the between test portion range and g is the number of samples.

$$s_w = \sqrt{\sum w_t^2/(2g)}$$

A)

and the between samples (*Ss*) standard deviation was calculated as 965 using equation (B) below where *Sx* is the standard deviation of sample averages and *Sw* is the within samples standard deviation.

$$s_s = \sqrt{s_x^2 - \left(s_w^2/2\right)}$$

B)

The samples are considered to be adequately homogeneous if the between samples standard deviation (*Ss*) is less or equal 0.3 times the standard deviation of the samples. (equation C)

C) $s_s \leqslant 0{,}3\,\hat{\sigma}$

As the SD between samples 965 is larger than 318 (0.3 times the SD) see figure 1, the criteria is not met and we conclude that the samples are not adequately homogeneous. When this is the case, the standard (ISO 13528) allows, for a number of samples to be distributed among the participants. Their results (fig. 2) may increase the within sample standard deviation and this can then be used to calculate the necessary number of replicate measurements for the criteria to be met.

ANALYST CODE	Averag	X-X*	*X*i*	it2	it3	it4	it5
20	468	7899	6618	6641	6651	6656	6658
24	5222	3145	6618	6641	6651	6656	6658
13	5693	2674	6618	6641	6651	6656	6658
18	6547	1820	6618	6641	6651	6656	6658
48	6547	1820	6618	6641	6651	6656	6658
3	6920	1447	6920	6920	6920	6920	6920
35	7293	1074	7293	7293	7293	7293	7293
49	7308	1059	7308	7308	7308	7308	7308
10	7413	954	7413	7413	7413	7413	7413
.....							
38	9467	1100	9467	9467	9467	9467	9467
47	9533	1166	9533	9533	9533	9533	9533
4	9622	1255	9622	9622	9622	9622	9622
22	9633	1266	9633	9633	9633	9633	9633
43	9667	1300	9667	9667	9667	9667	9667
34	9840	1473	9840	9840	9840	9840	9840
14	9880	1513	9880	9880	9880	9880	9880

Average *X*	8128		8316	8318	8319	8320	8320
SD *S*	1577		984	980	978	977	977
robust average *X**	8367	new *X**	8316	8318	8319	8320	8320
robust stdev *S**	1166	new *S**	1116	1111	1109	1108	1108
δ= 1.5*S**	1749		1674	1667	1664	1662	1661
*X**- δ	6618		6641	6651	6656	6658	6659
*X**+ δ	10116		9990	9985	9983	9982	9981
Between Samples SD	965	From homogeneity test					
new stdev for Homogeneity	1469						

Figure 2: Homogeneity test results by the participant laboratories

The new SD for the homogeneity test (σ_{r1}) 1469 cells/L (Fig.2) is calculated using equation (D) where σ_r is the robust standard deviation and (*Ss*) is the between samples Standard deviation

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

D)

Using σ_{r1} instead of σ_r in the equation (E) below to calculate the number of replicate measurements needed for the criteria to be met (see fig.3)

$$\sigma_r / \sqrt{n} \leqslant 0{,}3\,\hat{\sigma}$$

(E)

	$\sigma_{r1=}\sqrt{1108^2+965^2}$	
	σr1=1469	
criteria:	1469/√3 < 0.3x 1108	
	n=3	
	then 848 < 332	
	so n= 20 for the rule to be satisfied	

Figure 3: Number of replicate measurements

We find that for the rule to be satisfied each analyst would have to carry out 20 replicate measurements (n=20). As this is not very practical, the standard allows for the between sample SD to be added to the proficiency SD for each test item using equation (D) above to take into account the heterogeneity of the samples. In the case of the homogeneity test, we take the between sample SD calculated by the expert laboratory in fig.1 (965) and the SD calculated by the participants fig.2 (1108) to calculate the new standard deviation for the test (1469).

The stability study analysis (fig.4) was carried out on three samples a month after the homogeneity study by the expert laboratory to test the stability of the materials over time. A minimum number of replicate measurements was needed (n=3).

The results show that the sample average was 7403±436 cells/L compared to 7944 cells/L (fig. 1) for the homogeneity average To check whether the results meet the criteria, the following equation (F) below is applied.

$$\left|\bar{x}_{\cdot r} - \bar{y}_{\cdot r}\right| \leqslant 0{,}3\hat{\sigma}$$

F)

Where;

$\bar{x}_{\cdot r}$ = Homogeneity study average

$\bar{y}_{\cdot r}$ = Stability study average

As figure 4 indicates the criteria is not met because the absolute difference of the homogeneity and stability averages (541) is larger than 0.3 times the SD for the proficiency test (318).

CELLS / L							
	Date	Sample number	Test portion 1	Test portion 2	sample average	Between test portion range	*2
	07/07/2013	137	8169	7606	7888	563	316969
	07/07/2013	172	6855	7700	7278	845	714025
	07/07/2013	31	7606	6479	7043	1127	1270129
				Average:	7403	Sum	2301123
				SD	436	P=	3
			SD within samples:		619		
			SD between samples:		39		
stability check criteria			7944	7403	541	318	

Figure 4: Stability study results by expert laboratory

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 of ISO 13528. The robust estimates for this exercise have been derived by iterative calculation, that is, by convergence of the modified data (fig. 5).

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 must be obtained for the data to be included in the calculation of the average and SD for the exercise using the values from the participants. Otherwise, these results won't be included in the calculation of statistics that affect other laboratories but they may be used for the calculation of their own.

So, the results of analyst 41 for the homogeneity test are not included in the calculations as only one result was returned from a possible three on the homogeneity test. All other results are fine.

Calculate initial values for x^* and s^* as:

x^* = median of x_i $\quad (i = 1, 2, \ldots, p)$

$s^* = 1{,}483$ median of $|x_i - x^*|$ $\quad (i = 1, 2, \ldots, p)$

Update the values of x^* and s^* as follows. Calculate:

$\delta = 1{,}5s^*$

For each x_i $(i = 1, 2, \ldots, p)$, calculate:

$$x_i^* = \begin{cases} x^* - \delta, & \text{if } x_i < x^* - \delta \\ x^* + \delta, & \text{if } x_i > x^* + \delta \\ x_i, & \text{otherwise} \end{cases}$$

Calculate the new values of x^* and s^* from:

$$x^* = \sum x_i^* / p$$

$$s^* = 1{,}134 \sqrt{\sum \left(x_i^* - x^*\right)^2 / (p - 1)}$$

Figure 5: Iterative process

4.3 Analysts' Data

The results of the participants were collated using Excel spreadsheets for each test item. 47 analysts and 32 laboratories returned results from a total of 49 and 34 laboratories. There were four species of interest in the sample for this exercise: *C.granii* (fig.6), *C.diadema* (fig.7) *H.akashiwo* (fig.8) and *G.instriatum* (fig.9).

Figures 10-13 show the modified results of figures 6-9 using algorithm A in annex C of ISO13528. These tables show the robust averages and standard deviations that will be used to calculate the limits for the Z-scores for each item.

The new standard deviation for each test item is, then calculated taking into account the heterogeneity of the samples, that is, the between samples standard deviation calculated from the homogeneity test (965) and the robust standard deviation using equation (D). This new standard deviation will be used to set the 2 and 3 sigma limits of the robust averages for each test item.

ANALYST CODE	SAMPLE CODES			Coscinodiscus granii (cells/L)			Average
				sample 1	sample 2	sample 3	
3	53	183	101	3200	2760	3880	3280
23	16	37	171	2560	3040	1600	2400
16	7	31	236	1760	1800	2440	2000
40	19	28	216	2000	5500	500	2667
5	123	151	237	2000	3600	2000	2533
44	10	34	69	2440	2600	2200	2413
30	96	131	150	3160	2960	2640	2920
18	115	119	61	2800	2200	2960	2653
2	36	148	44	3040	2680	2560	2760
13	52	130	155	1840	1960	1840	1880
10	110	124	135	2520	2400	2480	2467
28	77	124	147	1960	2240	3000	2400
36	64	103	142	2200	2000	1200	1800
11	75	94	156	2600	2320	2200	2373
31	191	185	218	2000	1480	1800	1760
38	51	87	186	2320	2200	2280	2267
24	5	43	73	1330	3170	3130	2543
25	184	210	195	1920	1840	1280	1680
34	81	62	175	3240	2320	1800	2453
20	161	32	58	147	309	215	224
41	98	180	194	2600	2533	1200	2111
1	27	192	213	2391	1870	3174	2478
45	35	188	204	2783	1870	2565	2406
29	42	144	221	2600	2520	2600	2573
22	49	223	70	2800	2800	2300	2633
39	6	21	238	2360	2400	1880	2213
37	83	157	176	2200	2320	2400	2307
12	63	45	25	3222	3055	2652	2976
43	17	117	120	1960	2240	3440	2547
9	4	197	205	1520	1080	2360	1653
7	68	97	207	2360	2760	3240	2787
14	187	76	169	1640	2240	1520	1800
35	54	165	167	2920	2360	2440	2573
8	30	72	136	2440	2880	2960	2760
15	203	172	57	2440	2240	2800	2493
4	93	126	143	2800	2733	2567	2700
17	109	134	233	2520	2520	3080	2707
6	149	219	179	2800	2560	2600	2653
42	38	82	215	3640	2680	3080	3133
27	88	209	225	1800	2360	2440	2200
33	8	41	92	2640	3120	2560	2773
19	199	173	231	2120	1960	2400	2160
48	174	67	164	1308	1346	1808	1487
49	59	105	190	1692	1538	2308	1846
50	114	200	230	2038	2346	1615	2000
46	78	178	182	2520	2640	1160	2107
47	12	137	229	3400	3800	3000	3400

Figure 6: Participants results for *C.granii*. not id= not identified; nr= no result

ANALYST CODE	SAMPLE CODES			Chaetoceros diadema (cells/L)			Average
				sample 1	sample 2	sample 3	
3	53	183	101	6400	7800	6640	6947
23	16	37	171	11920	14800	11000	12573
16	7	31	236	14720	13840	15920	14827
40	19	28	216	not id	not id	not id	not id
5	123	151	237	3600	2600	2800	3000
44	10	34	69	5000	7880	2960	5280
30	96	131	150	9080	11720	10240	10347
18	115	119	61	4440	3400	7120	4987
2	36	148	44	14440	12400	13480	13440
13	52	130	155	8960	7360	7040	7787
10	110	124	135	13080	12000	16320	13800
28	77	124	147	8160	7040	8160	7787
36	64	103	142	5000	6640	5880	5840
11	75	94	156	6320	7760	9240	7773
31	191	185	218	10960	11200	15000	12387
38	51	87	186	25420	26650	24600	25557
24	5	43	73	not id	not id	not id	not id
25	184	210	195	12800	15000	12360	13387
34	81	62	175	13640	14360	16880	14960
20	161	32	58	529	559	nr	544
41	98	180	194	0	8200	1400	3200
1	27	192	213	13174	12305	12957	12812
45	35	188	204	10827	13870	10566	11754
29	42	144	221	10760	14520	10000	11760
22	49	223	70	16600	15400	21200	17733
39	6	21	238	10680	9320	10080	10027
37	83	157	176	13320	8600	11000	10973
12	63	45	25	17532	18282	18731	18182
43	17	117	120	10480	12280	10840	11200
9	4	197	205	15080	14840	14920	14947
7	68	97	207	6760	12560	7000	8773
14	187	76	169	8960	10320	6480	8587
35	54	165	167	640	560	1400	867
8	30	72	136	5920	6480	4840	5747
15	203	172	57	12000	12600	15120	13240
4	93	126	143	8900	8767	10600	9422
17	109	134	233	9680	11800	7160	9547
6	149	219	179	8520	6760	7040	7440
42	38	82	215	8720	9960	6480	8387
27	88	209	225	1880	1560	2440	1960
33	8	41	92	7640	6600	5640	6627
19	199	173	231	1080	1000	1160	1080
48	174	67	164	115	1231	1462	936
49	59	105	190	9923	17154	4154	10410
50	114	200	230	7885	6269	11346	8500
46	78	178	182	13680	11320	7200	10733
47	12	137	229	16000	8600	6200	10267

Figure 7: Participants results for *C.diadema*. not id= not identified; nr= no result

ANALYST CODE	SAMPLE CODES			Heterosigma akashiwo (cells/L)			Average
				sample 1	sample 2	sample 3	
3	53	183	101	8240	6480	9240	7987
23	16	37	171	7280	7000	4400	6227
16	7	31	236	7240	8120	5840	7067
40	19	28	216	5500	1500	2500	3167
5	123	151	237	not id	not id	not id	not id
44	10	34	69	1040	6360	1520	2973
30	96	131	150	5160	5880	6560	5867
18	115	119	61	3360	2720	3120	3067
2	36	148	44	8040	5840	3320	5733
13	52	130	155	4520	4880	2520	3973
10	110	124	135	7760	6240	7080	7027
28	77	124	147	9640	10600	9480	9907
36	64	103	142	9400	9520	8520	9147
11	75	94	156	7600	9400	6040	7680
31	191	185	218	7000	7960	10040	8333
38	51	87	186	4600	6080	6640	5773
24	5	43	73	1200	2100	700	1333
25	184	210	195	4640	3600	3840	4027
34	81	62	175	6640	8760	5920	7107
20	161	32	58	137	11	nr	74
41	98	180	194	0	1800	1000	933
1	27	192	213	8348	6087	6783	7073
45	35	188	204	8000	7739	6174	7305
29	42	144	221	7560	3360	5600	5507
22	49	223	70	4900	7200	5500	5867
39	6	21	238	6520	6520	8320	7120
37	83	157	176	6720	7240	8600	7520
12	63	45	25	7385	6445	6243	6691
43	17	117	120	7720	6960	6080	6920
9	4	197	205	5120	5080	5040	5080
7	68	97	207	2640	3560	5760	3987
14	187	76	169	7960	6320	5240	6507
35	54	165	167	not id	not id	not id	not id
8	30	72	136	4480	5160	5440	5027
15	203	172	57	13800	15760	19440	16333
4	93	126	143	8600	9733	5367	7900
17	109	134	233	10440	14280	10080	11600
6	149	219	179	9280	11280	10200	10253
42	38	82	215	15280	13800	10560	13213
27	88	209	225	8040	8000	9640	8560
33	8	41	92	14960	13560	13520	14013
19	199	173	231	3920	4680	3800	4133
48	174	67	164	1643	1096	548	1096
49	59	105	190	2192	5692	2538	3474
50	114	200	230	538	231	38	269
46	78	178	182	3400	5440	4160	4333
47	12	137	229	4400	6900	5800	5700

Figure 8: Participants results for *H.akashiwo*. not id= not identified; nr= no result

ANALYST CODE	SAMPLE CODES			Gyrodinium instriatum (cells/L)			Average
				sample 1	sample 2	sample 3	
3	53	183	101	8560	8200	10920	9227
23	16	37	171	8960	9800	8560	9107
16	7	31	236	9200	10550	9760	9837
40	19	28	216	5500	3000	2000	3500
5	123	151	237	6800	4200	5200	5400
44	10	34	69	9240	8480	8840	8853
30	96	131	150	8840	8440	7320	8200
18	115	119	61	9400	7360	10160	8973
2	36	148	44	11760	9880	10320	10653
13	52	130	155	8240	7080	6600	7307
10	110	124	135	10000	8920	9240	9387
28	77	124	147	9320	8160	8800	8760
36	64	103	142	8920	9320	8120	8787
11	75	94	156	9520	8720	7760	8667
31	191	185	218	8920	8640	7680	8413
38	51	87	186	8720	9400	7880	8667
24	5	43	73	4200	8067	7500	6589
25	184	210	195	8640	8400	8640	8560
34	81	62	175	10440	10600	8000	9680
20	161	32	58	676	988	529	731
41	98	180	194	7200	7600	3400	6067
1	27	192	213	9261	8696	9261	9073
45	35	188	204	8826	7870	9174	8624
29	42	144	221	8800	9960	9120	9293
22	49	223	70	9300	9100	10500	9633
39	6	21	238	9360	9760	8560	9227
37	83	157	176	8520	8560	8400	8493
12	63	45	25	8056	8190	8593	8280
43	17	117	120	8480	9560	9520	9187
9	4	197	205	8200	8000	8640	8280
7	68	97	207	9080	9600	8960	9213
14	187	76	169	7360	8200	6920	7493
35	54	165	167	7680	8400	10200	8760
8	30	72	136	8600	8400	8640	8547
15	203	172	57	7680	9080	10120	8960
4	93	126	143	8500	8500	10267	9089
17	109	134	233	7640	7760	8120	7840
6	149	219	179	7920	9320	8920	8720
42	38	82	215	8800	9520	8800	9040
27	88	209	225	7920	7200	8600	7907
33	8	41	92	10040	9880	9600	9840
19	199	173	231	9520	9000	9840	9453
48	174	67	164	6000	2923	5115	4679
49	59	105	190	8731	16654	7846	11077
50	114	200	230	8538	8423	8538	8500
46	78	178	182	8960	9680	7240	8627
47	12	137	229	7800	8700	9200	8567

Figure 9: Participants results for *G.instriatum*. not id= not identified; nr= no result

ANALYST CODE	Average	X-X*	X*i	it2
20	224	2230	1890	1890
48	1487	966	1890	1890
9	1653	800	1890	1890
25	1680	773	1890	1890
31	1760	693	1890	1890
36	1800	653	1890	1890
14	1800	653	1890	1890
49	1846	607	1890	1890
13	1880	573	1890	1890
50	2000	453	2000	2000
16	2000	453	2000	2000
46	2107	347	2107	2107
41	2111	342	2111	2111
19	2160	293	2160	2160
27	2200	253	2200	2200
39	2213	240	2213	2213
38	2267	187	2267	2267
37	2307	147	2307	2307
11	2373	80	2373	2373
23	2400	53	2400	2400
28	2400	53	2400	2400
45	2406	47	2406	2406
44	2413	40	2413	2413
34	2453	0	2453	2453
10	2467	13	2467	2467
1	2478	25	2478	2478
15	2493	40	2493	2493
5	2533	80	2533	2533
24	2543	90	2543	2543
43	2547	93	2547	2547
29	2573	120	2573	2573
35	2573	120	2573	2573
22	2633	180	2633	2633
18	2653	200	2653	2653
6	2653	200	2653	2653
40	2667	213	2667	2667
4	2700	247	2700	2700
17	2707	253	2707	2707
2	2760	307	2760	2760
8	2760	307	2760	2760
33	2773	320	2773	2773
7	2787	333	2787	2787
30	2920	467	2920	2920
12	2976	523	2976	2976
42	3133	680	3017	3017
3	3280	827	3017	3017
47	3400	947	3017	3017
Average *X*	2361		2406	2406
SD *S*	531		357	357
robust average *X**	2453		2406	2406
robust stdev *S**	376		529	529
δ= 1.5*S**	564		794	794
*X** - δ	1890		1612	1612
*X** + δ	3017		3200	3200
Between Samples SD	965	From homogeneity test		
new stdev for C.granii	1101			

Figure 10: Iteration for *C.granii*

ANALYST CODE	Average	X-X*	X*i	it2
20	544	9003	2814	2814
35	867	8680	2814	2814
48	936	8611	2814	2814
19	1080	8467	2814	2814
27	1960	7587	2814	2814
5	3000	6547	3000	3000
41	3200	6347	3200	3200
18	4987	4560	4987	4987
44	5280	4267	5280	5280
8	5747	3800	5747	5747
36	5840	3707	5840	5840
33	6627	2920	6627	6627
3	6947	2600	6947	6947
6	7440	2107	7440	7440
11	7773	1773	7773	7773
13	7787	1760	7787	7787
28	7787	1760	7787	7787
42	8387	1160	8387	8387
50	8500	1047	8500	8500
14	8587	960	8587	8587
7	8773	773	8773	8773
4	9422	124	9422	9422
17	9547	0	9547	9547
39	10027	480	10027	10027
47	10267	720	10267	10267
30	10347	800	10347	10347
49	10410	864	10410	10410
46	10733	1187	10733	10733
37	10973	1427	10973	10973
43	11200	1653	11200	11200
45	11754	2207	11754	11754
29	11760	2213	11760	11760
31	12387	2840	12387	12387
23	12573	3027	12573	12573
1	12812	3265	12812	12812
15	13240	3693	13240	13240
25	13387	3840	13387	13387
2	13440	3893	13440	13440
10	13800	4253	13800	13800
16	14827	5280	14827	14827
9	14947	5400	14947	14947
34	14960	5413	14960	14960
22	17733	8187	16279	16279
12	18182	8635	16279	16279
38	25557	16010	16279	16279
40	not id	not id	not id	not id
24	not id	not id	not id	not id
Average *X*	9474		9386	9386
SD *S*	5078		4098	4098
robust average *X**	9547		9386	9386
robust stdev *S**	4489		6077	6077
δ= 1.5*S**	6733		9115	9115
*X** - δ	2814		271	271
*X** + δ	16279		18501	18501
Between Samples SD	965	From homogeneity test		
new stdev for C.diadema	6153			

Figure 11: Iteration for *C.diadema*

ANALYST CODE	Average	X-X*	X*i	it2
20	74	6153	2015	2015
50	269	5957	2015	2015
41	933	5293	2015	2015
48	1096	5131	2015	2015
24	1333	4893	2015	2015
44	2973	3253	2973	2973
18	3067	3160	3067	3067
40	3167	3060	3167	3167
49	3474	2752	3474	3474
13	3973	2253	3973	3973
7	3987	2240	3987	3987
25	4027	2200	4027	4027
19	4133	2093	4133	4133
46	4333	1893	4333	4333
8	5027	1200	5027	5027
9	5080	1147	5080	5080
29	5507	720	5507	5507
47	5700	527	5700	5700
2	5733	493	5733	5733
38	5773	453	5773	5773
30	5867	360	5867	5867
22	5867	360	5867	5867
23	6227	0	6227	6227
14	6507	280	6507	6507
12	6691	464	6691	6691
43	6920	693	6920	6920
10	7027	800	7027	7027
16	7067	840	7067	7067
1	7073	846	7073	7073
34	7107	880	7107	7107
39	7120	893	7120	7120
45	7305	1078	7305	7305
37	7520	1293	7520	7520
11	7680	1453	7680	7680
4	7900	1673	7900	7900
3	7987	1760	7987	7987
31	8333	2107	8333	8333
27	8560	2333	8560	8560
36	9147	2920	9147	9147
28	9907	3680	9907	9907
6	10253	4027	10253	10253
17	11600	5373	10438	10438
42	13213	6987	10438	10438
33	14013	7787	10438	10438
15	16333	10107	10438	10438
5	not id	not id	not id	not id
35	not id	not id	not id	not id
Average *X*	6286		6130	6130
SD *S*	3438		2555	2555
robust average *X**	6227		6130	6130
robust stdev *S**	2808		3789	3789
$\delta = 1.5S^*$	4212		5684	5684
$X^* - \delta$	2015		446	446
$X^* + \delta$	10438		11814	11814
Between Samples SD	965	From homogeneity test		
new stdev for H.akashiwo	3910			

Figure 12: Iteration for *H.akashiwo*

ANALYST CODE	Average	X-X*	*X*i*	it2
20	731	7989	7682	7682
40	3500	5220	7682	7682
48	4679	4041	7682	7682
5	5400	3320	7682	7682
41	6067	2653	7682	7682
24	6589	2131	7682	7682
13	7307	1413	7682	7682
14	7493	1227	7682	7682
17	7840	880	7840	7840
27	7907	813	7907	7907
30	8200	520	8200	8200
12	8280	440	8280	8280
9	8280	440	8280	8280
31	8413	307	8413	8413
37	8493	227	8493	8493
50	8500	220	8500	8500
8	8547	173	8547	8547
25	8560	160	8560	8560
47	8567	153	8567	8567
45	8624	96	8624	8624
46	8627	93	8627	8627
11	8667	53	8667	8667
38	8667	53	8667	8667
6	8720	0	8720	8720
28	8760	40	8760	8760
35	8760	40	8760	8760
36	8787	67	8787	8787
44	8853	133	8853	8853
15	8960	240	8960	8960
18	8973	253	8973	8973
42	9040	320	9040	9040
1	9073	353	9073	9073
4	9089	369	9089	9089
23	9107	387	9107	9107
43	9187	467	9187	9187
7	9213	493	9213	9213
3	9227	507	9227	9227
39	9227	507	9227	9227
29	9293	573	9293	9293
10	9387	667	9387	9387
19	9453	733	9453	9453
22	9633	913	9633	9633
34	9680	960	9680	9680
16	9837	1117	9758	9758
33	9840	1120	9758	9758
2	10653	1933	9758	9758
49	11077	2357	9758	9758
Average *X*	8335		8704	8704
SD *S*	1778		660	660
robust average *X**	8720		8704	8704
robust stdev *S**	692		978	978
δ= 1.5*S**	1038		1467	1467
*X**- δ	7682		7237	7237
*X**+ δ	9758		10171	10171
Between Samples SD	965	From homogeneity test		
new stdev for G.instriatum	1374			

Figure 13: Iteration for *G.instriatum*

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 (Figs. 10-13). The standard uncertainty of the assigned value can then be calculated using the equation (G) below;

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

Where;

u_x= Standard uncertainty of the assigned value,

s^*= robust standard deviation for the test

p= number of analysts

	C.granii	C.diadema	H.akashiwo	G.instriatum	Homogeneity
Robust mean x*	2406	9386	6130	8704	8320
Robust Stdev s*	529	6077	3789	978	1108
Standard Ux	97	1132	706	178	204
n=	47	45	45	47	46
if Ux < 0.3xSTdev	159	1823	1137	293	332
then Ux is negligible	neg	neg	neg	neg	neg
The equation is satisfied in all cases					

Figure 14: Assigned value and standard uncertainty for the test.

If *Ux* is less than 0.3 times the standard deviation for the test, then this uncertainty is negligible for the test material. In our case, all our test materials satisfy the equation.

4.5 Comparison of the assigned value

When the consensus values from the participants are used to calculate the standard uncertainty of the assigned values, the values can then be compared against a reference value from an expert laboratory. We used the homogeneity test result to compare this value against the value calculated by the participants using equation (H) below:

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

Where;

u_x= Standard uncertainty of the assigned value,

s^*= robust standard deviation for the test

p= number of analysts

If the difference is more than twice its uncertainty, then possible reasons need to be sought regarding bias. In this case the difference (376) is less than twice the uncertainty (577), so the rule is satisfied.

	C.granii	C.diadema	H.akashiwo	G.instriatum	Homogeneity		Homogeneity test
Robust mean x*	2406	9386	6130	8704	8320	Reference value mean	7944
Robust Stdev s*	529	6077	3789	978	1108	Reference value stdev	1061
Standard Ux	97	1132	706	178	204		
n=	47	45	45	47	46		
if Ux < 0.3xSTdev	159	1823	1137	293	332		
then Ux is negligible	neg	neg	neg	neg	neg		
The equation is satisfied in all cases							

Comparison with assigned value		
*x *-X*	376	
Uncertainty	289	577

If diff. Is more than twice its Uncertainty then rule is not satisfied

Figure 15: Comparison of the assigned value

4.6 Calculation of performance statistics

4.6.1 Estimates of laboratory bias

Estimates of laboratory bias indicate results are normally distributed around zero for all measurands (Fig. 16). Most results are within one standard deviation of each other. The percentage difference graph by measurand (fig. 17) suggests that the spread of results across zero is larger on the *C.diadema* and *H.akashiwo* counts and tighter on the rest. It also shows green lines and red lines which correspond to warning and action signal limits.

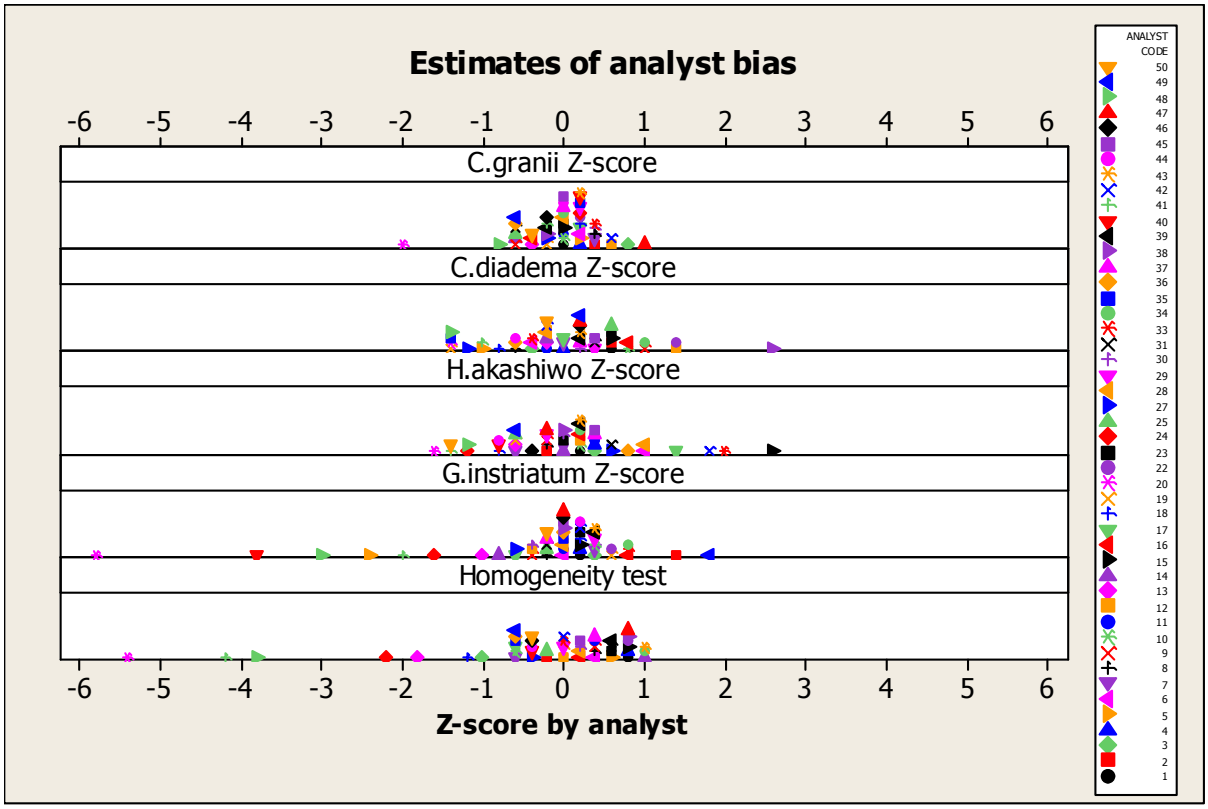


Figure 16: Estimates of analyst bias using Z-scores by test item

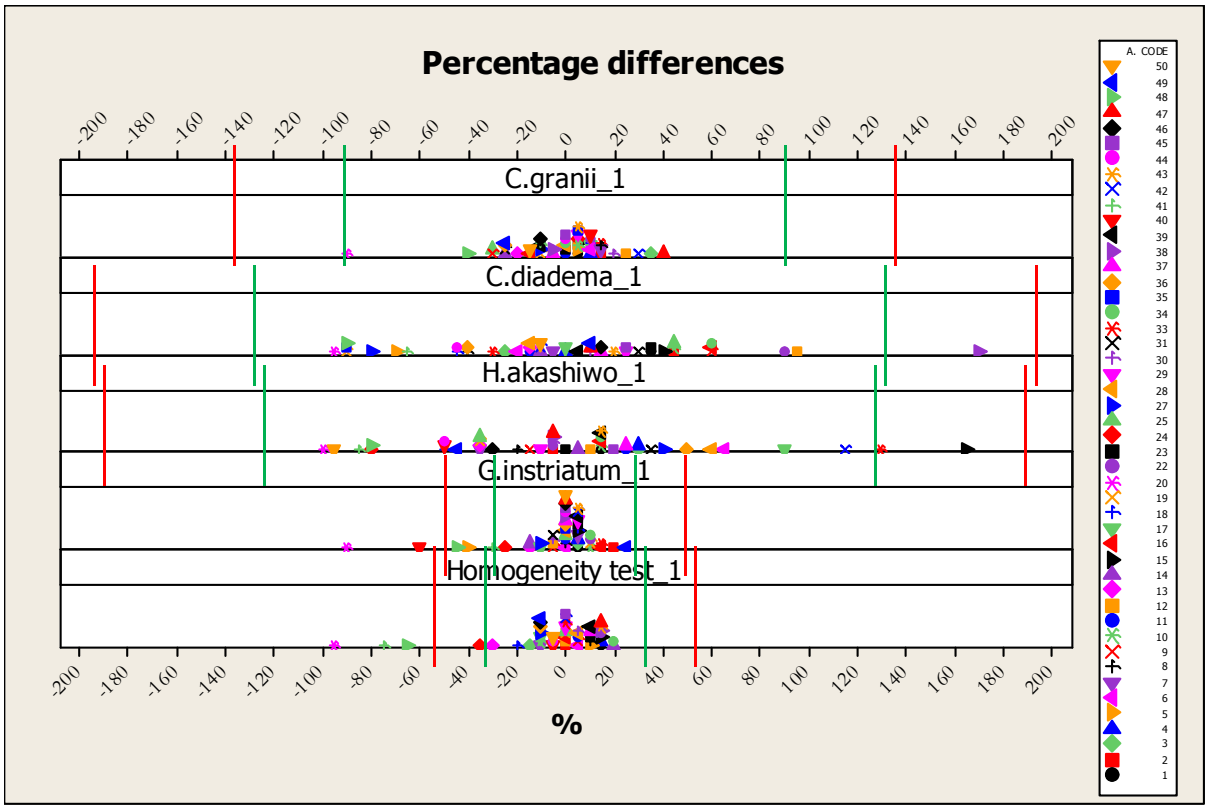


Figure 17: Percentage difference by test item

4.6.2 Probability plots by % rank

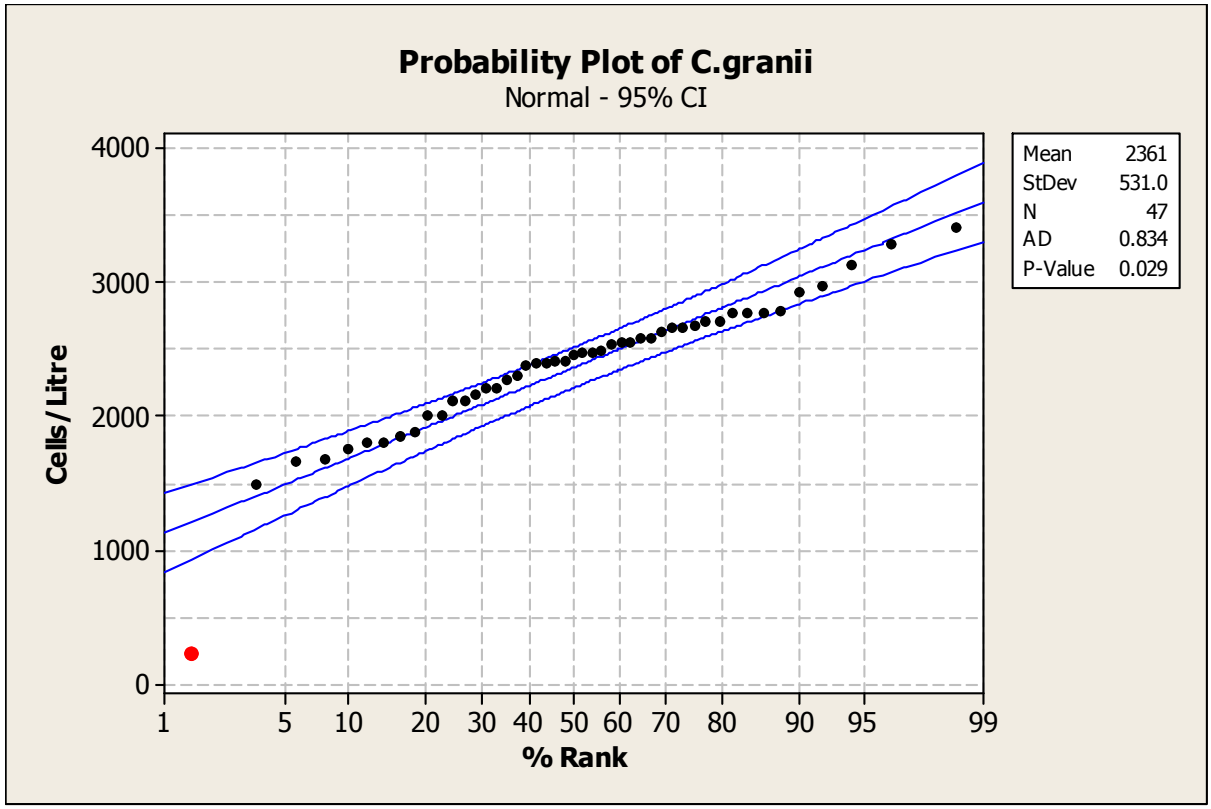


Figure 18: Probability plot for *C.granii*

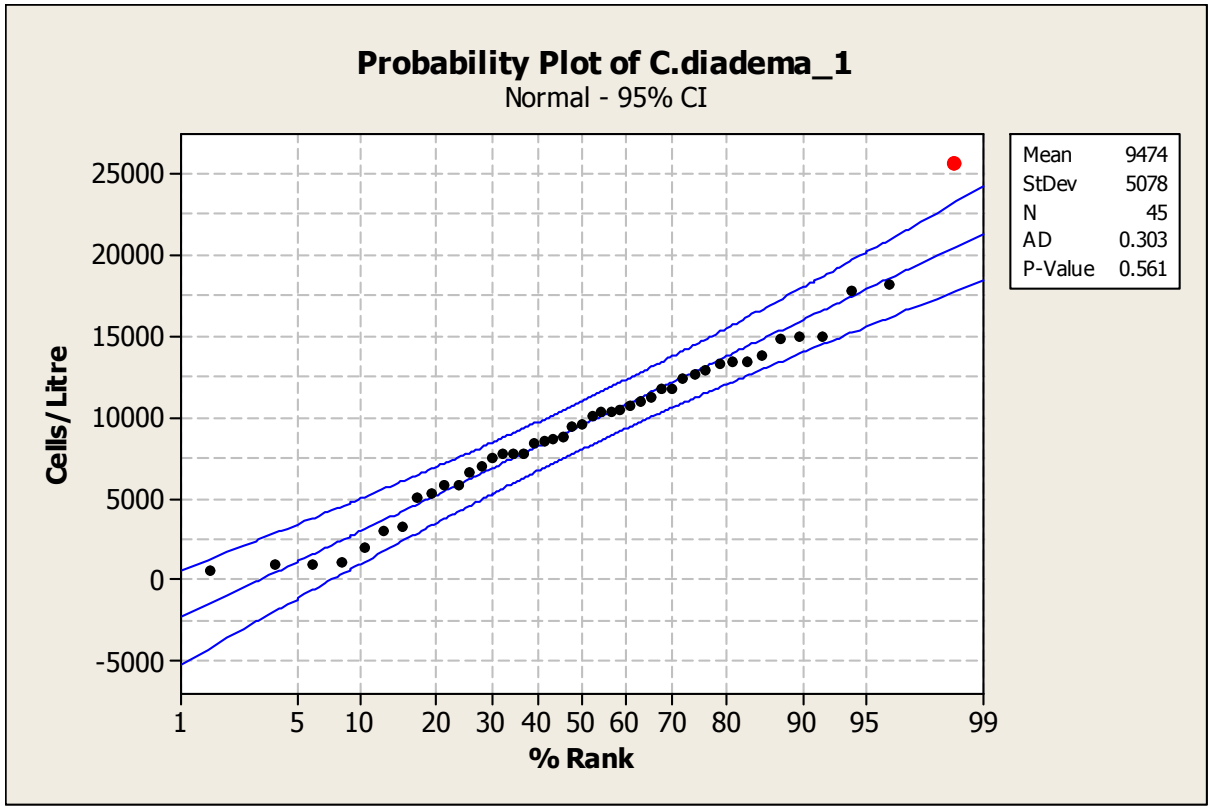


Figure 19: Probability plot for *C.diadema*

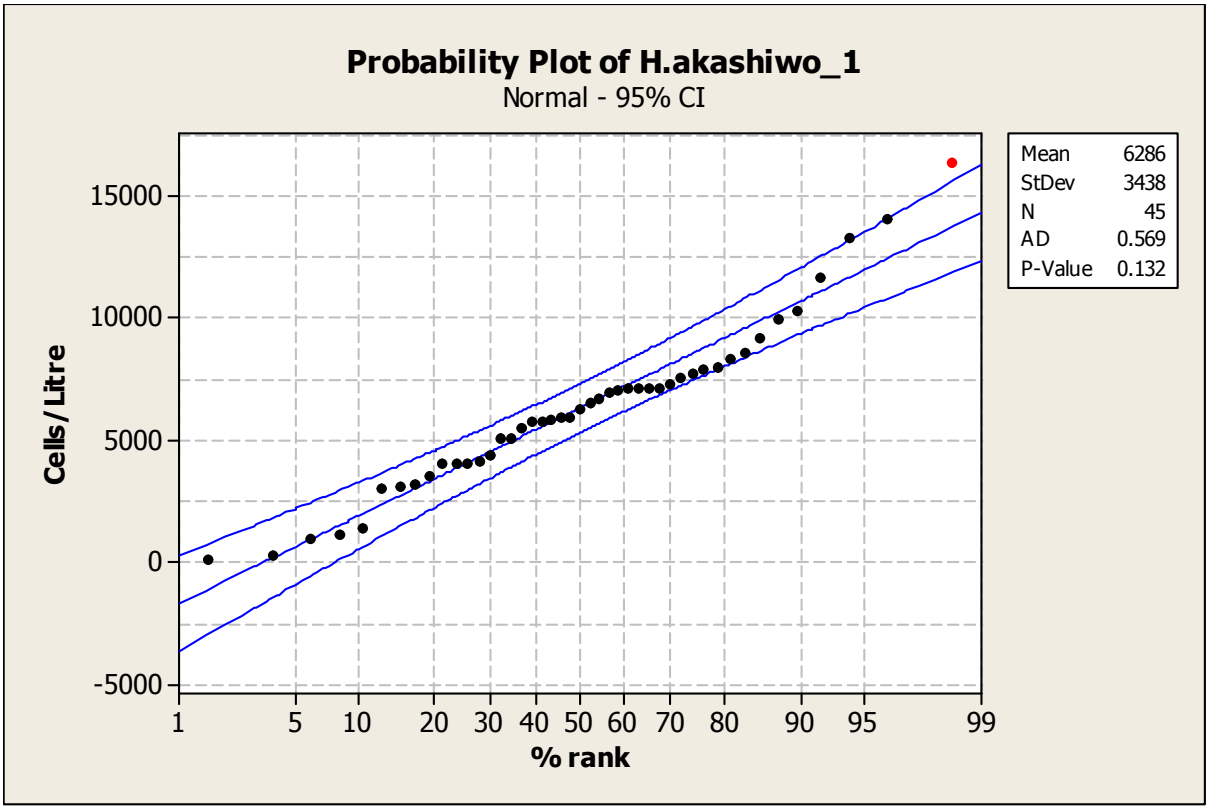


Figure 20: Probability plot for *H.akashiwo*

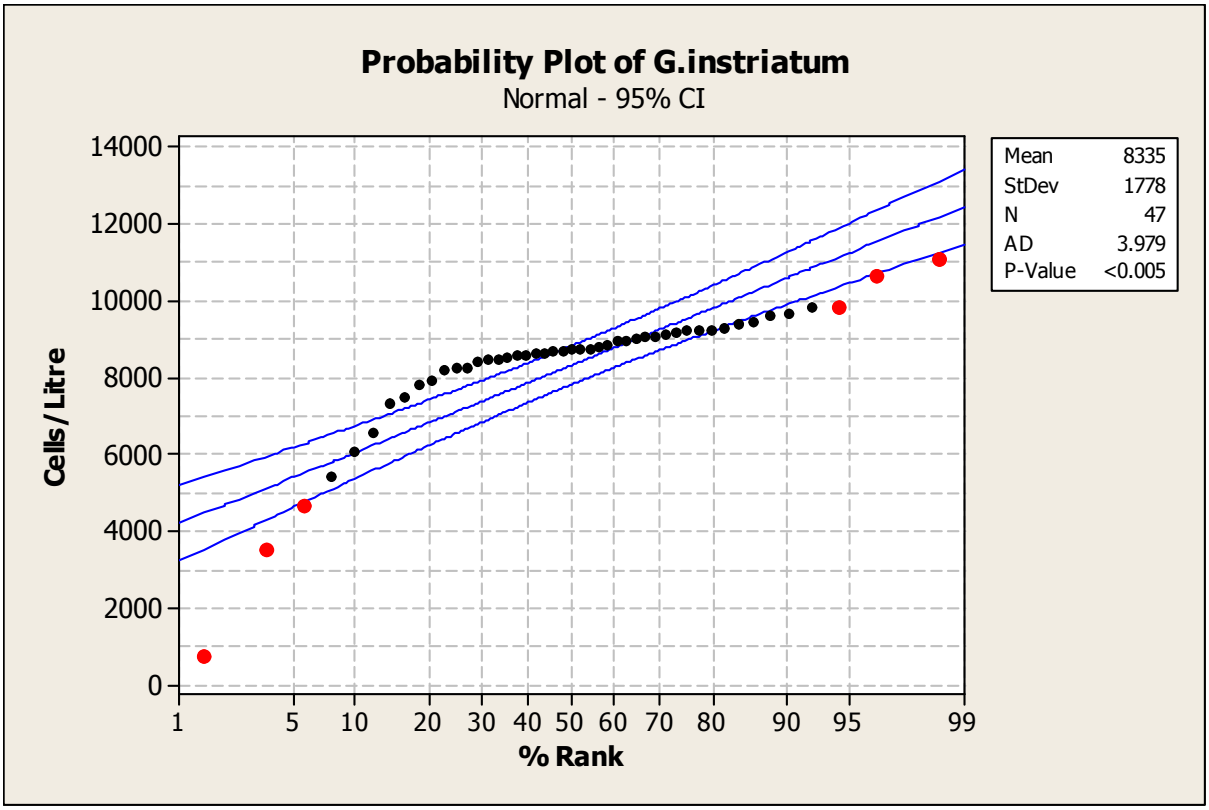


Figure 21: Probability plot for *G.instriatum*

The probability plots using percentage rank in the x axis is an easy way to show the laboratories reporting the most extreme results for each measurand. The laboratory with the lowest result is assigned rank 1, the next lowest result is rank 2 and so on until the laboratory ranked with the highest result. This analysis doesn't assume that the data follows any particular probability distribution.

4.6.3 Z-scores

The z-scores derived using the robust averages and standard deviations (figs. 10-13) are tabulated and found in annex IX. Figure 22 shows the warning (2SD) and action (3SD) limits for each measurand using the robust standard deviations and taking into account the heterogeneity of the samples. The graphs (figs. 23-26) show the Z-scores of each analyst using this data.

	C.granii	C.diadema	H.akashiwo	G.instriatum
Robust mean	**2406**	**9386**	**6130**	**8704**
new Stdev	**1101**	**6153**	**3910**	**1374**
σ*3.0	3303	18459	11730	4122
σ*2.0	2202	12306	7820	2748
σ*-2.0	-2202	-12306	-7820	-2748
σ*-3.0	-3303	-18459	-11730	-4122

Figure 22: Robust mean and standard deviation limits for Z-scores

There is a warning signal in the *C.diadema* count for analyst 38, two action signals for analysts 33 and 15 in the *H.akashiwo* count and two warning (analysts 48, 5) and two action (analysts 20, 40) signals in the *G.instriatum* count.

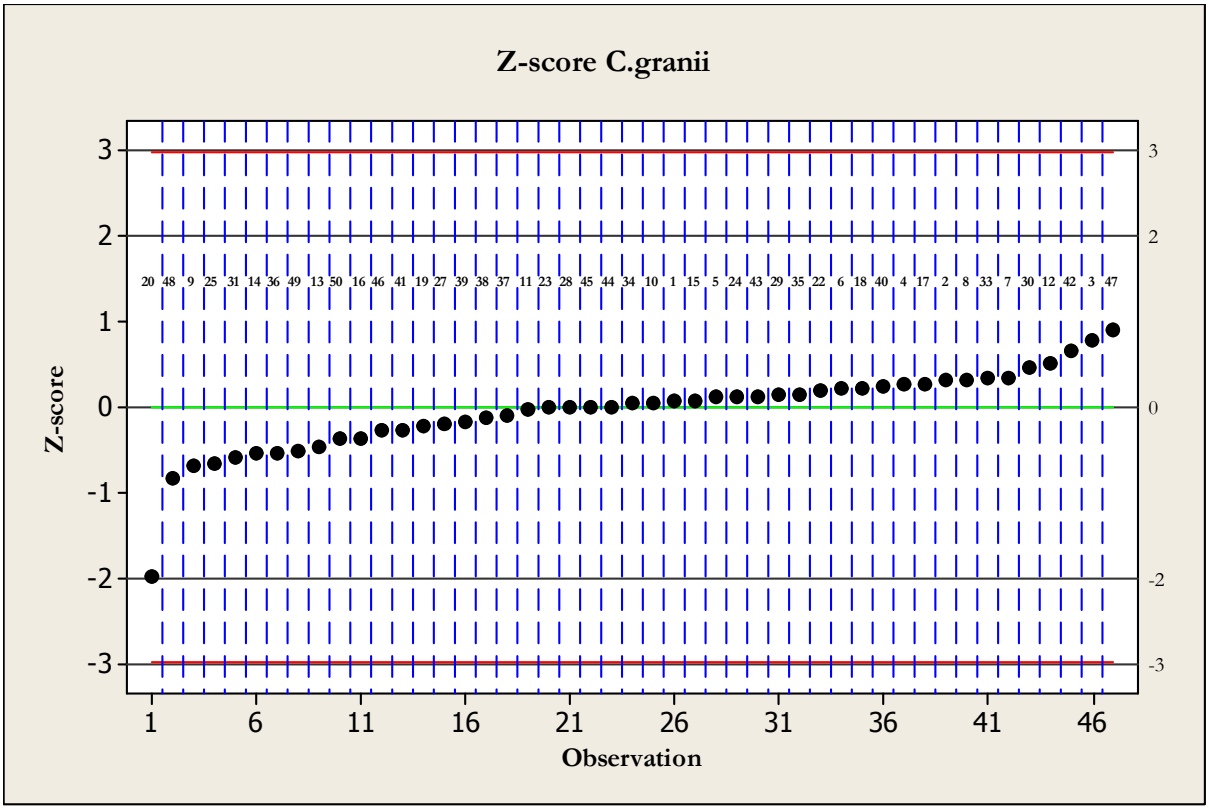


Figure 23: Z-scores for *C.granii*

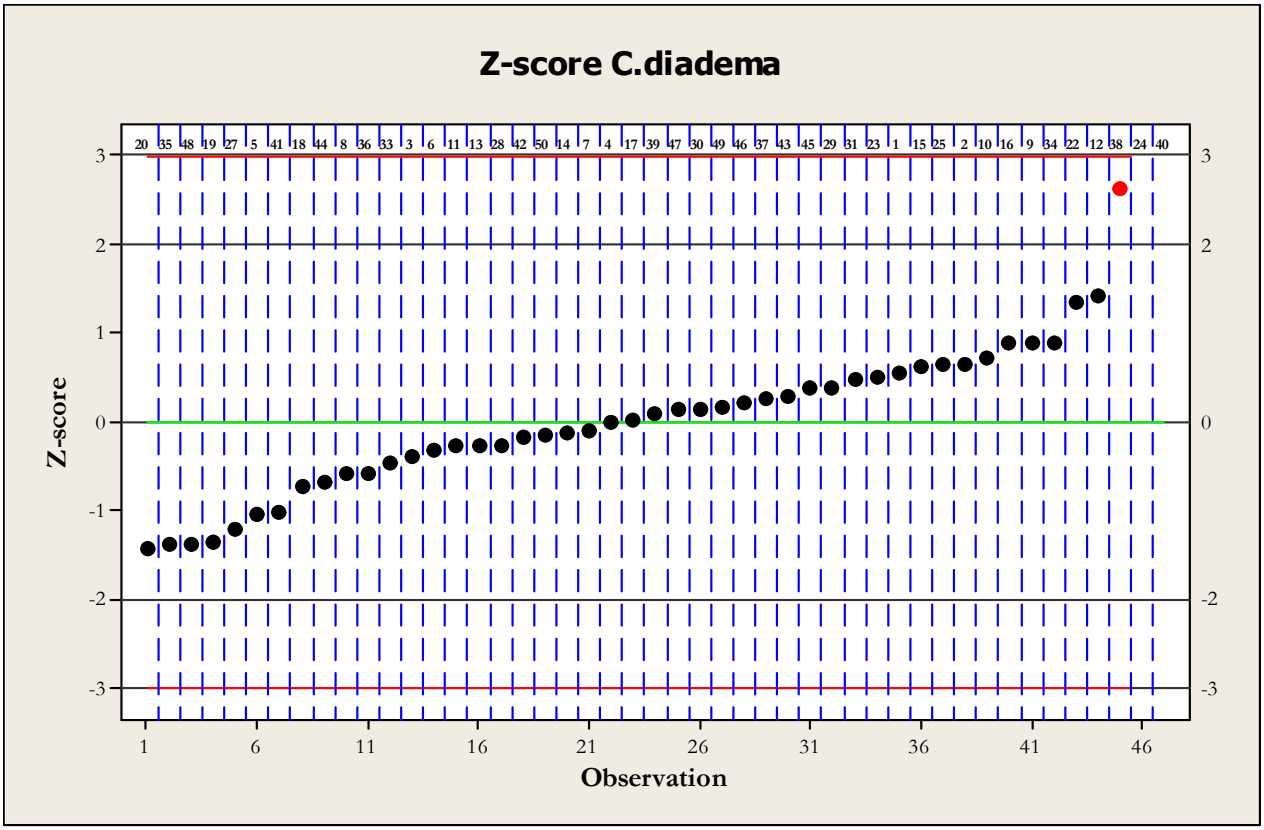


Figure 24: Z-scores for *C.diadema*

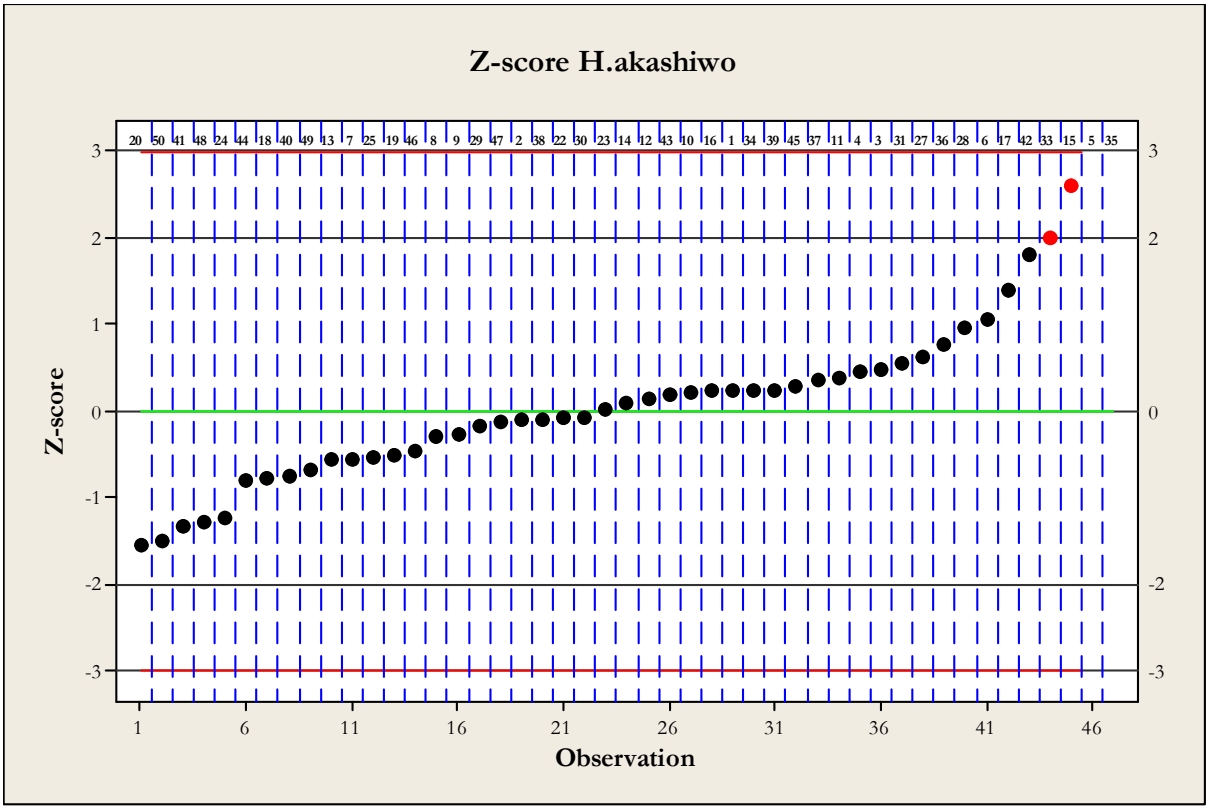


Figure 25: Z-scores for *H.akashiwo*

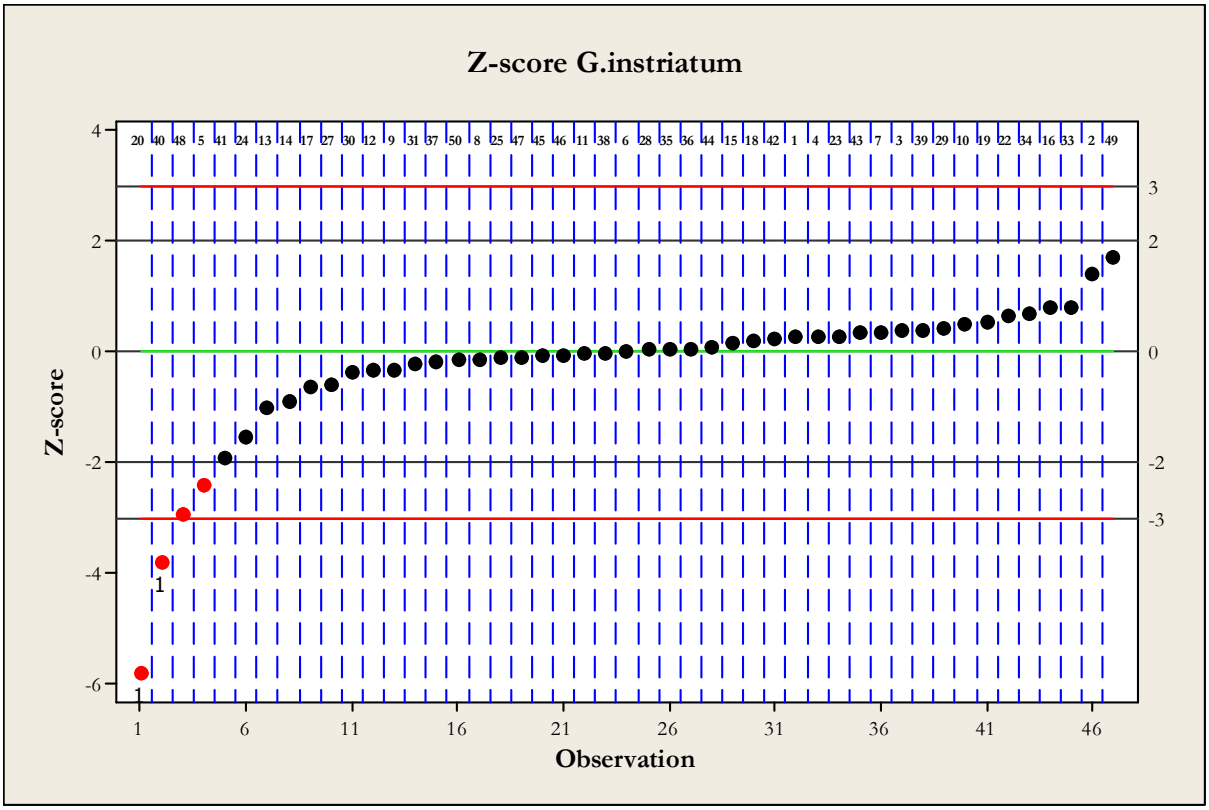


Figure 26: Z-scores for *G.instriatum*

4.7 Combined performance scores

4.7.1 Histograms

The histograms in figures 27-30 show the frequency of warning and action signals by measurand.

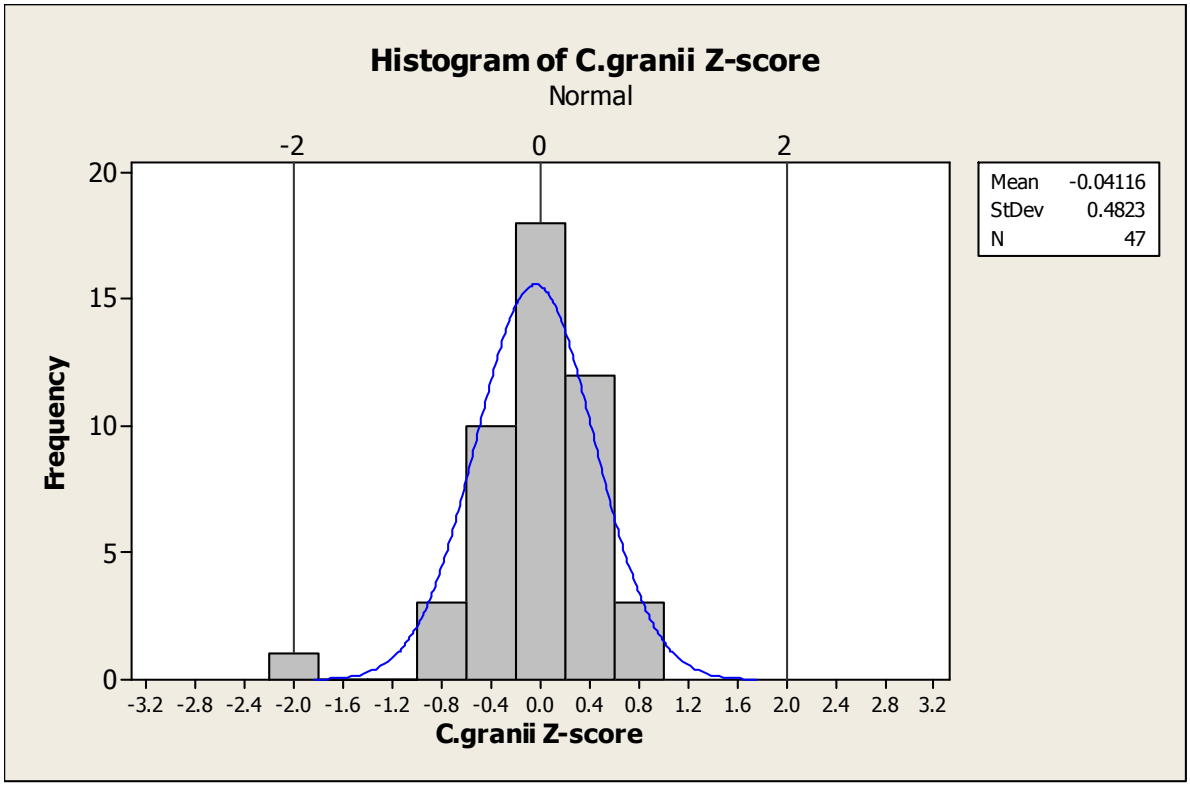


Figure 27: Histogram of *C.granii*

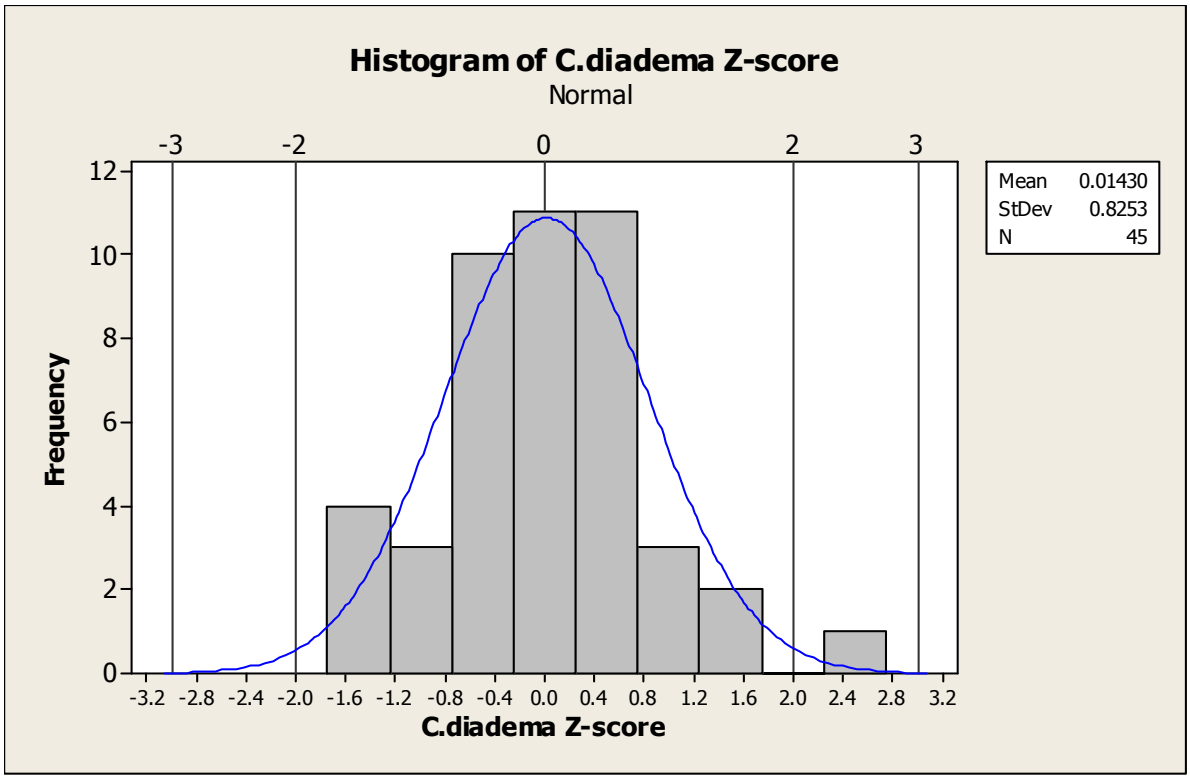


Figure 28: Histogram of *C.diadema*

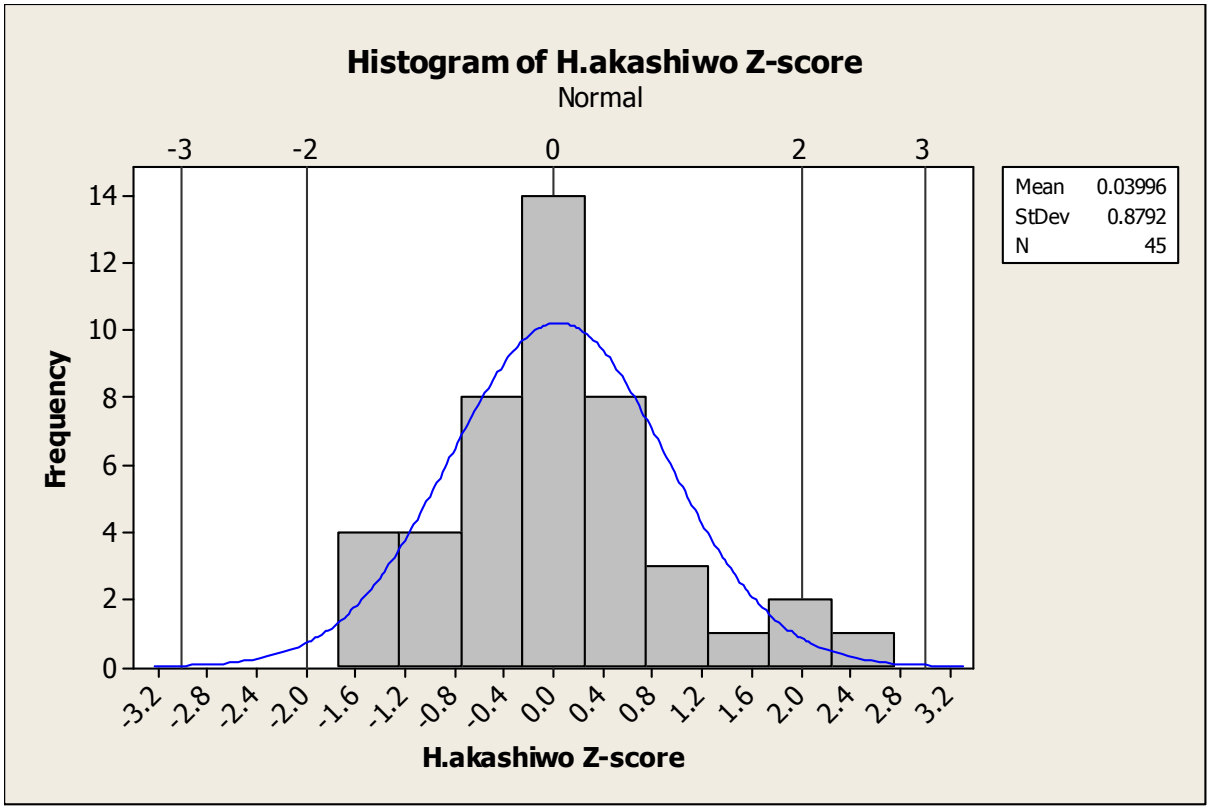


Figure 29: Histogram of *H.akashiwo*

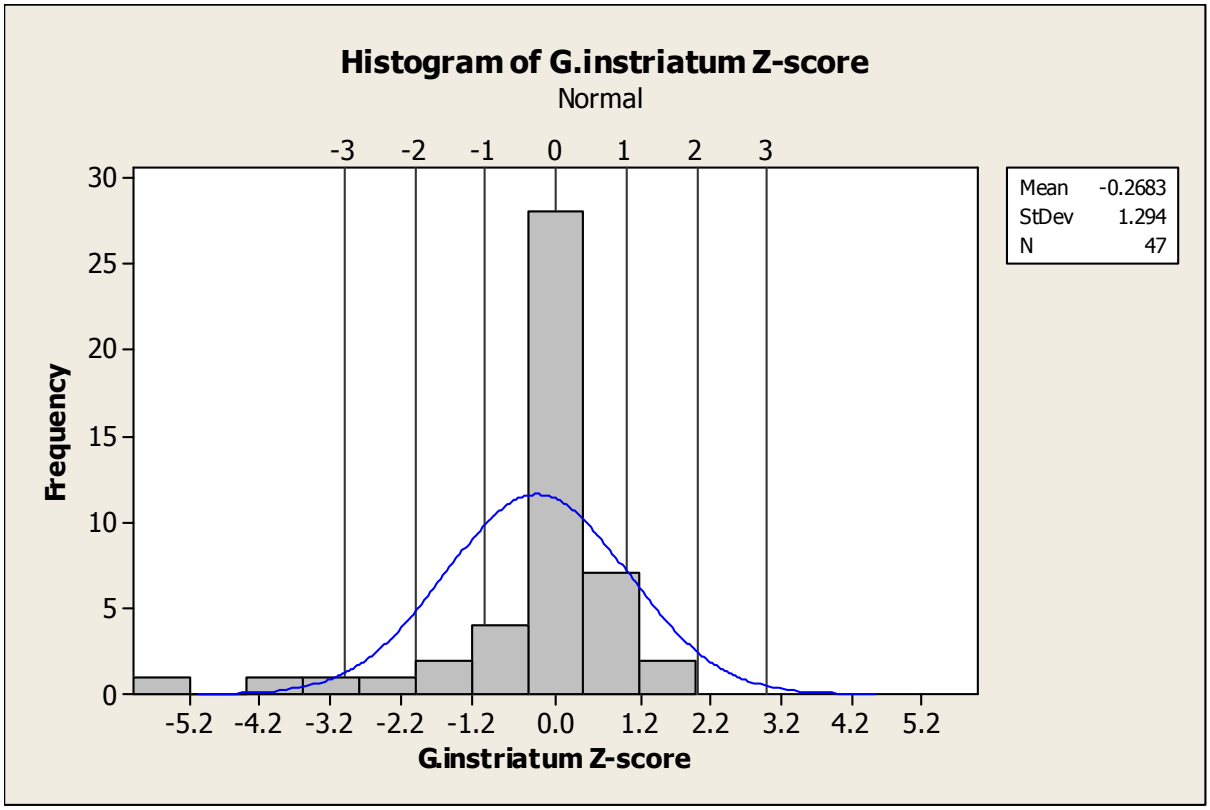


Figure 30: Histogram of *G.instriatum*

4.7.2 Bar plots of standardized laboratory bias

This bar plot charts the z-scores of all measurands by analyst. This plot reveals a cause of bias for analysts 20, 41 and 48. These three analysts have tended to underestimate all their counts. In some cases, these are above the warning and action signals. These analysts should study the cause of this as it could point out to a methodology issue.

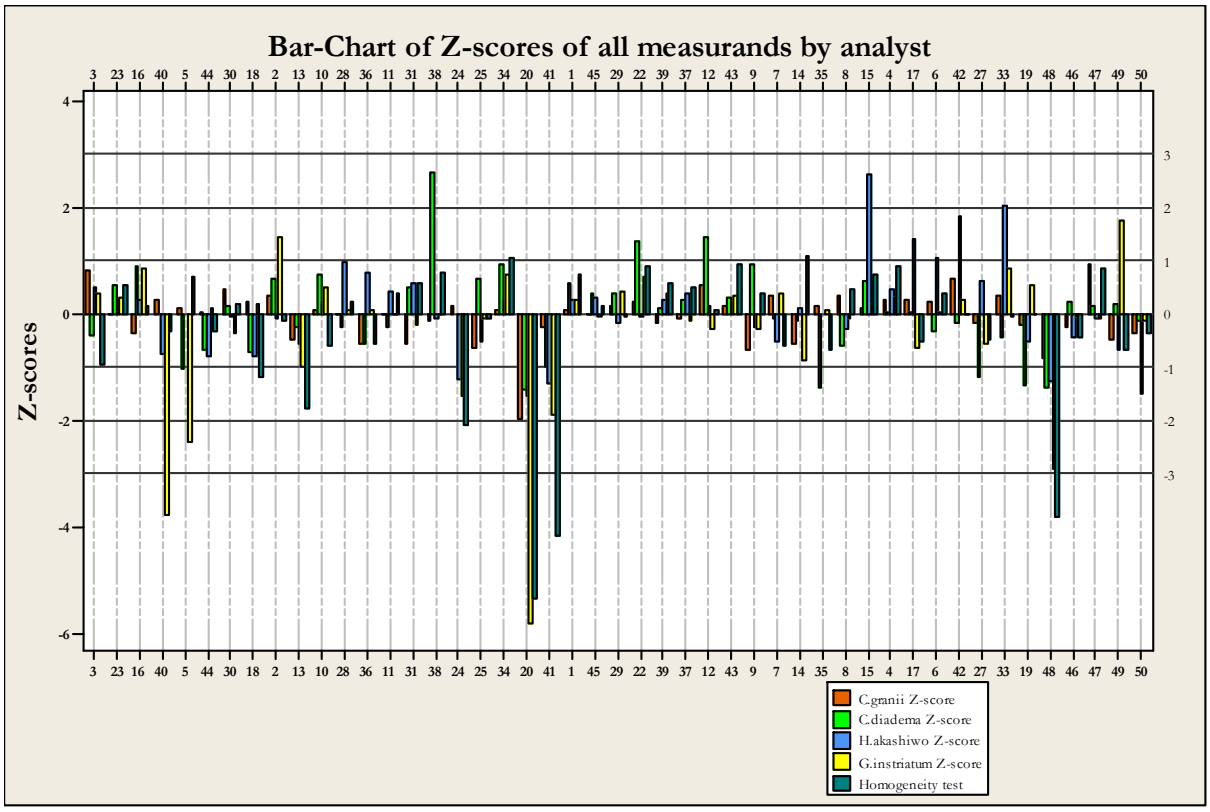


Figure 31: Bar plot of Z-scores of all measurands by analyst

4.7.3 Plots of repeatability standard deviation

The plots of repeatability standard deviations are used to identify analysts whose average and standard deviation are unusual. They assume that the data is normally distributed and the null hypothesis is that there are no differences between the analyst means and standard deviations using the van Nuland circle technique (figs. 32-35) for each measurand. It shows that the averages for *C.diadema* (fig. 33) and *H.akashiwo* (fig. 34) are unusually spread across the consensus mean with a wide spread of results suggesting a difficulty assessing the density of these cell counts in the samples.

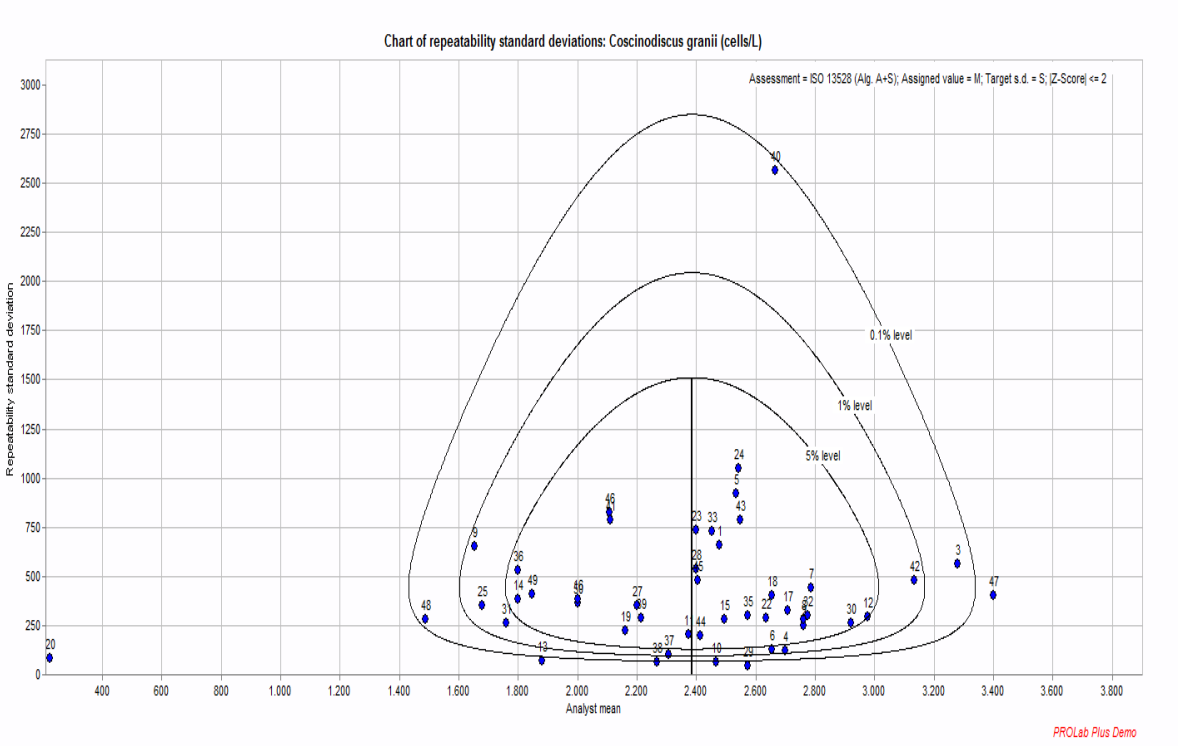


Figure 32: Plot of repeatability standard deviation of *C.granii*

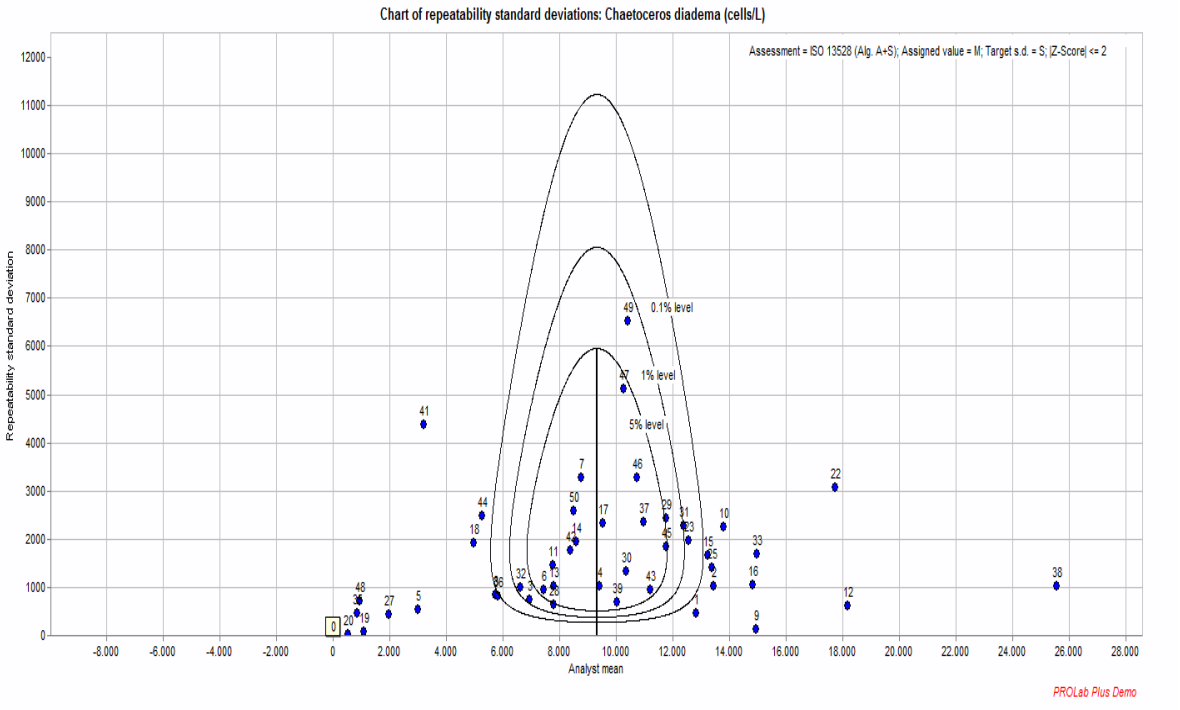


Figure 33: Plot of repeatability standard deviation of *C.diadema*

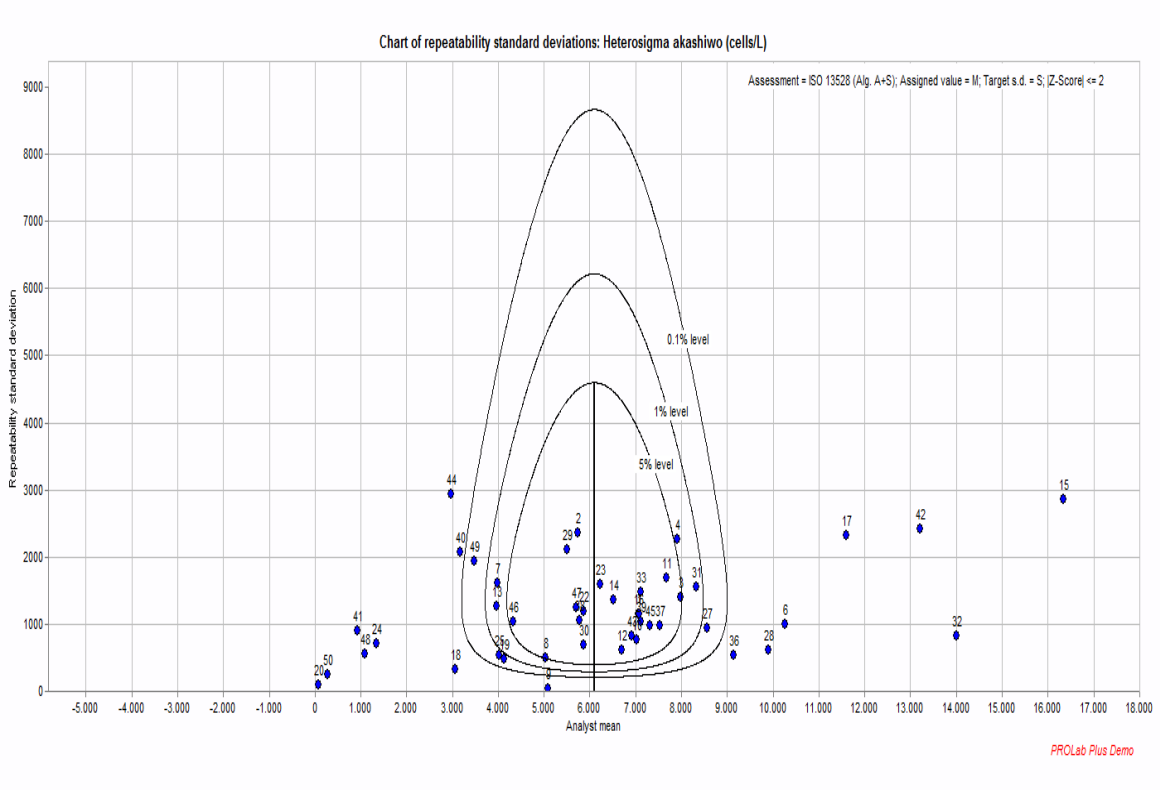


Figure 34: Plot of repeatability standard deviation of *H.akashiwo*

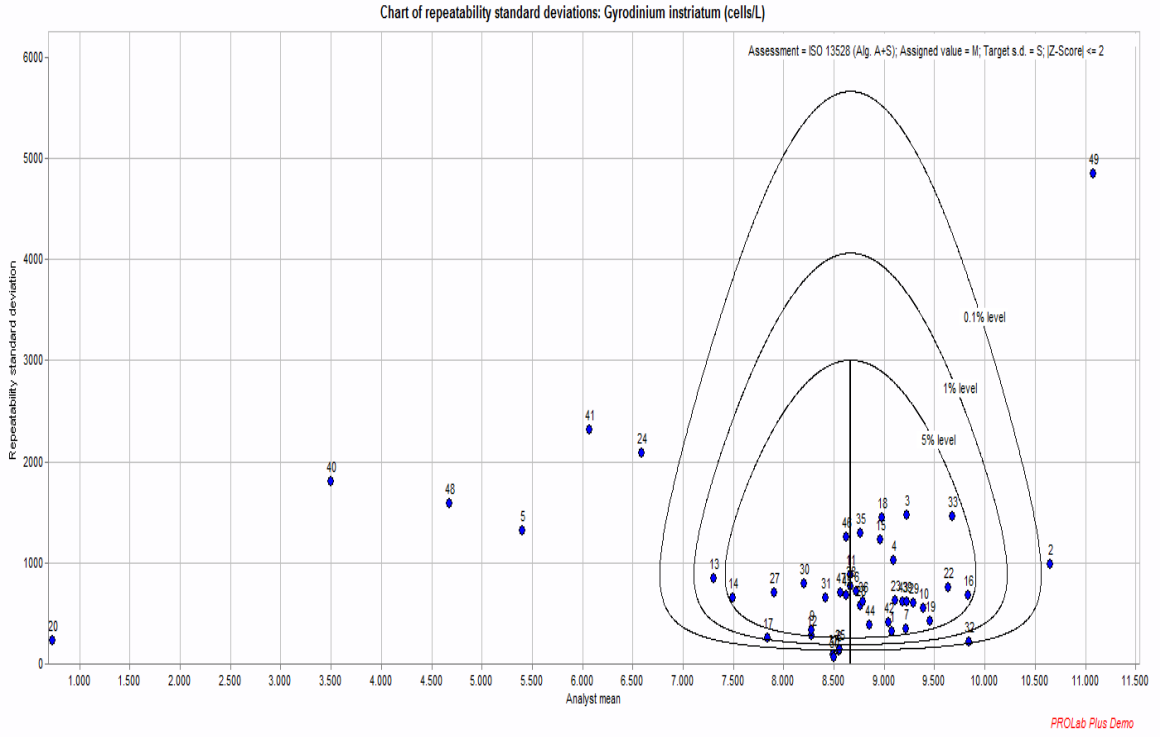


Figure 35: Plot of repeatability standard deviation of *G.instriatum*

4.8 Qualitative data

Figure 36 shows the answers given by analysts to the identification of the species spiked in the samples. Participants found the species *C.granii* and *H.akashiwo*, the easiest to identify with nearly perfect scores for both. *C.diadema* was one of the most difficult species to identify with most participants deciding to go to genus level only and 12 participants to species level. Six identified correctly the species but there were no incorrect answers at genus level. *G.instriatum* was the most difficult of all the species to identify with a total of 18 correct answers to species level and 37 to genus level. Also, ten incorrect answers were given, eight as *Gimnodinium catenatum* and two as *Karenia mikimotoi*.

	np	Answers	genus correct	Species	not id	other answers				
Coscinodiscus granii	2	47	47	46	0	1 Concinnus				
Chaetoderos diadema	2	47	45	6	2	33 Hyalochate		5 lorenzianus		1 didymus
Heterosigma akashiwo	2	47	45	45	2	0				
Gyrodinium instriatum	2	47	37	18	0	19 sp.	8 G.catenatum		2 K.mikimotoi	

Np= not participated Not id= not identified

Figure 36: Qualitative data by measurand

4.9 Ocean Teacher online HAB quiz

The online HAB quiz consisted of 14 questions, annex X shows the results of each analyst by question and the final grade by analyst and the statistics of each question at the bottom. Below (fig 37) shows the final grades by analyst and laboratory.

Analyst code	% correct	Analyst code	% correct
40	100.0	48	100.0
13	100.0	31	100.0
25	100.0	16	100.0
45	100.0	35	100.0
49	100.0	18	98.6
30	100.0	41	97.7
43	100.0	11	92.9
17	100.0	47	92.9
34	100.0	20	92.9
8	100.0	9	92.9
15	100.0	24	92.9
27	100.0	6	92.9
46	100.0	36	92.9
38	100.0	29	92.9
44	100.0	28	92.9
42	100.0	10	92.9
50	100.0	39	85.7
12	100.0	2	85.7
33	100.0	3	85.7
23	100.0	37	85.7
4	100.0	5	82.9
19	100.0	14	71.4
1	100.0		

Figure 37: Oceanteacher HAB quiz grades by analyst

The results (fig 37) suggest a high rate of perfect scores for this quiz. The cumulative frequency of scores (fig. 38) shows that 27 analysts, that is 60% of all analysts had a 100% score with another 12 analysts above 90% (29% of all analysts) and only 13.3 % of analysts below this mark. This suggests a high standard for most analysts involved.

Variable	Grade	Count	N	N*	CumN	Percent	CumPct
ANALYST CODE	71.4	1	1	0	1	2.2222	2.222
	82.9	1	1	0	2	2.2222	4.444
	85.7	4	4	0	6	8.8889	13.333
	92.9	10	10	0	16	22.2222	35.556
	97.7	1	1	0	17	2.2222	37.778
	98.6	1	1	0	18	2.2222	40.000
	100.0	27	27	0	45	60.0000	100.000

Figure 38: Cumulative percentage of correct answers by analyst

Figure 39 a value plot of correct answers by question show that the majority of the questions average above 90% except for Q8. This was the worst answered question with a 73% on average correct responses (see annex X).

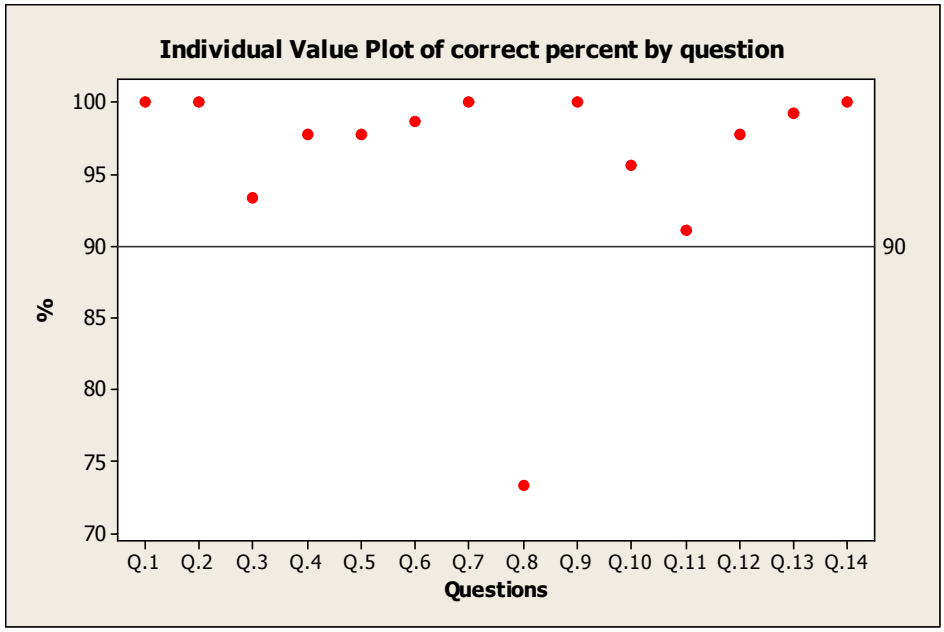


Figure 39: Individual value plot of % correct answers by question

There were some problems with scores arising from 'short answer' question types where grammar errors, punctuation or similar answers were given as incorrect. This is a software related problem not easily resolved as the answers given by analysts have to match perfectly the one written in the programme. Therefore, the results had to be filtered in Excel to update some scores. There was another issue regarding the naming authority and use of synonyms in answers as in Q10 where the name *Zygabikodinium* currently regarded as a synonym of *Preperidinium* was accepted as correct.

5. Discussion

The present format of this intercomparison exercise has been in use since 2010 and it appears to be a successful working model. This test is divided into two clearly defined sections; an online HAB quiz test set up in a remote platform accessed via the web and the identification and quantification of marine algae in lugol's preserved water samples. These are generally spiked with cultured material, which allows for a better control of the spiked material in terms of their cell concentration and their identity.

Identification and enumeration on preserved water samples

The identification and enumeration exercise has been prepared in a similar fashion to previous years but a number of changes have taken place in relation to the use of statistics, this time, we are following the statistical methods laid out in ISO13528 to calculate the performance statistics for the test. Also, some of the forms used to write the results of the test have been re-vamped. The enumeration and identification logsheets (See Annex II and III), which in previous years were set up as a Word document where analysts entered their results and calculations, this time were set up as an Excel spreadsheet.

The Excel spreadsheet contains an embedded reduced marine phytoplankton species list which is linked to the identification logsheet table and appears as a dropdown menu list, where analysts must choose the right entries for each sample. The advantages of using the forms set up in this way to include the analysts' results are various but primarily, the results are always readable, numerical transcription errors are avoided and no interpretation of the results is needed as it avoids identifications like e.g. unidentified armoured dinoflagellate, centric diatom, naked dinoflagellates, etc. There are also some disadvantages, as the reduced list can be construed to be an aid to the identification of the species and a deviation to the method.

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

The new way of calculating these values using the robust averages and standard deviations from ISO 13528 is a definitive departure from previous years. ISO 13528 is the standard used for statistical methods in proficiency testing by interlaboratory comparisons. It describes sound statistical methods and recommendations of their use which can be applied to demonstrate unacceptable levels of laboratory bias. It

gives the statistical guidelines for the interpretation of tests and it is to be used as the reference document in future exercises. This standard is only applicable to quantitative data but not qualitative.

Homogeneity and stability test

A homogeneity and stability test showed that the samples didn't meet the assessment criteria set out in ISO 13528. The standard, however, gives various ways of working around this. The first step is to check the sample preparation of the materials. The materials are homogenized manually and this procedure for sample homogenization it is best practice and widely used. Lots of work has gone into the preparation of these materials over the years and while this methodology is not perfect, it is the best and simplest available protocol.

The data also shows that the average and standard deviation of the homogeneity samples either analysed by the expert laboratory (7944 ±1061) (fig. 1) or the participants (8320±1108) (fig. 2) are reasonably close, a standard deviation of 1000 cells/L is the equivalent of 25 cell difference between samples, which in my view doesn't look like a big difference. We need to think also in terms of the difficulties in homogenizing different materials in terms of size, shape, their fragility, cells in chains, preservation integrity and so on. In conclusion phytoplankton species are not easy test materials to homogenize and perhaps this is the best we can do. There are, other methods for homogenizing samples automatically and we could in future exercises use and compare these automated techniques against our manual method, see if homogeneity and stability improves.

ISO 17043 in note 3 says: "In some cases, materials that are not sufficiently homogeneous or stable are the best available; in such cases, they can still be useful as proficiency test items, provided that the uncertainties of the assigned values or the evaluation of results take due account of this". We have calculated the standard uncertainty of the assigned values (fig. 14) and we have found that in all the test items used in this round the standard uncertainty is negligible. Also, when the consensus values form the participants are used, the assigned value can be compared with a reference value in order to ascertain that there is no bias in the method. We have used the data generated in the homogeneity test and proved that this is the case (fig.15).

The second step is to calculate the number of replicate measurements per participant needed so that the assessment criteria are met and in this case we had calculated 20 (fig. 3) as the desirable number, which is too large to be a practical option. The third option is to include the between sample standard deviation to the assigned value standard deviation for each test item which is what we have done here.

Calculation of performance statistics

The consensus values from the participants were used to calculate the performance statistics for the test. These values take into account the heterogeneity of the samples from the homogeneity test, that is the between sample standard deviation, and the assigned values for the test materials used in this round were calculated using the robust algorithm A in annex C of ISO13528 which are derived by an iterative calculation using the new modified averages and standard deviations until the process converges (figs.10-13). This method takes care of outliers in the dataset and missing values.

The assigned values for each measurand were then used to calculate estimates of laboratory bias (fig.16), percentage differences (fig. 17), ranking (figs. 18-21) and finally Z-scores (figs.23-26). 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, results shown are rounded around the zero which suggests no bias. Percentage difference is another way of showing data similar to the estimates of laboratory bias plots by using percentages instead of Z-scores. Ranking used in figures 18-21 uses probability plots of percentage ranks, this type of plots do not involve assumptions above the normality of the data and simply identifies the laboratories/analysts that report the most extreme results for each measurand.

Z-scores are used to assign the results to each analyst. These Z-scores have been produced using the robust averages and standard deviations for each measurand through iteration and adding the standard deviation calculated from the heterogeneity of the samples. The results show that Z-scores are generally within the specification of the test for most analysts with a number of warning and action signals. A warning signal is a result between 2 and 3SD of zero and an action signal is a result outside 3SD. Two warning signals in consecutive intercomparisons give rise to an action signal. An action signal signifies that an investigation of the causes by the laboratory should be carried out.

There are only two action signals in one count (analysts 20 and 40) and five warning signals (analysts 38, 48, 5, 33, 15) in total in all the counts.

Combined performance scores

It is common in any rounds of a proficiency testing exercise to obtain results from several test items or measurands, in our case each species found in the samples is a test item or measurand. As this is generally

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

The Z-scores plotted in figures 23-26 can also be represented through histograms in figures 27-30. Histograms represent a quick way to see how many laboratories/analysts fail to satisfy the assessment criteria. Also, this frequency failure can help to assess whether the actual criteria is too relaxed or too tight.

Bar plots of standardized repeatability bias as in figure 31 is a good way to see all Z-scores of each participant plotted together, which can reveal common features for an analyst like tendencies to over or underestimate cell counts which could point out to methodological or counting issues. It is up to individual laboratories to investigate the causes which may cause anomalies.

The plot of repeatability standard deviations shown in figures 32-35 uses a modified approach to the circle technique of van Nuland. This plot uses the average and standard deviation of each laboratory/analyst and plots one against the other. Because of this modified approach, the critical region drawn doesn't have the shape of a circle anymore. This critical region corresponds to a significance level of 5% for the inner layer, 1% and 0.1% for the most outer layer. This plot determines which laboratories/analysts are having unusual averages and standard deviations.

There are two counts (*C.diadema*, *H.akashiwo*) where many averages appear to be outside the significance region and there could be several causes for this. It definitely points out to a difficulty counting these two species. In the case of *H.akashiwo*, belonging to the raphydophites, the cause could be found in the lugol's iodine preservation of the species as this group of organisms tends to lose the shape or even lyse upon preservation, so it is possible to suggest that some cells had not preserved well in the samples. *C.diadema* on the other hand is a chain forming organism and the culture used for the test was mostly made up of small chains of two-three cells per chain. A possibility here is that sometimes it is hard to ascertain whether a cell half-broken or that looks half-empty of contents should be counted or not. Definitely, a consideration on using more chain former species in future exercises to ascertain whether there is a tendency to count differently this type of species or whether there is an extra difficulty here.

These combined scores over several rounds of a proficiency test can also be combined in future exercises using other performance statistics and graphical representations like Shewhart or Cusum control charts that allow for trends and other features of the results over time.

Qualitative data

ISO13528 doesn't deal with qualitative results, but the correct identification of the organisms in the samples is still a very important part of the exercise, as correct or incorrect flags will be given as a result in each statement performance certificate. The composition of species changes from year to year and in 2013 we have spiked four species. The data received from the analysts shows that analysts are highly skilled in the identification of marine phytoplankton and the results show near perfect scores for all identifications. The most difficult species was *G.instriatum* perhaps because as a naked dinoflagellate the shape of this organism suffers as a consequence of preservation and the details needed for a good identification are not so obvious. Also, *C.diadema* was difficult to identify to species level, the culture of this chain forming diatom was probably not at its best and the chains found in the samples were quite short (2-3 cells) making it difficult for analysts to go to species level. No problems were found identifying the other two species with close to perfect scores.

Only four analysts in total failed to identify one of the species in the samples which indicates an overall high standard of correct identification. The flags for correct identifications are based on a correct genus answer rather than on species taxon, as this sometimes is nearly an impossible task using light microscopy alone. However, for the purpose of the intercomparison we ask analysts to go to species level which gives us a better insight on the analyst and laboratory approach to identification and while this is not used for final marks, the information is still valuable for discussion among the participants. It also gives the co-ordinator of the scheme invaluable data towards species selection in future exercises.

Online HAB quiz

The online HAB quiz has proven very successful and original problems with the software have been ironed out as much as possible. There are still a small number of issues, specifically with 'short answer' type questions where analysts must write the answer to the question exactly as it has been written into the programme by the coordinators. If the answer doesn't match exactly letter by letter, down to the punctuation, the question will be marked as incorrect. Also, it is quite difficult for the quiz manager to re-correct scores within the programme itself and modifications are easier after downloading the data in a

different format like Excel for example. We must not forget that we are working with freeware and we have no control over the development of the software. Even, with these considerations in mind, the HAB quiz is otherwise a good addition to the exercise and this online facility helps greatly the administration and reporting of results.

The standard of the analysts in the HAB quiz over the years has been quite high which demonstrates that analysts have a very good theoretical grounding on marine phytoplankton taxonomy. This year, the results show that this trend continues with 60% of analysts in 100% grades and another 29% of analysts with over 90% grades, which corresponds to over 89% of the participants achieving a proficiency mark.

The quiz this year focused on a group of algae that normally doesn't get enough attention because of their size and therefore inconspicuity; the nanoflagellates, five questions of a total of 14 focused on marine nanoflagellates, one question on raphidophytes, seven questions focused on armoured dinoflagellates taxonomy, life cycle or ecology and one question on general diatoms/dinoflagellates identification.

6. **Recommendations from workshop 2013**

- Form 2 needs to be updated to take into consideration that not all samples may be analysed in the same day, there is no provision for adding this information in the present format.

- Results and provisional Z-scores should be handed out before the workshop, so analysts have time to study them and be able to ask relevant questions about them at the workshop

- Accreditation to ISO 17043: Conformity assessment- General requirements for proficiency testing. One main goal of this intercomparison exercise is to become an accredited proficiency testing scheme. This carries over from 2012. There is quite a bit of work to be done in order to accredit the scheme but the first steps in relation to the use of standard methods for statistics, the formation of an expert group and the fulfilling of the technical requirements is in place.

- The workshop is to continue in its present format of 2-3 days but we may consider having a bi-annual workshop instead. Workshop attendance was good with representation from 17 laboratories and a total of 26 participants.

- Participants did not bring any samples from their areas to the workshop but it should be encouraged that any samples that may be of interest or that you may have difficulty with to bring along to analyse and discuss at the workshop.

- Also, to encourage other participants to present their work at the next workshop. This could be a small 10-20 mins presentation on a particular and relevant topic of interest.

- As statistical analysis becomes standardized through the use of ISO13528. There may not be any need to describe these statistics each year in the report and instead a summary report will be presented of the final results and any warning and action limits that may have arisen for particular analysts flagged.

- Discussion on improvements on how to prepare the samples including the possibility of using an automated system and compare results between manual and automatic homogenization.

ANNEX 1: Form 1 return slip and checklist

Bequalm Intercomparison PHY-ICN-13-MI1

FORM 1: RETURN SLIP AND CHECKLIST

Please ensure to complete the table below upon receipt of samples, and fax or scan and e-mail immediately to the Marine Institute. + 353 91 387237 or rafael.salas@marine.ie			
Analyst Name:			
Laboratory Name:			
Analyst Code Assigned :			
Contact Tel. No. / e-mail			
CHECKLIST OF ITEMS RECEIVED (Please circle the relevant answer)			
Sample numbers **Homogeneity test** (black ink)_____		YES	NO
Sample numbers id. test (red ink)_____		YES	NO
Set of Instructions		YES	NO
Enumeration and identification result log sheet **(Form 2 +3)**		YES	NO

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

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

SIGNED: _____

DATE: _____

ANNEX 2: Form 2 Enumeration and identification results log sheet

Bequalm Intercomparison PHY-ICN-13-MI1

FORM 2: ENUMERATION AND IDENTIFICATION RESULTS LOGSHEET

Analyst Name:	
Laboratory Code:	
Analyst Code :	

Settlement date:	**Analysis date:**	**Volume Chamber (ml)=**		**Sample No:**				
Organism		**Cell count**	**Cell count**	**Cell count**	**Multiplication factor**	**Number cells/L**	**Number cells/L**	**Number cells/L**	**Average**
									#DIV/0!
									#DIV/0!
									#DIV/0!
									#DIV/0!
									#DIV/0!
Comments:									

ANNEX III: Form 3: Homogeneity test results log sheet

Bequalm 2013 Phytoplankton Intercomparison Exercise

Analyst Name:	
Laboratory Code:	
Analyst Code :	

Settlement date:	**Sample No:**				
Analysis date:	**Cell count**				
Volume Chamber (ml)=	**Multiplication factor**				**Average**
	Number cells/L				#DIV/0!

Form 3: Homogeneity test

Comments:

ANNEX IV: Test instructions

Marine Institute-IOC- BEQUALM-NMBAQC Phytoplankton Proficiency Test PHY-ICN-13-MI1 Vr2.0

Instructions

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

1. **Introduction**

1. **Preliminary checks, deadlines and use of forms**

2. **Test method**

3. **Equipment**

4. **Sedimentation chambers and sample preparation**

5. **Counting strategy**

6. **Samples**

7. **Conversion calculations of cell counts**

8. **Online HABs quiz**

9. **Points to remember**

2. Introduction

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

Reports from previous exercises can be obtained in the NMBAQC website (www.nmbaqcs.org) and information on all the Bequalm intercomparison schemes can be found in their website (www.bequalm.org). Registration to the exercise is through the Marine institute. You need to contact our administrator Maeve Gilmartin at maeve.gilmartin@marine.ie .

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

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

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

This Phytoplankton Ring Test this year is set up to test the homogeneity and stability of the materials sent to the participants to investigate methodology issues. Also, to determine the variability within and between laboratories in the abundance and composition of marine phytoplankton species from a number of samples spiked with cultured material.

Participants are asked to carry out counts on a set of three samples and also to identify and count all the organisms present in a second set of samples. Each analyst will receive an

envelope containing seven samples: a set of three samples and a set of four samples in 30ml sterilin tubes preserved in lugol's iodine

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

3. Preliminary checks, deadlines and use of forms

Upon receipt of the samples, every analyst must make sure that they have received everything listed in the Return Slip and checklist form (Form 1). Make sure that all the samples are intact and sealed properly and check that you have received the enumeration and identification results log sheet (Form 2) and homogeneity test (form 3) in the same Excel workbook. Please complete form 1: Return slip and checklist form and send it by fax (+353 91 387237) or scan it and send it via e-mail to Rafael.salas@marine.ie A receipt of fax/e-mail is necessary for the Marine Institute to validate the test process for each analyst.

Once samples have been receipt, analysts have four weeks to complete the exercise and return the results to Rafael Salas, Marine Institute, Phytoplankton laboratory, Rinville, Oranmore, Co. Galway, Ireland by e-mail (Rafael.salas@marine.ie). The enumeration and identification results log sheet (Form 2 + 3) **must be received** in the Marine Institute by **July 15th, 2013**.

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.

This year we are using an Excel workbook named 'Enumeration and identification logsheet' for you to input your results. The worksheets Form 2 and Form 3 are included in this workbook. In both forms, first fill in your name, analyst and laboratory code at the top of both forms. In form 2: Logsheet you must enter the identification and enumeration results of sample set 2 (Read section 8. Samples) there is a table that you need to fill in with the name of the species identified and the cell count for each species and sample. In the green cells you write the sample number and under the cell named 'organism' a drop down menu will appear with a list of possible species names. You must choose from this list your answer. The list of species is a reduced list and is designed to have more entries than

species are in the samples, you must choose which ones you think have been spiked in the samples and provide a cell count.

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

Form 3: Homogeneity test is in the next tab, Here you must include the results of your sample Set 1 (read section 8. Samples). There is a simple table here. You must enter your sample number in the green cells, the settlement, analysis date and volume used.

Under cell count, write the number of cells found in the sample, the multiplication factor used and the final cell density in cells per litre. There is a formula already embedded under 'Average' to calculate the mean of your three measurements.

In the comments box in both forms you can write information about the test method you used if deviates from the Utermöhl test method and how you did your calculations if necessary.

4. Test method

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

We advise the use of 25ml sedimentation chambers for the purpose of this intercomparison exercise if these are available. If not, other sub-sample volumes and/or chambers may be used. If a different method is used, please state all this information in your results.

5. Equipment

The following are the equipment requirements to complete this exercise:

Sedimentation chambers (25ml volume if possible).

Inverted Microscope: This should be equipped with long distance working lenses up to 40 x objective or higher and condenser of Numerical Aperture (NA) of 0.3 or similar and capable for bright field microscopy.

Tally counters

6. Sedimentation chambers and sample preparation

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

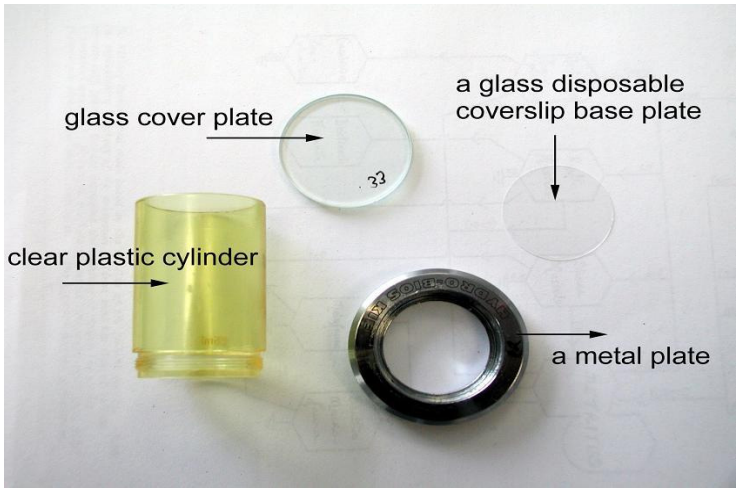


Fig 1: Sedimentation counting chamber

5.1 All sedimentation chambers should be cleaned before start

5.2 Place a new not used disposable cover slip base plate inside a cleaned metal plate.

5.3 Screw the plastic cylinder into the metal plate. Extra care should be taken when setting up chambers. Disposable cover slip base plates are fragile and break easily causing cuts and grazes.

5.4 **Important:** Once the chamber is set up, it should be tested for the possibility of leaks by filling the completed chamber with sterile seawater and allowing it to rest for a few minutes. If no leakage occurs, pour out the water, dry out completely and proceed with the next step.

5.5 To set up a sample for analysis or sub-sample. Firmly invert the sample 100 times to ensure that the contents are homogenised properly.

5.5.1 Pour the sample into the counting chamber. (samples must be adapted to room temperature before hand to reduce the risk of air bubbles in the chambers)

5.5.2 There should be enough sample volume in each sample to fill a 25ml sedimentation chamber. Top up the sedimentation chamber and cover with a glass cover plate to complete the vacuum and avoid air pockets.

5.5.3 Label the sedimentation chamber with the sample number from the sterilin tube.

5.6 Use a horizontal surface to place chambers protected from vibration and strong sunlight.

5.6 Allow the sample to settle for a minimum of twelve hours.

5.7 Set the chamber on the inverted microscope and analyse.

5.8 Enumeration and identification results for each sample are to be entered in Excel workbook **Form 2 enumeration and identification results log sheet and Form 3 Homogeneity and stability test.**

5.9 If using a different method to the Utermöhl test method, please send the Standard Operating Procedure for your method with your results. Explain briefly

how it works and how samples are homogenized, set up, analysed, counted and how you calculate the final concentration.

7. Counting strategy

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

8. Samples

Analysts will have to analyse 6 samples to complete the test. This comprises two set of samples. The sample sets have been prepared in separate envelopes.

Set 1 is composed of three samples. The numbers are written in black ink. These are made up in sterile filtered Seawater. One organism has been added to the samples to the required cell density. Participants are asked to carry out a whole chamber count on each of the three samples. This data will be use to test the homogeneity and stability of the samples. **No identification of the organism is needed.**

Set 2 consist of four samples but only three need to be analysed, one is just a spare sample. These have been made up with sterile filtered seawater as in the previous set but this time a number of cultured species have been added. The cultures come from the Marine Institute Phytoplankton culture collection, and the IOC Science and communication centre for Harmful Algae culture collection in Denmark. All the materials have been preserved using lugol's iodine and then homogenized following the IOC Manual on Harmful Marine Algae technique of 100 times sample inversion to extract sub-samples.

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

It is very important to spend some time becoming familiar with the samples and how the cells appear on the base plate before any count is done as part of the test. The reason for

this is that cultured cells could be undergoing division or fusion and look different to the known standard vegetative cell type. See figure 1.

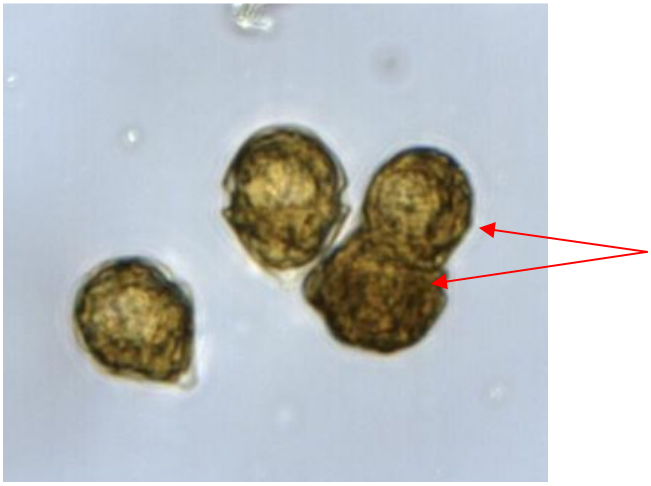


Figure 1: Two Cells fusing

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

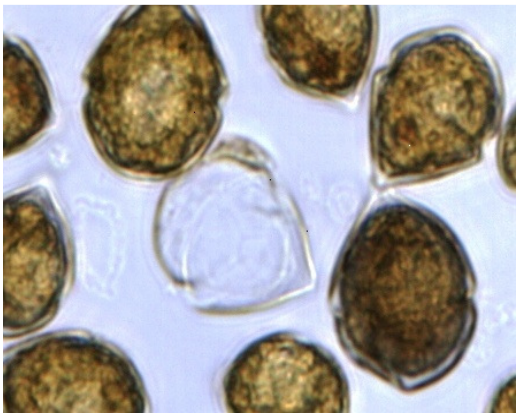


Figure 2: Empty theca

Cells may also vary in size, some cells will appear smaller than others, this is normal in culture conditions (see figure 3). Sometimes Plasmolysis may occur and the cells appear naked and rounded (see figure 4). Aberration of cell morphology can occur also in culture conditions and upon preservation of samples with lugol's iodine.

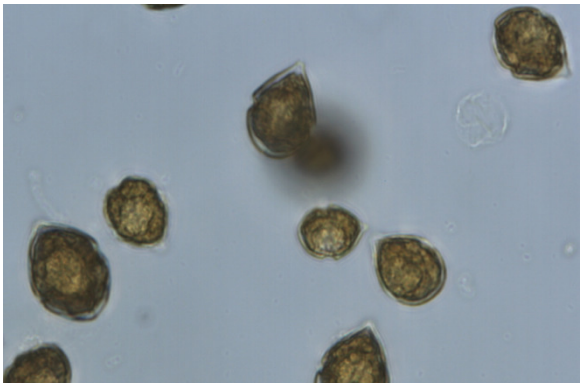


Figure 3: Big versus small cells

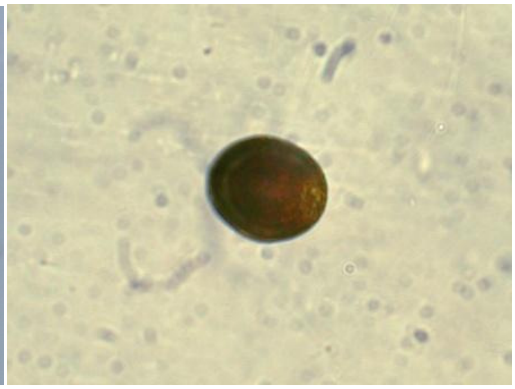


Figure 4: Plasmolised cell

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

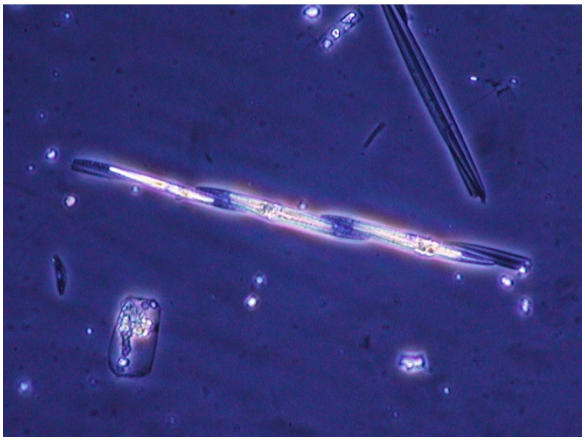


Figure 5

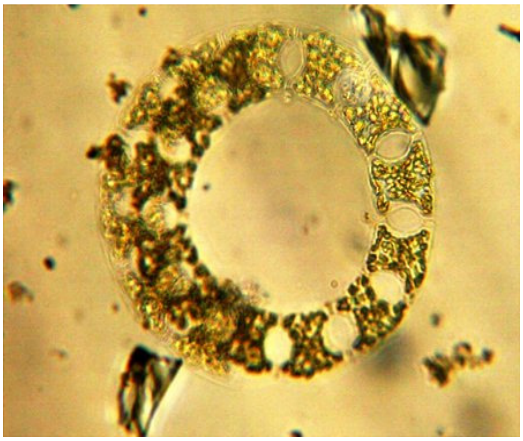


Figure 6

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

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

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

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

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

d) Plasmolised cells should not be counted

e) Aberrant forms should be counted

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

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

h) Participants should name phytoplankton species according to the current literature and scientific name for that species. Where species have been named using a synonym to the current name and if this synonym is still valid or recognized the answer will be accepted as correct.

These rules are only applicable to this intercomparison exercise.

9. Conversion calculations of cell counts

The number of cells found should be converted to cells per litre.
Please show the calculation step in Form 2 + 3: enumeration and identification results log sheet.

10. Online HABs quiz

A HAB taxonomic quiz will be developed in the web platform 'Ocean teacher' and it should be ready by July 2013. All participants will need access to the internet to complete this part of the exercise. More information on when participants will be able to access this exercise will be sent to you by e-mail later on.

In order to access the exercise you need to go to the webpage http://classroom.oceanteacher.org/ and login. Analysts which took part in the exercise in 2011 or 2012 will already have a username and password which is still active, those using this facility for the first time need to register first.

When you go to the page http://classroom.oceanteacher.org/ in the top right hand corner of this page, you'll see a link to login. Press login and in the next page if you already have registered in 2011 or 2012, enter your username and password to access the course, if you forgot your password press the forgotten password link. If this is your first time using this system, then go to create new account and register your details. Once you register your

details we will be able to activate your account. This year as in 2012 participants will be able to self-enrol for this exercise, so once you are registered and logged in you must supply an enrolment key to access the exercise. This key is **Beq2013**. We will tell you the exact date the exercise is opened.

So, how do you do access the course?, Once you are all logged in, in the main page scroll down to the bottom and under interdisciplinary courses, click courses, on the next page and under categories click Harmful Algal Bloom (HAB). The Harmful algal bloom programme Bequalm 2013 link will appear, click on it, enter your key (**Beq2013**) and start your quiz. Make sure you enter the right course.

Analysts will have 4 weeks to complete the exercise once it opens (dates to be decided). Only one attempt to the exercise is allowed and once the exercise is submitted analysts won't have access to it, only to review. So, make sure you review all your answers before submitting.

There are a number questions and a maximum grade of 100% for a perfect score. All questions have the same score.

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

11. Points to remember

1. **All results must be the analysts own work. Conferring with other analysts is not allowed.**
2. The Excel worksheets Form 2: Enumeration and identification results log sheet and Form 3: Homogeneity test results, must be received by the Marine Institute, Phytoplankton unit by **July 15th, 2013**.

ANNEX V: Workshop agenda

Agenda Bequalm Phytoplankton Intercomparison workshop

Marine Institute, Rinville, Oranmore, County Galway, Ireland 7-9 Oct 2013.

Monday 7 –Wednesday 9 Oct 2013

	Morning 9.30am-13.00pm	**Afternoon 14.00pm-17.30pm**
Monday, 7 Oct	Intercomparison exercise results Enumeration and identification exercise results. Ocean teacher online HABs quiz exercise results. (R.Salas) ISO13528 statistical methods (R.Salas) Discussion of exercise and ideas for 2014 (All)	Community analyses of North Sea phytoplankton (R.Van Wezel) Calculating Phytoplankton Biovolume, Biomass and Carbon – How and Why! (Lars Edler) Field samples from participants (microscopy and identification) All
Tuesday, 4 Dec	Lecture and microscope demonstration of the Raphidophytes group (J.Larsen)	Lecture and microscope demonstration of the nanoflagellates group (J.Larsen)
Wednesday 9 Oct	Lecture and microscope demonstration of naked dinoflagellates with emphasis on Gyrodinium and Gymnodinium genera (J.Larsen)	Departure

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

Lunch 13:00-14:00 pm

ANNEX VI: Participating Laboratories

Number of Laboratories	Company Name	Address
1	Isle of Man Government Laboratory	Dept of Environment, Food and Agriculture Ballakermeen Road, Douglas, Isle of Man, IM1 4BR
2	Scottish Marine Institute	SAMS Research Services Ltd, Oban, Argyll, PA37 1QA, Scotland
3	IMARES	Korringaweg 5 4401 NT Yerseke The Netherlands
4	Nostoca Algae Laboratory	7770 Springridge Road N.E., Bainbridge Island Washington 98110 USA
5	Cefas Laboratory	Pakefield Road Lowestoft Suffolk NR33 0HT
6	Institute of Oceanography and Fisheries	Laboratory of Plankton and Shellfish toxicity,Šetalište I. Meštrovića 63, 21000 Split, Croatia
7	Marine Scotland Marine Laboratory	Inshore Ecosystems, 375 Victoria Road, Aberdeen, AB11 9DB, UK.
8	Laboratorio de Control de calidad de los recursos pesqueros	Agencia de gestion agraria y pesquera de Andalucia
9	Laboratorio de Medio Ambiente de Galicia (LMAG)	Iglesia 19 36153 Lourizán (Pontevedra) Spain
10	DHI Laboratory	200, Pandan Loop #08-02 Pantech21 Singnapore 128388
11	Jacobs UK Limited	Kenneth Dibben House, Enterprise Road, Southhampton Science Park SO16 7NS UK
12	Stazione Zoologica Anton Dohrn Villa Comunale	80121 - Napoli Italy
13	IVL Swedish Environmental Institute	Rosviksgatan 12 SE-45330 Lysekil Sweden
14	Australian Shellfish Quality Assurance Program (SASQAP)	Port Lincoln, South Australia, Australia
15	Biopol Sjávarlíftæknisetur / Marine Biotechnology	Einbúastíg 2 545 Skagaströnd Iceland
16	LIENSs, CNRS, University of La Rochelle	Bâtiment ILE, 2 rue Olympe de Gouges, 17000 La Rochelle, FRANCE
17	Eidikos Logariasmos Kondilion Erevnas	Ktirio KE.D.E.A- 3 Septemvriou - Panepistimioupoli P.C : GR 54636 Thessaloniki Greece
18	Phytoplankton Monitoring Program National Direction of Aquatic Resources	Constituyente 1497 11200 Montevideo, Uruguay
19	IFREMER Station de biologie marine	Place de la Croix BP 40537 29185 Concarneau Cedex France
20	Fisheries and Aquatic Ecosystems Branch	Agri-Food & Biosciences Institute Newforge Lane Belfast BT9 5PX
21	Koeman en Bijkerk bv	Oosterweg 127, 9751PE HAREN 9750AC HAREN The Netherlands
22	Plymouth Marine Laboratory	Prospect Place The Hoe Plymouth PL1 3DH UK
23	Corben Ltd	Loch Melfort , Arduaine, Argyll Scotland PA34 4XQ
24	Laboratoire des sciences de l'environment Marin (LEMAR)	Institut Universitaire Européen de la Mer Technopôle Brest-Iroise rue Dumont d'Urville 29280 Plouzané - France
25	Université Bordeaux	1 UMR CNRS EPOC 5805 Station Marine d'Arcachon 2 rue du Prof. Jolyet F 33120 Arcachon France
26	IRTA E-43540 Sant Carles de la Ràpita (Tarragona) Spain	Ctra. de Poble Nou, Km 5,5
27	CNRS-UPMC - Service Mer et Observation	FR2424 Place Georges Teissier CS90074 29688 ROSCOFF FRANCE
28	CLS	c/o Marine Institute, Rinville Galway
29	Microalgal Services	308 Tucker Road Ormond VIC 3204 Australia
30	Marine Institute Galway	Rinville, Oranmore, County Galway, Ireland
31	Marine Institute Bantry	Gearhies pier, Bantry, County Cork, Ireland
32	SEPA	Clearwater House, Heriot-Watt Research Park, Edinburgh, EH14 4AP
33	Laboratoire d'Océanologie et de Géosciences	UMR CNRS 8187 LOG 32 av. Foch 62930 Wimereux France
34	APEM Limited	Riverview, Embankment Business Park, Heaton Mersey, Stockport, SK4 3GN

ANNEX VII: Statement of Performance

Biological Effects Quality Assurance in Monitoring Programmes /
National Marine Biological Analytical Quality Control Scheme /
Marine Institute
STATEMENT OF PERFORMANCE
Phytoplankton Component of Community Analysis
Year 2013

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

Statement Issued:
Statement Number: MI-BQM-13-

Summary of results:

Component Name	Subcontracted	Results		identification
		Z-score (+/- 2 Sigma limits)		
Phytoplankton abundance and composition PHY-ICN-13-MI1	Marine Institute	*Coscinodiscus granii*		
		Chaetoceros diadema		
		Heterosigma akashiwo		
		Gyrodinium instriatum		
Overall Result Taxonomic quiz (Pass Mark 70%, over 90% proficient)				
Phytoplankton Taxonomy quiz PHY-ICN-13-MI1	IOC Science and communication Centre on Harmful algae			

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

Notes:

Details certified by:

Joe Silke
Section manager

Rafael Gallardo Salas
Scientific Technical Officer

Description of Scheme components and associated performance standards

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

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

ANNEX VIII: Ocean Teacher HAB Quiz

Question 1

Correct

Mark 1.0 out of 1.0

Nanoflagellates (<20 µm) are common in the marine environment and most species can be identified only in live material; in some cases electron microscopy is required for species id. However, many nanoflagellates can be assigned to order or class by Light Microscopy. Below are shown schematic line-drawings of the most important groups of marine flagellates - assign them to groups. **Please note : Figures are shuffled randomly by the programme and do not follow a particular order (Figs A to J). If you can't see all the drawings in this plate, change browser zoom level from 100% to 75-80%**

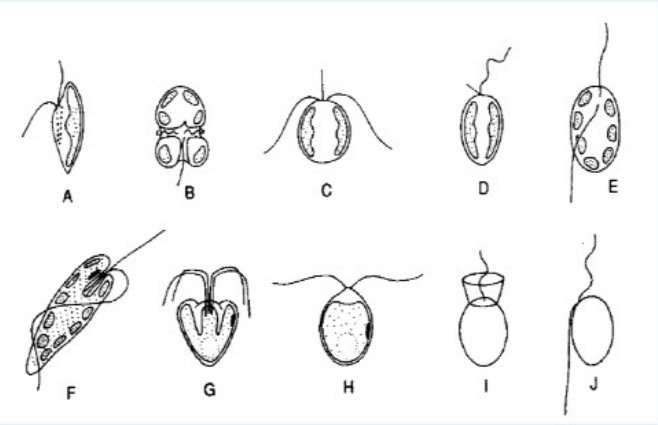


Fig. B Dinoflagellates ✓

Fig. D Chrysophytes ✓

Fig. E Raphidophytes ✓

Fig. A Cryptophytes ✓

Fig. G Prasinophytes ✓

Fig. I Choanoflagellates ✓

Fig. C Haptophytes ✓

Fig. F Euglenophytes ✓

Fig. J Kinetoplastids ✓

Fig. H Chlorophytes ✓

The correct answer is: Fig. B – Dinoflagellates, Fig. D – Chrysophytes, Fig. E – Raphidophytes, Fig. A – Cryptophytes, Fig. G – Prasinophytes, Fig. I – Choanoflagellates, Fig. C – Haptophytes, Fig. F – Euglenophytes, Fig. J – Kinetoplastids, Fig. H – Chlorophytes

Question 2

Correct

Mark 1.0 out of 1.0

Nanoflagellates (<20 µm) are common in the marine environment. In the question 'Marine flagellates 1', you have identified the most important groups of flagellates. Which of the following groups comprise toxic members in the marine environment?

Chrysophytes, cryptophytes, chlorophytes, euglenophytes, haptophytes, prasinophytes, raphidophytes, unarmoured dinoflagellates

Select one or more:

- ☑ a. Haptophytes ✓
- ☐ b. Cryptophytes
- ☐ c. Chlorophytes
- ☐ d. Euglenophytes
- ☐ e. Chrysophytes
- ☑ f. Unarmoured dinoflagellates ✓
- ☑ g. Raphidophytes ✓
- ☐ h. Prasinophytes

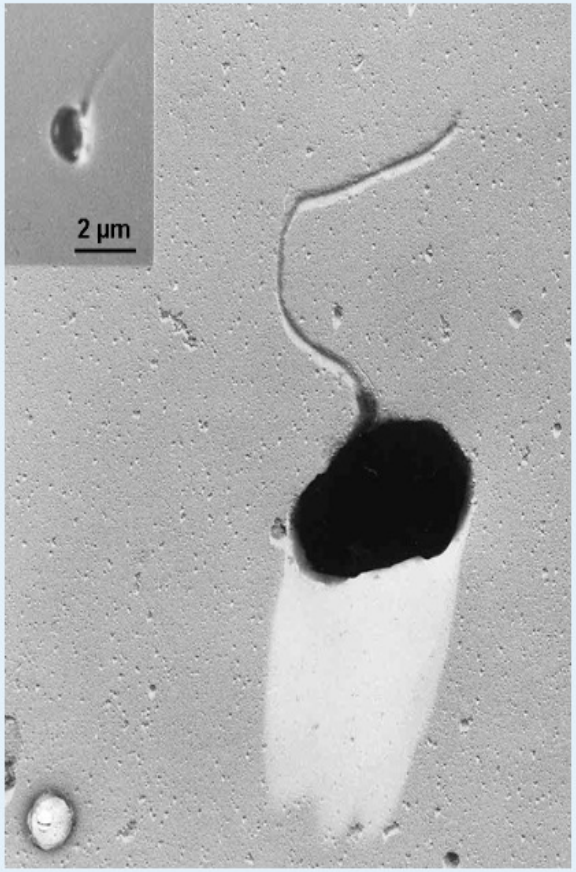
The correct answer is: Haptophytes, Raphidophytes, Unarmoured dinoflagellates

Question 3

Correct

Mark 1.0 out of 1.0

The illustrated flagellate belongs to the Prasinophytes and is one of the smallest eukaryotic organisms known. It is a common and presumably important primary producer in marine environments, but overlooked and rarely identified because of its small size. What is the name of this flagellate?. Identify to genus level. Capitalize the first letter of the generic name



Answer: Micromonas

The correct answer is: Micromonas

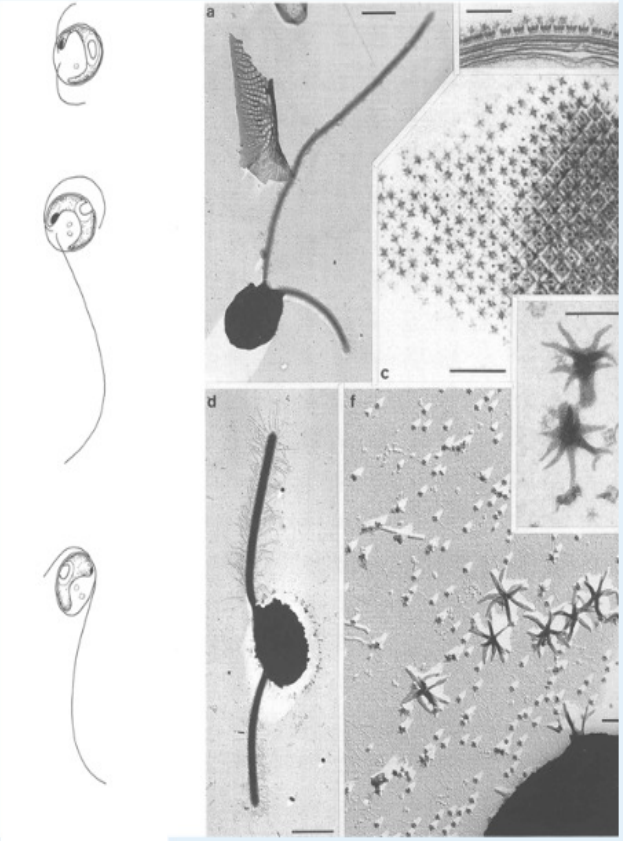
Question 4

Correct

Mark 1.0 out of 1.0

The illustrations show different species of a green flagellate belonging to the class Prasinophyceae. The illustrated species also belong to the same genus. What is the name of this genus? Capitalize first letter of genus name.

Figs A & D scale bar= 5µm.



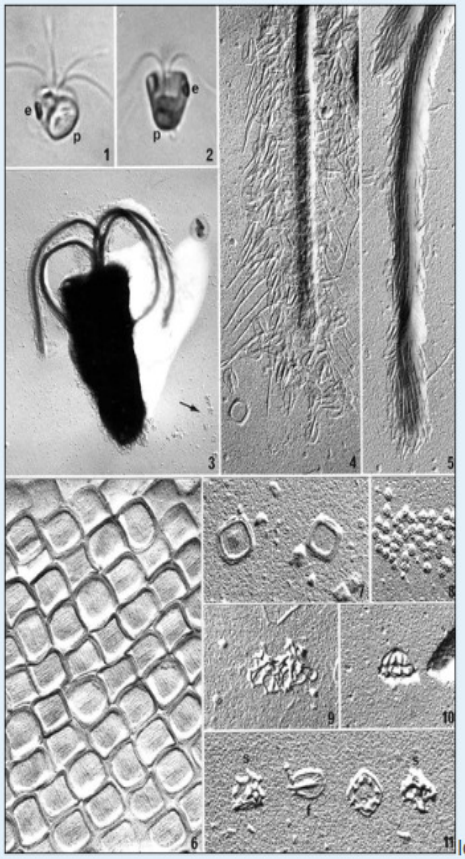
Answer: Nephroselmis

The correct answer is: Nephroselmis

Question **5**

Correct

Mark 1.0 out of 1.0



Identify the illustrated organism to species level if possible, if not, identification to genus level is sufficient and will be given as correct. Please note: the first letter of the generic name should be capitalized.

Answer: Pyramimonas ✓

Pyramimonas or Pyramimonas orientalis are both correct answers

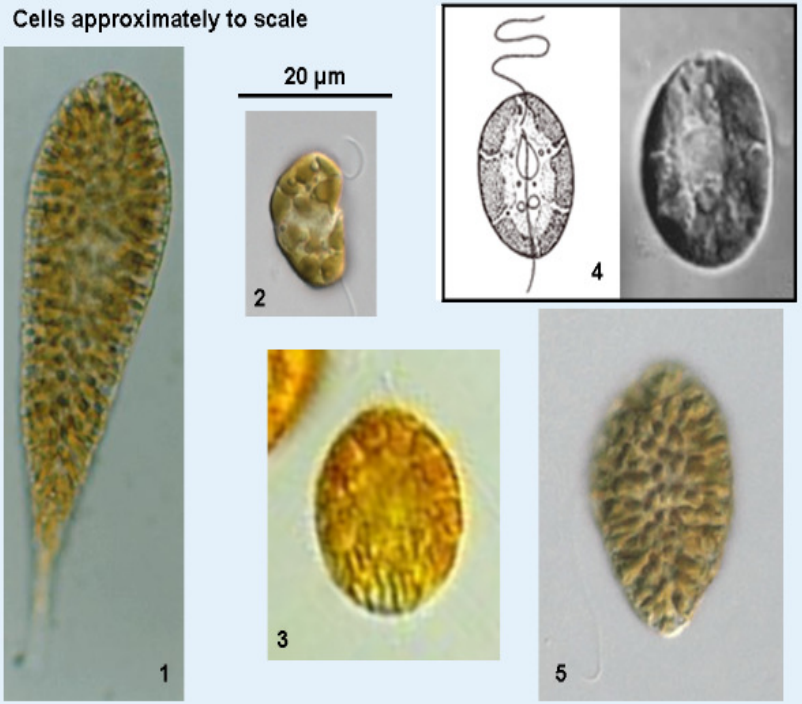
The correct answer is: Pyramimonas

Question **6**

Correct

Mark 1.0 out of 1.0

The illustrations show 5 different species of Raphidophytes. Identify the species. **Please note : Figure numbers are shuffled randomly by the programme and do not follow a particular order. If you can't see all images in this plate, change browser zoom level from 100% to 75-80% or less.**



Species 3 Fibrocapsa japonica ✓

Species 4 Olisthodiscus luteus ✓

Species 5 Chattonella subsalsa ✓

Species 1 Chattonella antiqua ✓

Species 2 Heterosigma akashiwo ✓

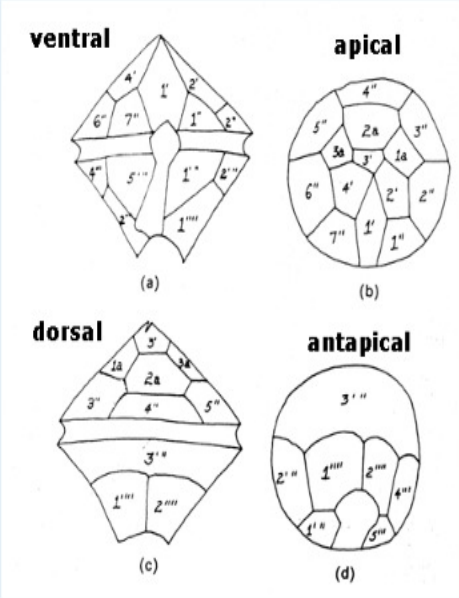
The correct answer is: Species 3 – Fibrocapsa japonica, Species 4 – Olisthodiscus luteus, Species 5 – Chattonella subsalsa, Species 1 – Chattonella antiqua, Species 2 – Heterosigma akashiwo

Question 7

Correct

Mark 1.0 out of 1.0

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



The plates marked 1""-2"" indicate: The antapical plates

The plates marked 1"'-5"' indicate: The postcingular plates

The plates marked 1a-3a indicate: The anterior intercalary plates

The plates marked 1'-4' indicate: The apical plates

The plates marked 1"-7" indicate: The precingular plates

smile

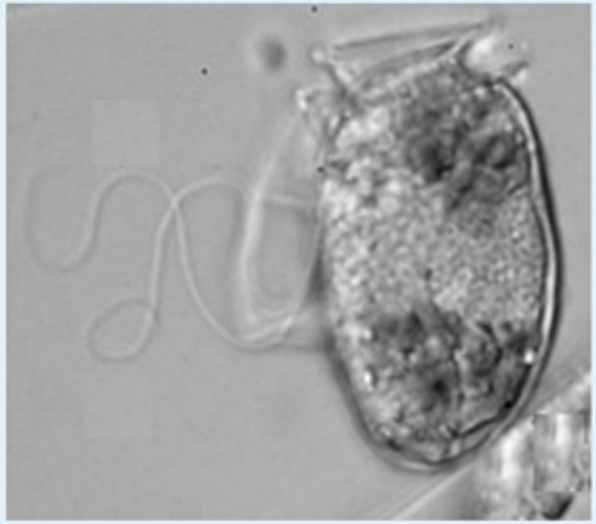
The correct answer is: The plates marked 1""-2"" indicate – The antapical plates, The plates marked 1"'-5"' indicate – The postcingular plates, The plates marked 1a-3a indicate – The anterior intercalary plates, The plates marked 1'-4' indicate – The apical plates, The plates marked 1"-7" indicate – The precingular plates

Question 8

Correct

Mark 1.0 out of 1.0

The image shows a.....



Select one:

- a. Planozygote ✓
- b. Vegetative cell
- c. Haploid gamete
- d. Cyst
- e. Hypnozygote

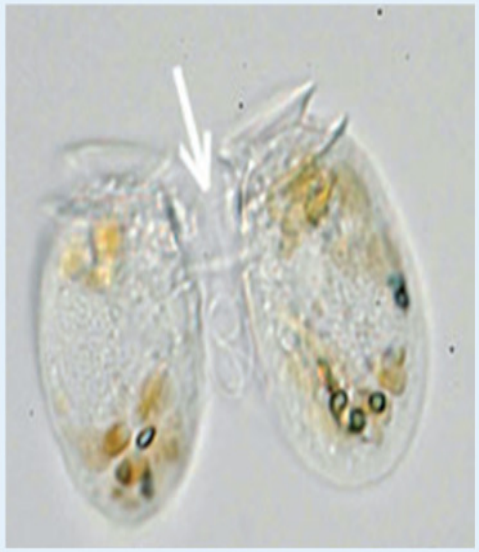
The correct answer is: Planozygote

Question 9

Correct

Mark 1.0 out of 1.0

The image shows dinoflagellate gametes fusing during sexual reproduction. When the gamete pair are the same size, this is called.....



Select one:

- a. Isogamy ✓
- b. Anisogamy
- c. Oogamy
- d. Heterothally
- e. Homothally

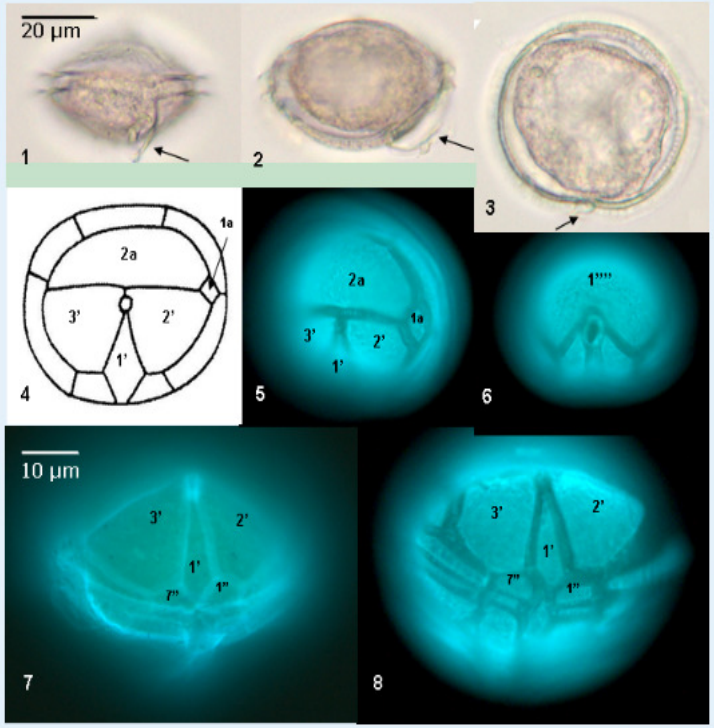
The correct answer is: Isogamy

Question 10

Correct

Mark 1.0 out of 1.0

The illustrations show a species of dinoflagellate belonging to the Diplopsalis-group. Identify the genus. Please note: Case sensitive. Capitalize first letter of genus name



Answer: Preperidinium

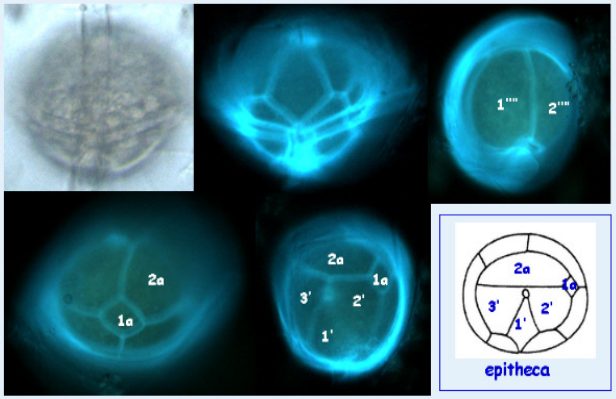
The correct answer is: Preperidinium

Question 11

Correct

Mark 1.0 out of 1.0

The illustrations show a species of dinoflagellate belonging to the Diplopsalis-group. Identify the genus. Please note: case sensitive answer. Capitalize first letter of genus name.



Answer: Diplopelta

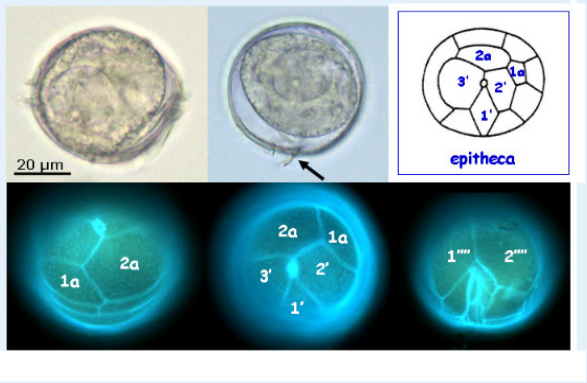
The correct answer is: Diplopelta

Question 12

Correct

Mark 1.0 out of 1.0

The illustrations show a species of dinoflagellate belonging to the Diplopsalis-group. Identify the genus. Please note: case sensitive answer. Capitalize first letter of genus name.



Answer: Diplopsalopsis

The correct answer is: Diplopsalopsis

Question 13
Correct
Mark 1.0 out of 1.0

Identify the following marine phytoplankton species to genus level. **Please note : Figures are shuffled randomly by the programme and do not follow a particular order (A to F). Please note: There are more species in the drop down list than images, some don't apply.**

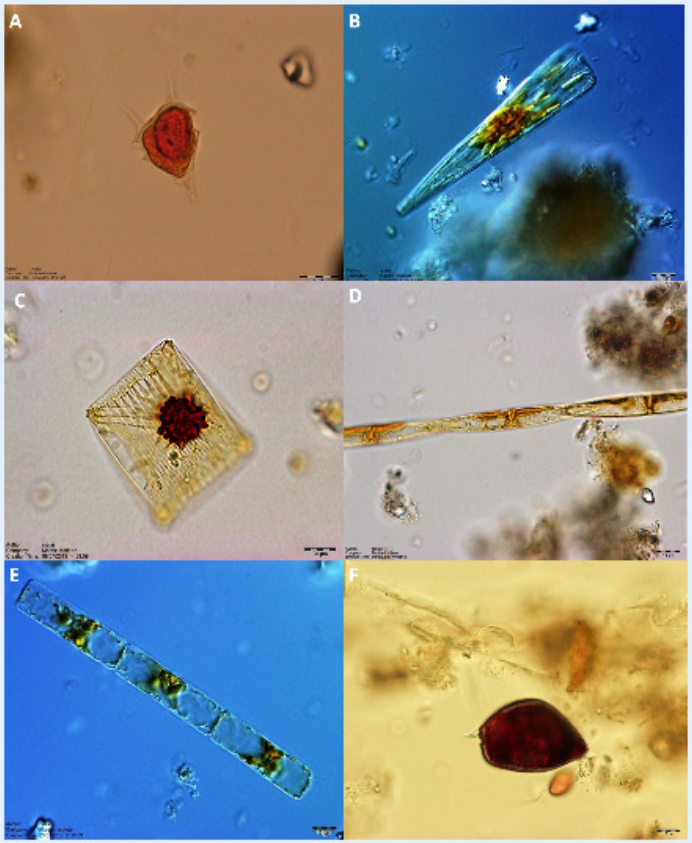


Image C: Striatella ✓
Image A: Protoperidinium ✓
Image D: Pseudo-nitzschia ✓
Image E: Guinardia ✓
Image F: Prorocentrum ✓
Image B: Licmophora ✓

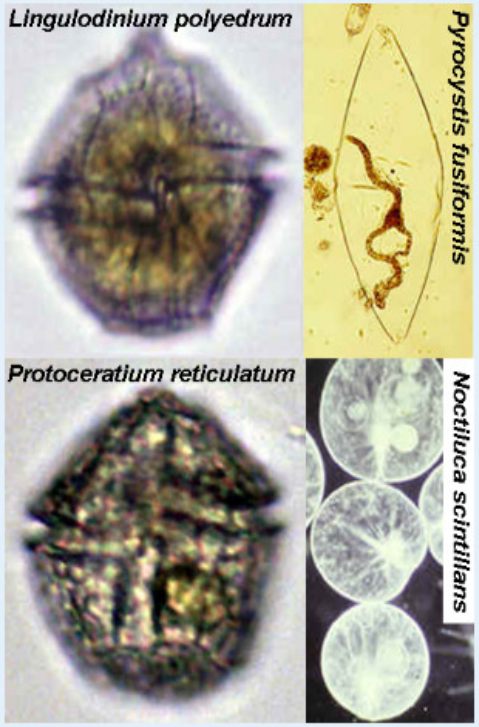
The correct answer is: Image C: – Striatella, Image A: – Protoperidinium, Image D: – Pseudo-nitzschia, Image E: – Guinardia, Image F: – Prorocentrum, Image B: – Licmophora

Question 14

Correct

Mark 1.0 out of 1.0

The dinoflagellates in this picture (*Pyrocystis fusiformis, Lingulodinium polyedrum, Protoceratium reticulatum and Noctiluca scintillans*) belonging to different genera share a particular trait (a biochemical reaction). Do you know the name of this biochemical reaction?



Answer: Bioluminescence

The correct answer is: Bioluminescence

ANNEX IX: Z-scores

ANALYST CODE	SAMPLE CODES			C.granii Z-score	C.diadema Z-score	H.akashiwo Z-score	G.instriatum Z-score
3	53	183	101	0.79	-0.40	0.47	0.38
23	16	37	171	-0.01	0.52	0.02	0.29
16	7	31	236	-0.37	0.88	0.24	0.82
40	19	28	216	0.24	not id	-0.76	-3.79
5	123	151	237	0.12	-1.04	not id	-2.40
44	10	34	69	0.01	-0.67	-0.81	0.11
30	96	131	150	0.47	0.16	-0.07	-0.37
18	115	119	61	0.22	-0.71	-0.78	0.20
2	36	148	44	0.32	0.66	-0.10	1.42
13	52	130	155	-0.48	-0.26	-0.55	-1.02
10	110	124	135	0.06	0.72	0.23	0.50
28	77	124	147	-0.01	-0.26	0.97	0.04
36	64	103	142	-0.55	-0.58	0.77	0.06
11	75	94	156	-0.03	-0.26	0.40	-0.03
31	191	185	218	-0.59	0.49	0.56	-0.21
38	51	87	186	-0.13	2.63	-0.09	-0.03
24	5	43	73	0.12	not id	-1.23	-1.54
25	184	210	195	-0.66	0.65	-0.54	-0.10
34	81	62	175	0.04	0.91	0.25	0.71
20	161	32	58	-1.98	-1.44	-1.55	-5.80
41	98	180	194	-0.27	-1.01	-1.33	-1.92
1	27	192	213	0.07	0.56	0.24	0.27
45	35	188	204	0.00	0.38	0.30	-0.06
29	42	144	221	0.15	0.39	-0.16	0.43
22	49	223	70	0.21	1.36	-0.07	0.68
39	6	21	238	-0.17	0.10	0.25	0.38
37	83	157	176	-0.09	0.26	0.36	-0.15
12	63	45	25	0.52	1.43	0.14	-0.31
43	17	117	120	0.13	0.29	0.20	0.35
9	4	197	205	-0.68	0.90	-0.27	-0.31
7	68	97	207	0.35	-0.10	-0.55	0.37
14	187	76	169	-0.55	-0.13	0.10	-0.88
35	54	165	167	0.15	-1.38	not id	0.04
8	30	72	136	0.32	-0.59	-0.28	-0.11
15	203	172	57	0.08	0.63	2.61	0.19
4	93	126	143	0.27	0.01	0.45	0.28
17	109	134	233	0.27	0.03	1.40	-0.63
6	149	219	179	0.22	-0.32	1.05	0.01
42	38	82	215	0.66	-0.16	1.81	0.24
27	88	209	225	-0.19	-1.21	0.62	-0.58
33	8	41	92	0.33	-0.45	2.02	0.83
19	199	173	231	-0.22	-1.35	-0.51	0.55
48	174	67	164	-0.83	-1.37	-1.29	-2.93
49	59	105	190	-0.51	0.17	-0.68	1.73
50	114	200	230	-0.37	-0.14	-1.50	-0.15
46	78	178	182	-0.27	0.22	-0.46	-0.06
47	12	137	229	0.90	0.14	-0.11	-0.10

ANNEX X: HABs Oceanteacher quiz results

ANALYST CODE	Q. 1	Q. 2	Q. 3	Q. 4	Q. 5	Q. 6	Q. 7	Q. 8	Q. 9	Q. 10	Q. 11	Q. 12	Q. 13	Q. 14	Grade
40	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
13	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
25	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
45	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
49	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
43	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
17	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
34	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
15	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
27	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
46	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
38	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
44	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
42	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
50	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
12	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
33	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
23	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
19	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
48	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
31	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
16	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
35	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
18	100.0	100.0	100.0	100.0	100.0	80.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.6
41	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	67.6	100.0	97.7
11	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	92.9
47	100.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	92.9
20	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	92.9
9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	92.9
24	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	92.9
6	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	92.9
36	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	92.9
29	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	92.9
28	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	92.9
10	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	92.9
39	100.0	100.0	0.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	85.7
2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	0.0	100.0	100.0	100.0	85.7
3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	0.0	100.0	100.0	100.0	85.7
37	100.0	100.0	0.0	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	85.7
5	100.0	100.0	100.0	100.0	0.0	60.6	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	82.9
14	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0	0.0	0.0	0.0	100.0	100.0	71.4
	100.0	100.0	93.3	97.8	97.8	98.7	100.0	73.3	100.0	95.6	91.1	97.8	99.3	100.0	96.0