United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

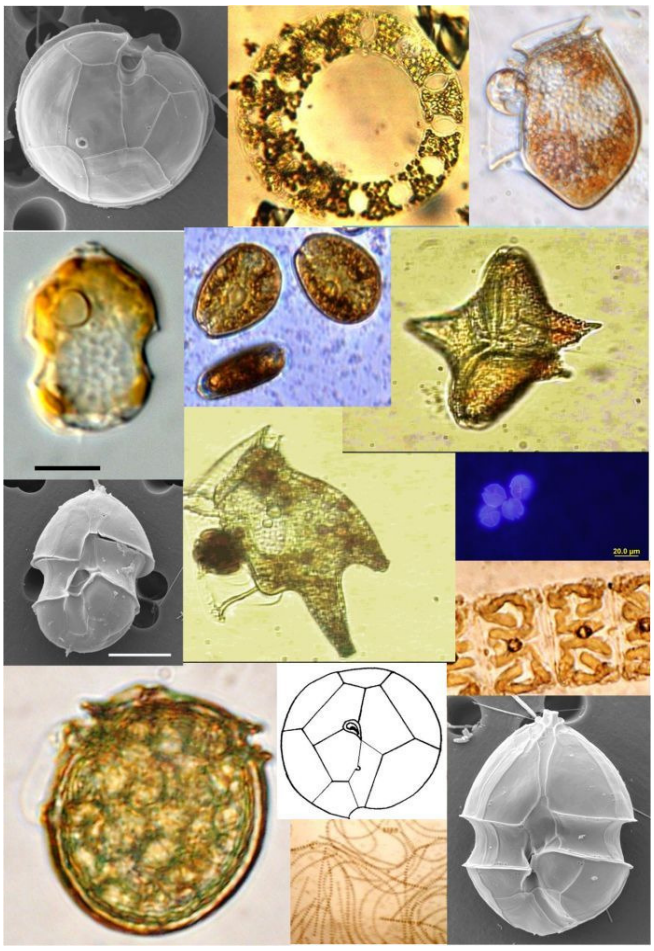
Marine Institute
Foras na Mara

BEQUALM

NMBAQC SCHEME

BEQUALM Phytoplankton proficiency test in the abundance and composition of marine microalgae 2012 report.

PHY-ICN-12-MI1 VR 2.0



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1. Summary of results

- 54 analysts from 29 laboratories from around the world took part in this intercomparison. 51 analysts and 28 laboratories returned results. This year, there are laboratories from Australia and North Africa taking part in this exercise for the first time. There are also two laboratories from South America.

- The bulk (24 laboratories) comes from across Europe: Ireland (4), Northern Ireland (1), Scotland (3), England (7), Netherlands (2), Sweden (2), Spain (4) and Greece (1).

- There were five species of interest in this intercomparison exercise. These were: *Dinophysis acuminata* Ehrenberg*, Phalacroma rotundatum* (Claparéde & Lachmann) Kofoid & Michener*, Lingulodiniun polyedrum* (Stein) Dodge*, Karenia selliformis* A.J.Haywood, K.A.Steidinger & L.MacKenzie *and Coolia monotis* Meunier. The statement of performance certificate, Z-score and identification only takes into account three counts: *D.acuminata, L.polyedrum and K.selliformis.*

- The other two counts are not used in the final statement for the reasons outlined here: *C.monotis* is not considered a toxic producing alga and analysts were asked to count only toxic and harmful species in the samples. *P.rotundatum* counts cannot be used because the cell density of this species was found at the limit of detection of the method of 1 cell in 25ml, so we cannot ascertain that all samples contained at least one cell.

- There were other toxic and harmful species found in the samples but these are not considered in this report as these were at very low cell densities and not possibly found in all samples.

- The descriptive statistics for each count using the Anderson-Darling Normality test suggests, that the data follows a normal distribution for most counts once outliers are taken out. The Individual charts and Z-scores suggest most analysts performed within the 2 standard deviation of the mean/median of the other analyst's results.

- The median was used to calculate the confidence intervals of the *L.polyedrum* and *Karenia* counts and the mean was used for the *D.acuminata, C.monotis* and *P.rotundatum.* The Z-scores were calculated using these numbers.

- *D.acuminata* and *L.polyedrum* were the easiest species to identify by the analysts and the identification should be correct to species level in this case. *C.monotis* and *K.selliformis* were the most difficult species to

identify in the samples. In the case of *Karenia* identification to species level is very difficult so identification to genus is sufficient for a correct answer. This is also the case for *C.monotis*, which should be identified to genus level only.

- While *C.monotis* is not a toxic organism, the *Coolia* genus includes toxic species, so analysts should probably have used the precautionary principle in this case and identify to genus only and count the cells in the samples. Those which decided not to count these species in the sample based on the non-toxic status of *C.monotis* and using light microscopy for their reliable identification tended to over-identify.

- A reliability qualitative measure calculated for the method indicates that the method in 2012, is more sensitive (93%) than specific (65%) and its efficiency based in the data is 86%. The false positive rate is higher (35%) than the false negative rates (7%) indicating that we are more likely to mis-identify a non-toxic species than the other way around.

- Most analysts performed above the 80% mark for the 'Ocean Teacher' Bequalm Hab quiz exercise. Questions 5 to 10 were nearly perfectly answered by all analysts. Q2 was dropped from the exercise due to the uncertainty regarding its correct answer. The worst answered questions were 4 and 12.

2. Introduction

The Phytoplankton Bequalm intercomparison study in 2012 was designed to test the ability of analysts to identify and enumerate correctly toxic and harmful marine phytoplankton species in preserved water samples. This year, samples have been designed using field material, which have been spiked also with laboratory cultures. We were interested only in toxic and harmful species found in the samples. The use of the term 'harmful' is a departure from previous exercises and one that poses uncertainty to the analysts. This term has not been purposely defined to allow the analysts to decide what they believe is or may be 'harmful' rather than preclude the outcome from a list of species.

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, 54 analysts from 29 laboratories around the world took part in this intercomparison. 51 analysts and 28 laboratories returned results. This year, there were laboratories from Australia and North Africa taking part in this exercise for the first time. There are also two laboratories from South America. The bulk (24 laboratories) comes from across Europe: Ireland (4), Northern Ireland (1), Scotland (3), England (7), Netherlands (2), Sweden (2), Spain (4) and Greece (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-12-MI1. PHY standing for phytoplankton, ICN for intercomparison, 12 refers to the year 2012, 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 2012.

3. Materials and Methods

Analysts were instructed to use the Utermöhl method (Utermöhl 1931, 1958) to analyse the water samples for abundance and composition of toxic/harmful marine phytoplankton. Samples were sent to analysts in 30ml plastic sterilin tubes which were preserved using neutral Lugol's iodine solution. A 25ml volume was set as the preferred volume for analysis but other sub-sample volumes were allowed (see Instructions: 3.test method). Analysts were given four weeks from sample receipt to analyse and return the results to the Marine Institute phytoplankton laboratory.

3.1 Field Sample and Culture material selection

The field sample used in this exercise was collected from North West Spain during a DSP outbreak caused by *Dinophysis acuminata* Claparéde & Lachmann, the field material was observed to contain as well other toxin producing species, There was interest on using *Phalacroma rotundatum* (Claparéde & Lachmann) Kofoid & Michener, although this species were only found at low cell densities, other toxic and harmful species found weren't in sufficient quantities to be of consideration this time. The laboratory cultures used were sourced from the Marine Institute culture collection (CCMI) in Ireland (*Coolia monotis* Meunier and *Lingulodinium polyedrum* (Stein) Dodge) and the Scandinavian Culture Collection of Algae and Protozoa (SCCAP) in Denmark (*Karenia selliformis* A.J.Haywood, K.A.Steidinger & L.MacKenzie).

This list was decided to study toxic species like *D.acuminata* and *P.rotundatum* (DSP), *L. polyedrum* (YTX), harmful species like *Karenia* and potentially toxic species like *Coolia.* The case of *Coolia* is particularly interesting because, although the species *C.monotis* is not considered to be toxic anymore, the species *C.tropicalis* is. So, some species in the genus produce toxins.

3.2 Cell concentrations

The cell concentration of *D.acuminata* and *P.rotundatum* were critical for this particular study. The cell densities from the field sample were typical of what we expect to find generally in monitoring samples. The cell concentration for *D.acuminata* worked out around 10 cells per sample or approximately 400 cells/litre while the cell concentration of *P.rotundatum* worked out to be 1 cell per sample or 40 cells/L. The abundance of *L. polyedrum* was the highest at 350 cells per sample or approximately 14000 cells/L. *C.monotis* at 50 cells per sample (2000 cells/L) was used at this concentration to make sure analysts would be able to identify

them apart from *L. polyedrum*, which could be hard at lower concentrations and finally *K. selliformis* at 50 cells per sample (2000 cells/L).

Generally the cell concentrations were low and ranging from approximately 350 cells for *L. polyedrum* to 1 cell for *P.rotundatum* in each 30ml sample. Preliminary cell counts 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 required.

3.3 Treatments and replicates

There was only one sample type for this study and this was sent in triplicates to analysts. Each analyst have to analyse three samples randomly selected from the sample population. An extra sample was sent as a spare.

3.4 Sample preparation, homogenization and spiking

All samples were prepared following the same protocol. The seawater used in this experiment was natural field water collected in Rinville, Oranmore, 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) and a concentrated field sample from North West Spain in a 50ml glass Schott bottle.

The sterilin tubes were made up to the required volume with sterile filtered seawater containing lugol's. This was carried out using a 25ml serological pipette (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.

A mixture of each organism was prepared separately using 50ml screw top Schott glass bottles (Duran®, Mainz, Germany). Then, a Master mix was prepared on a 500ml bottle containing all the species at the required concentration from each 50ml bottle. The master mix was inverted 100 times to homogenate the sample 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.

300 samples were produced for this study. 216 samples were sent to 54 analysts at 4 samples per analyst.

3.5 Sample randomization

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

3.6 Bequalm online HAB quiz

This year 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 activities in collaboration like 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).

Analysts had to register in the following web address: http://classroom.oceanteacher.org/ if they were registering for the first time, before they could access the content, but analysts that had registered to this platform last year, did not need to repeat this step. This year, analysts used self-registration through a password sent to them. Some of the technical issues that were raised last year were also resolved.

Three weeks were allowed for analysts to register, complete and submit the test. The course itself was found under the courses tab in the main menu page and under the section called interdisciplinary courses. Analysts could link to the Harmful Algal Bloom programme BEQUALM 2012 and quiz content from here.

The test itself consisted of 12 questions (see Annex 7). There were different question types used in this quiz, 4 true/false questions, 4 matching questions, 1 numerical, 1 multiple choice and 2 short answer questions. In the true/false questions analysts have to choose between true or false, the matching questions had drop – down menus with the answers and they had to choose the right one, the numerical question needed a numerical answer, there was a tolerance given to the answer and in the short answer questions the analysts had to write the answers.

3.7 Forms and instructions

The instructions for the exercise (Annex 3) were sent to all participants. All analysts were asked to read and follow the instructions before commencing the test. Two forms were also send to the analysts, Form 1 (Annex 1) was a form to confirm the receipt of materials; number and condition of samples and correct sample code. Form 2 (Annex 2) is a hardcopy to write the results of the test, both abundance and composition. These forms were sent to the analysts via e-mail, with their respective laboratory and analyst codes.

The samples were 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.

3.8 Statistical analysis

Statistical analysis was carried out on Minitab® Statistical Software Vr16.0 and Microsoft office Excel 2007 on the data returned. A graphical summary of the data for each species was carried out using the Anderson-Darling test which doesn't assume the normality of the data. This test allows for a graphical representation and a number of useful descriptive statistics of the data.

The analysts' results were represented by Individual charts for each analyst and every species. This data was plotted against the mean/median and 2 standard deviations of the analysts' results. A whiskers and box plot was used to visualize the variability of the data for each species.

Z-scores using Excel were plotted to show the final score of each analyst and every identification in the sample using the mean/median and 2 standard deviation of all the analysts results.

The sample identification results had been used to build a qualitative reliability measure for the test method. This measure gives an indication of how fit for purpose this method is for the correct identification of microalgae in preserved water samples. It doesn't give any information on the abundance results. It has been solely developed to give information on the qualitative side of the method.

As a qualitative test, the degree of correctness of the organism identification has been measured for the method in terms of false positive and negative rates. These positive and negative rates based on false positive and negative responses have been combined and expressed as a Bayesian likelihood ratio. The

sensitivity and specificity of the method has been calculated as a Youden index (Youden, 1975). In order to calculate the positive and negative rates of this intercomparison, a definition was needed to describe what makes a false positive rate and what makes a false negative rate. The definition of a false positive rate is the number of false positive results divided by the number of true negative results + false positive results. Equally the false negative rate is the number of false negative results divided by the number of true positive results + false negatives results.

A **true positive** (TP) result in this case is the number of toxic/harmful species correctly identified. A **false positive** (FP) result is the number of non-toxic/non-harmful species identified incorrectly or identified as toxic/harmful. A **true negative** (TN) is the number of non-toxic/non-harmful species correctly identified and a **false negative** (FN) is the number of toxic/harmful species identified incorrectly or identified as non-toxic/non-harmful.

This gives us a very powerful reliability measure for the intercomparison (Table below). These rates then can be used to construct a measure of how sensitive, specific and efficient the method is.

Table 1: Expression of reliability measure for identification

Realiability measure	Expression
False Positive Rate	FP/(TN+FP)
False Negative Rate	FN/(TP+FN)
Sensitivity	TP/(TP+FN)
Specificity	TN/(TN+FP)
Efficiency	TP+TN/(TP+TN+FP+FN)
Youden Index	Sensitivity+Specificity-1
Likelihood ratio	1-False Negative rate/False
Bayes Posterior probability	Bayes Rule

The correct identification of the following organisms *D.acuminata, P.rotundatum, K.selliformis and L.polyedrum* were considered to be true positives, the correct identification of *C.monotis*, were considered to be true negatives. If an analyst, did not identified or misidentified a true positive organism, then the response was considered a false negative response, equally, if an analyst did not identified a true negative organism, then the response was considered a false positive response.

4. Results

4.1 Enumeration results

4.1.1 Data

51 analysts from 28 laboratories returned abundance and composition results on three replicate marine phytoplankton field samples collected in the North West coast of Spain and spiked with cultured material.

There were five species of interest in the sample for this exercise: *D.acuminata*, *P.rotundatum* (found in the field sample) and *C.monotis*, *L.polyedrum* and *K.selliformis* spiked in the samples from culture collections. The data is confined to these species.

The mean of the three replicates for each analyst and each species has been used to generate z-scores, I charts and descriptive statistics of the data. The data and the graphs generated was manipulated using Microsoft Excel 7 and Minitab vr.16.0.

The Z-scores in the final statement of performance only applies to three species: *D.acuminata*, *L.polyedrum* and *K.selliformis*. There is no Z-score for *C.monotis* because analysts were asked to count the toxic/harmful species only and there is no score for *P.rotundatum* because at the cell density of these species in the samples was at the limit of detection (1 cell/25ml) but we cannot ascertain that analysts used 25ml chambers or that there was 1 cell in each sample. However, we are showing this data in the report because it is of interest to analysts what happens when the analyte, in this case *P.rotundatum*, is at the limit of detection of the method.

4.1.2 Analysts' results

Table 2 shows the mean results returned by the analysts for this intercomparison for each species of interest. N/A means no results returned by an analyst. 51 out of 54 analysts that took part in the intercomparison returned results to the organizing laboratory. The results are expressed in cells per litre.

Table 2: Analysts' results

ANALYST CODE	LAB CODE	SAMPLE CODES			L.polyedrum	K.selliformis	D.acuminata	C.monotis	P.rotundatum
20	9	37	127	12	14522	not id	449	2261	not id
11	6	136	150	298	13600	1973	373	2267	27
22	6	164	203	245	13520	1667	467	2387	13
7	5	286	128	66	10107	not id	360	2627	not id
47	5	55	94	204	12613	not id	360	2560	not id
16	5	214	153	33	12653	not id	293	2680	not id
25	5	224	41	122	12547	not id	400	3147	40
15	5	212	92	251	9427	13	333	2800	not id
12	5	208	134	59	14187	not id	360	2293	not id
44	8	213	52	188	13955	233	356	2256	22
31	12	281	35	160	13467	7693	507	2187	27
24	23	58	221	132	13960	1827	427	not id	120
1	11	165	103	178	14160	547	373	2267	13
34	11	172	8	112	13733	1640	440	1720	40
39	22	260	16	169	n/a	n/a	n/a	n/a	n/a
50	20	60	87	243	12347	2107	320	not id	not id
6	3	234	249	275	12067	1333	427	2200	not id
46	3	80	129	39	12667	2427	360	1933	27
2	3	180	17	42	12287	1227	173	2187	13
51	16	158	177	116	14600	2547	413	2013	53
10	14	242	32	285	11507	120	320	3093	80
23	14	176	81	137	13880	93	307	2253	27
30	17	225	257	10	15387	3747	480	2693	40
49	17	24	231	21	13613	3493	453	2627	not id
3	18	182	199	235	9240	1547	467	2147	27
26	24	108	147	7	16965	2595	519	not id	33
36	25	47	54	267	14467	1693	493	2360	27
9	1	218	179	290	8558	1068	352	1333	38
19	4	139	141	266	16520	987	560	not id	13
48	7	65	246	197	11707	853	440	2613	53
43	7	29	48	61	8744	178	178	1911	not id
41	7	118	297	230	14507	173	240	2787	27
5	19	173	64	36	11000	not id	333	2500	not id
38	27	74	38	114	16433	3533	650	2533	27
42	10	1	140	201	not id	8640	360	not id	not id
32	13	268	219	262	16373	2547	453	not id	40
28	21	73	207	174	9547	5987	493	not id	not id
18	21	264	220	209	n/a	n/a	n/a	n/a	n/a
45	21	270	295	45	13880	13360	507	not id	27
21	21	76	67	171	13600	11053	400	not id	13
14	2	277	250	274	7733	233	267	not id	13
17	2	107	133	46	4633	133	200	not id	not id
8	2	28	65	78	4400	277	277	not id	not id
13	26	125	256	185	3507	80	133	413	not id
37	26	280	124	255	13507	160	547	2040	13
29	26	62	248	196	16587	not id	453	not id	27
35	26	211	120	239	n/a	n/a	n/a	n/a	n/a
40	26	287	100	126	2053	27	147	173	13
33	26	240	215	51	7133	27	213	1240	40
27	26	26	72	238	14267	413	507	not id	27
52	15	159	237	9	13731	652	536	1287	not id
4	15	75	170	50	14750	2767	383	1767	33
53	28	3	167	244	9920	387	253	2187	53
54	29	181	261	292	14347	1373	387	2880	40
					1 id as alexandrium	15 x id mikimotoi	3x id D.sacculus	5 x id alexandrium	
						5 x id Brevis		1 x id L.polyedrum	
						16 x id sp.		1 x id G.foliaceum	
						6 x id selliformis			
						1 id naked dino			

Not i.d.= not identified; n/a= not participated

4.1.3 Descriptive statistics

Figures 1 to 5: Anderson-Darling normality test (Analysts' results) for *D.acuminata, L.polyedrum, C.monotis, P.rotundatum* and *K.selliformis*.

Figure 1.

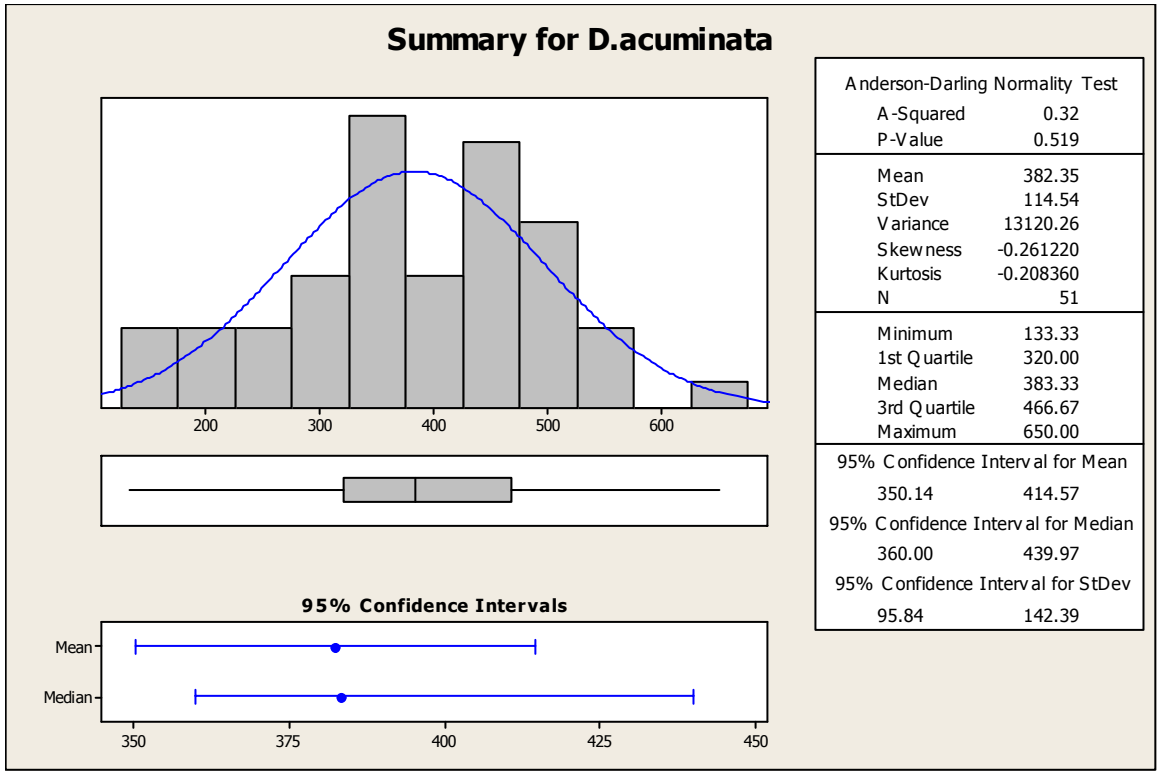


Figure 2.

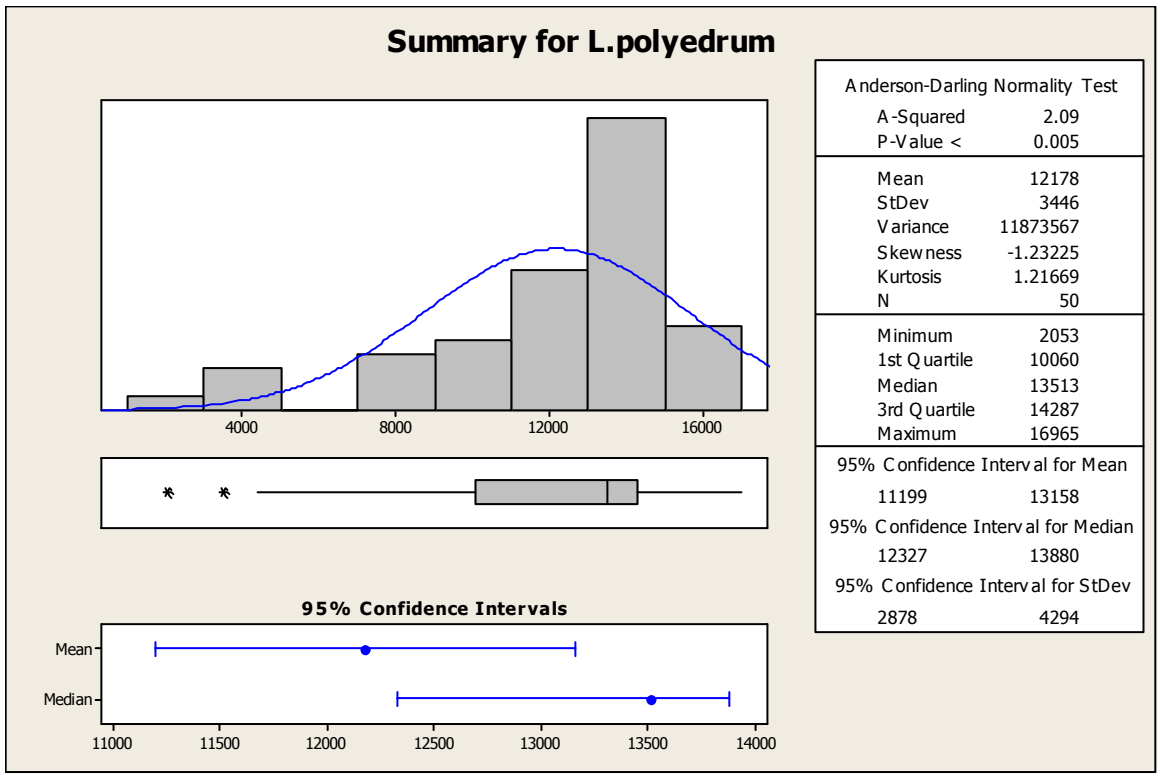


Figure 3.

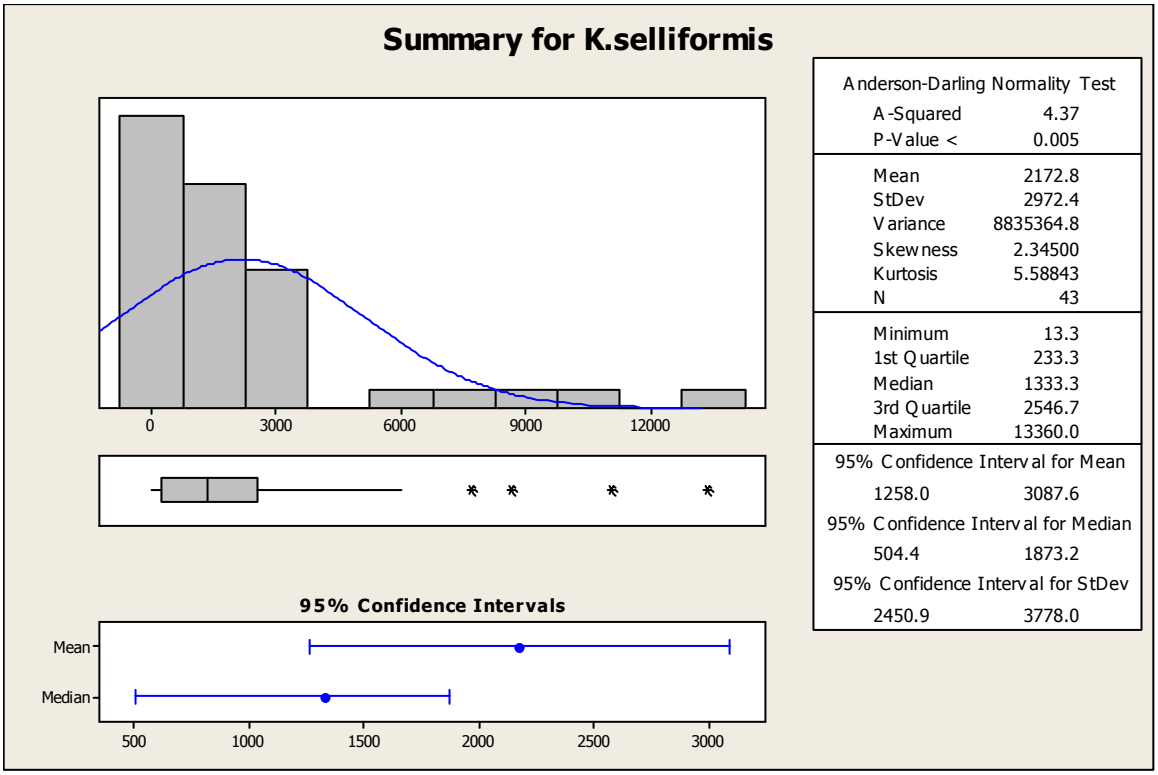
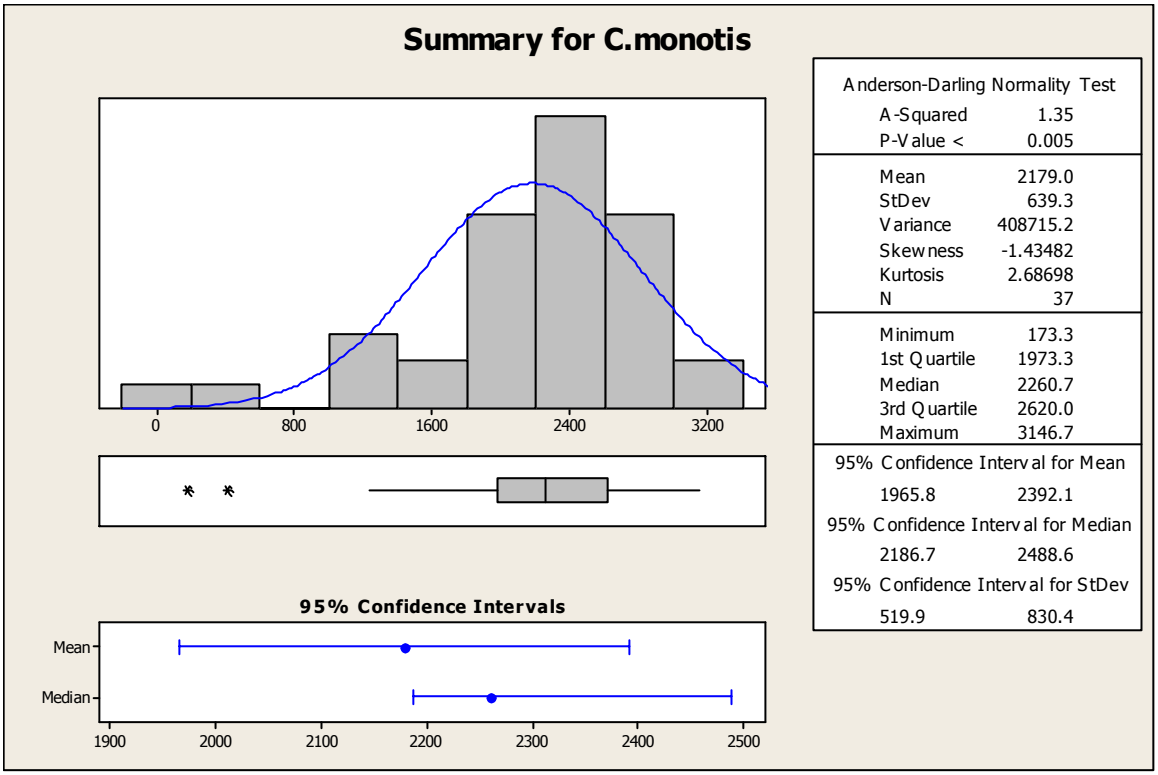


Figure 4.



Figures 1 to 5 are the Anderson-Darling normality tests for each species. In some cases, the data doesn't seem to follow a normal distribution, except for the *D.acuminata* count. When outliers are taken out (data not shown) the test follows a normal distribution for the *C.monotis* and *L.polyedrum* counts but still not for the *K.selliformis* and *P.rotundatum* counts. Figure 6 shows the box plot of the five counts.

Figure 5.

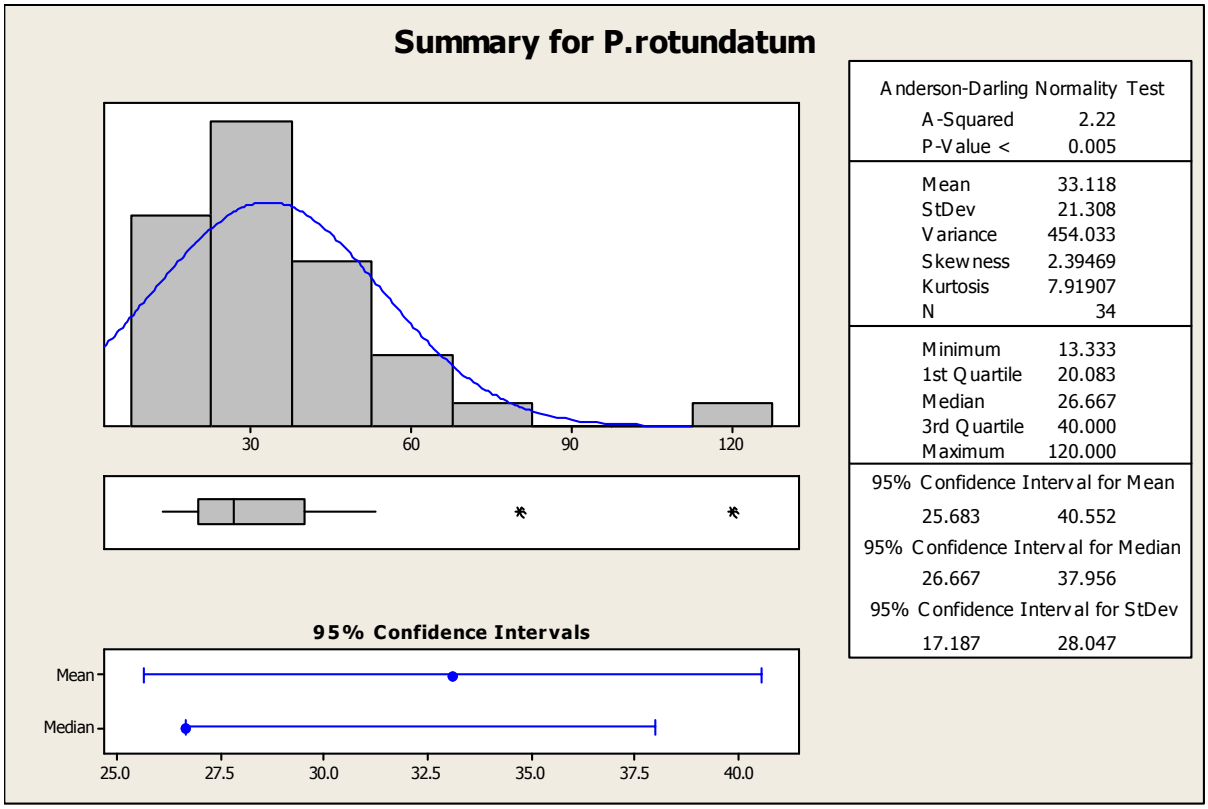
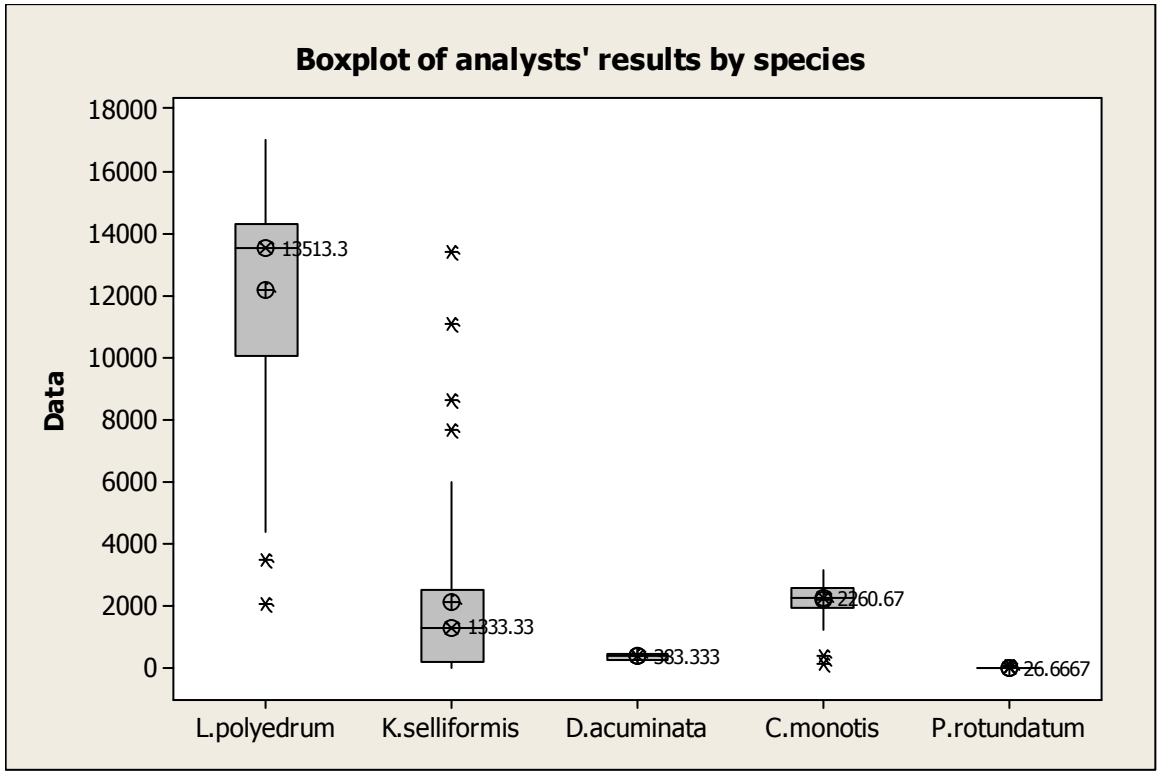


Table 3 shows the mean, median and the standard deviation of each count. The median was used to generate z-scores and confidence limits for *L.polyedrum & K.selliformis* counts. The mean was used in the other three counts.

Table 3: Mean, Median and Standard deviation of each count

mean		12178	2173	382	2179	33
median		13513	1333	383	2261	27
sd		3446	2972	115	639	21
		L.polyedrum	K.selliformis	D.acuminata	C.monotis	P.rotundata

Figure 6: Box plot of analysts' results by species



4.1.4 Individual charts and Z-scores

The individual charts (figures 7 to11) show the individual mean values for each species by laboratory code. Analysts 38 (lab 27) and 13 (lab 26) were outside the 2 standard deviation for the *D.acuminata* cell count (figure 7), also, laboratory 2 identified the organism as *D.sacculus*. Most laboratories performed within the specifications for this count.

Figure 8 shows the results of the *L.polyedrum* count. Again, most laboratories performed well with a small number of out of specification results mainly in two laboratories: Lab 2 (analysts 17 and 8) and Lab 26 (analysts 13 and 40), also laboratory 10 did not identify the species and laboratory 4 identified the species as *Alexandrium*, which is incorrect.

The *Coolia monotis* count (figure 9) returned a more complex set of results, while most analysts were well within the 2 Standard deviations of the mean count of all analysts, except for lab 26 (analysts 13 and 40). A number of laboratories failed to identify the species (laboratories 23, 20, 24, 4, 2 and 26), laboratories 13 and 21 did not identify the species but that is because they did not consider *C.monotis* to be toxic. Other laboratories identified incorrectly the species (17, 18, 7 only one analyst, 15 and 28).

Figure 10 is the *Karenia* count, Most laboratories are within the required confidence limits, Laboratories 12, 10 and 21 are outside the 2SD. Five out six analysts in laboratory 5 did not identify the species and one analyst in laboratory 3 identified them as unarmored dinoflagellate, which is not correct. Most analysts identified correctly to genus level but there were differences of opinion in relation to the species taxon. Most analysts opted for *Karenia sp.* as the main option, the next most popular choices were *K.mikimotoi* and *K.selliformis*. Some analysts chose *K.brevis*.

The *P.rotundatum* count (figure 11) is more straightforward with most analysts scoring well, two analysts outside the limits (labs 23 and 14 (10)) and 17 analysts not identifying the species.

Figures 7 to 11: I-charts of individual results labeled by Laboratory code for *D.acuminata, L.polyedrum, C.monotis, K.selliformis* and *P.rotundatum*.

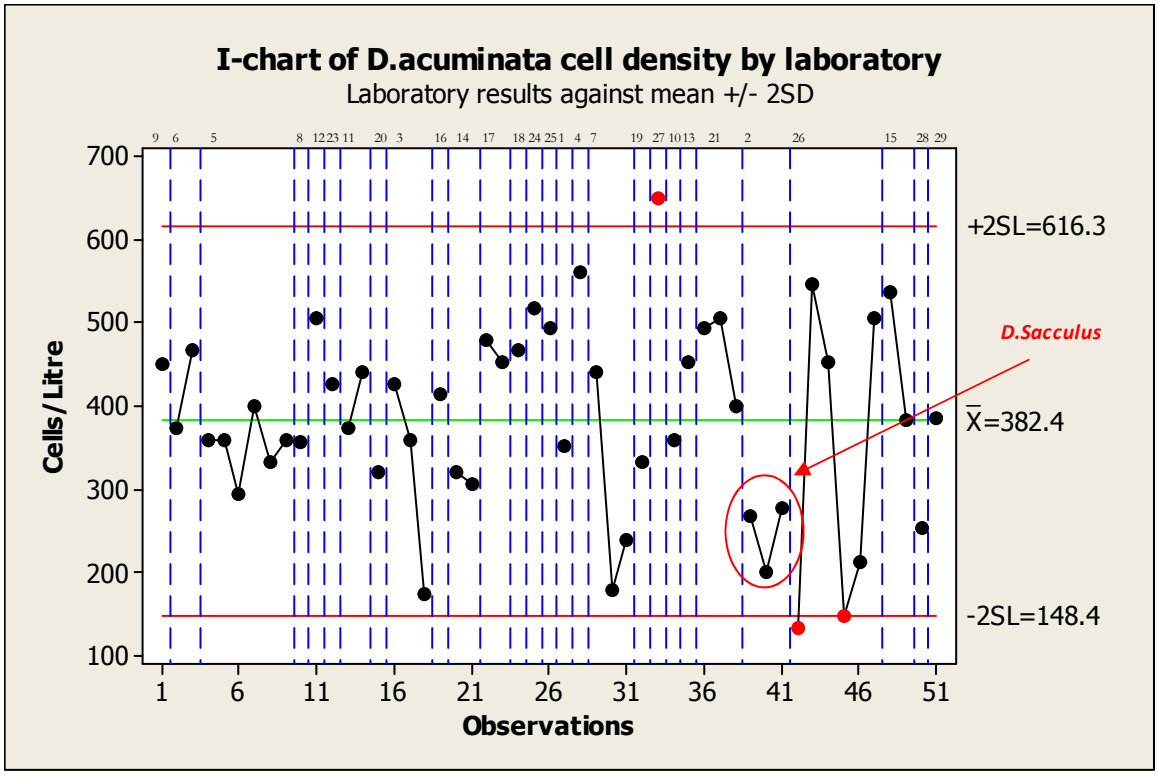


Figure 8.

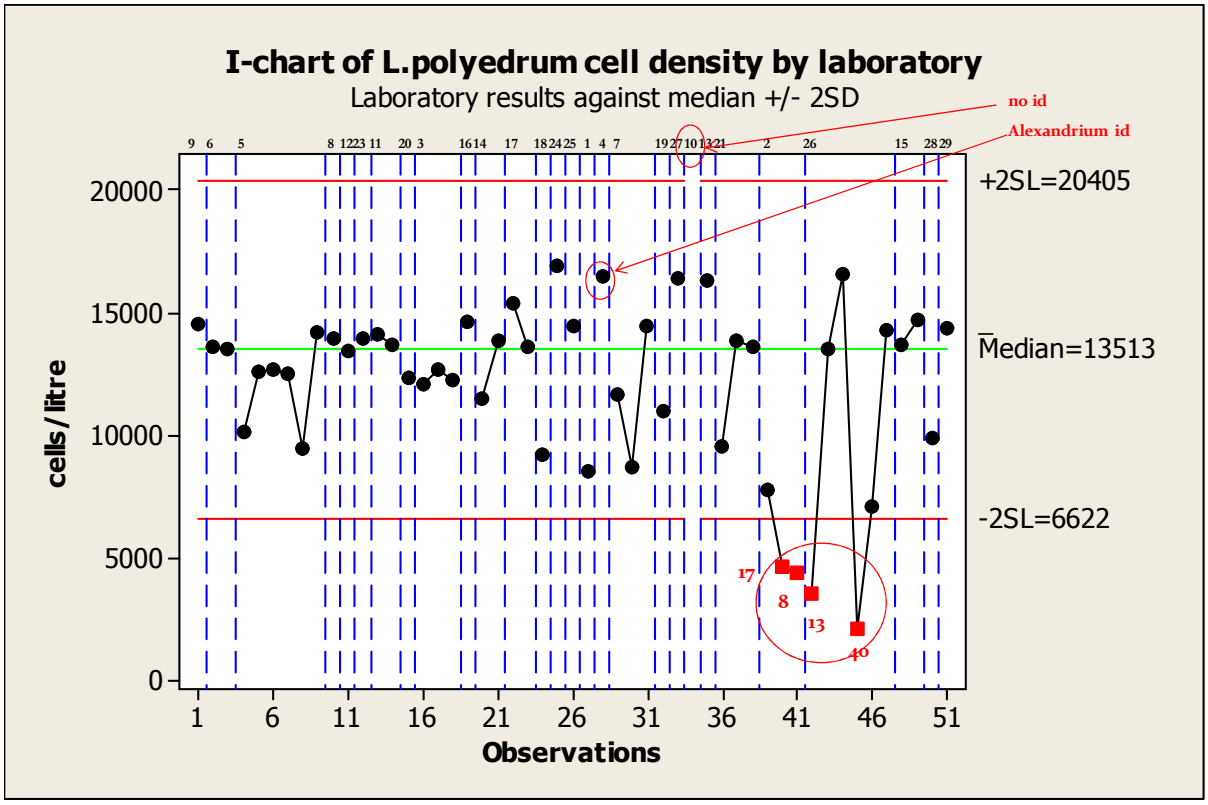


Figure 9.

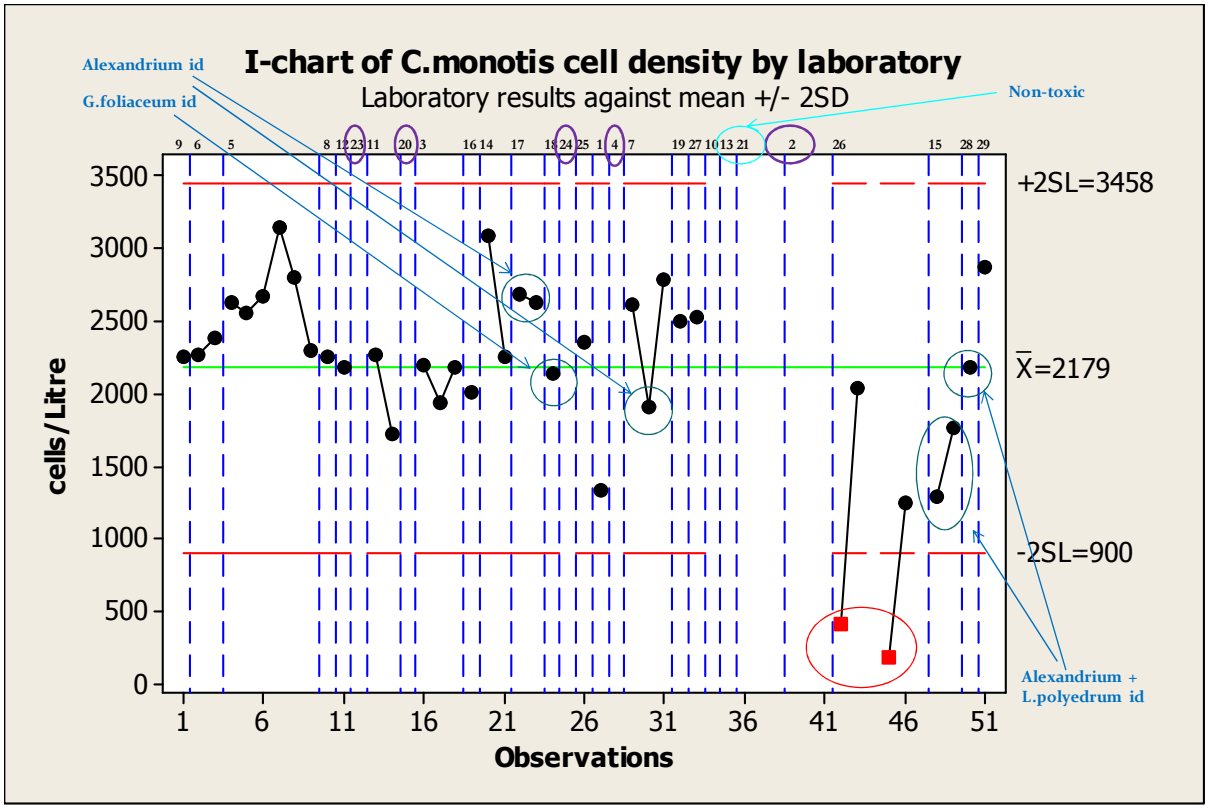


Figure 10.

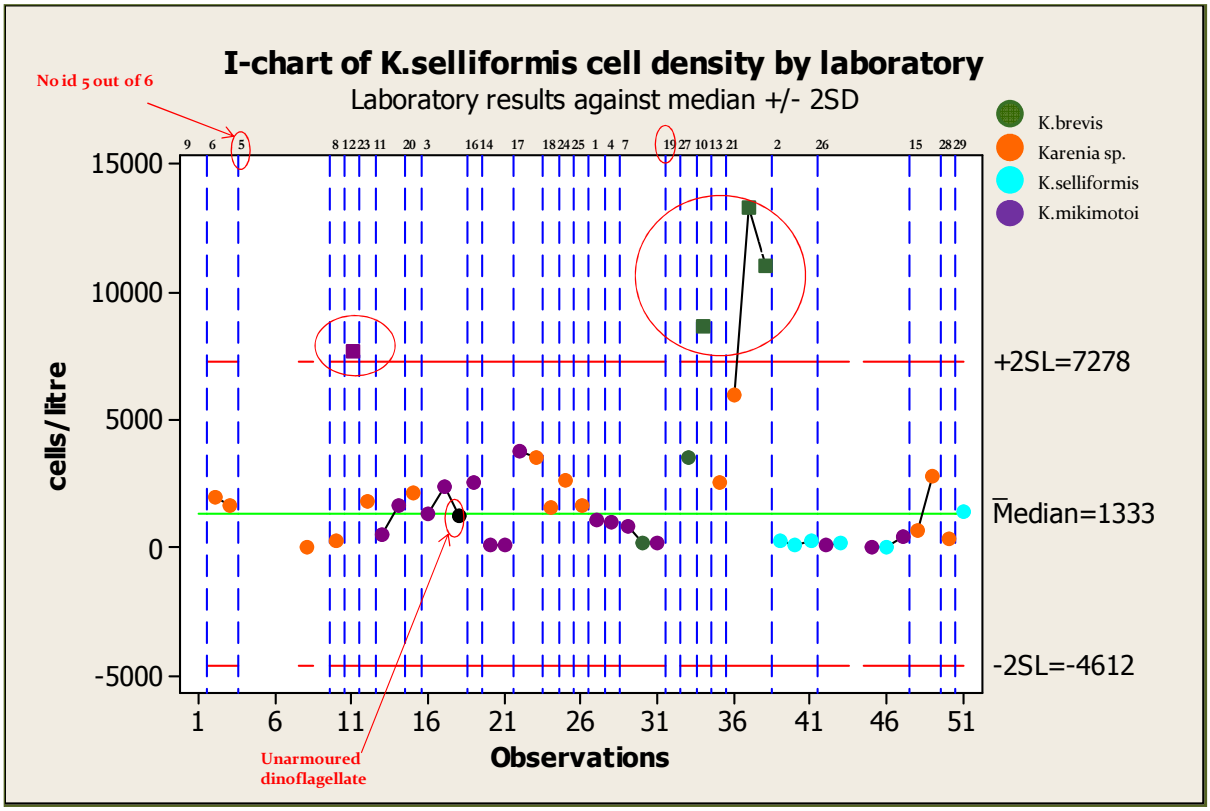
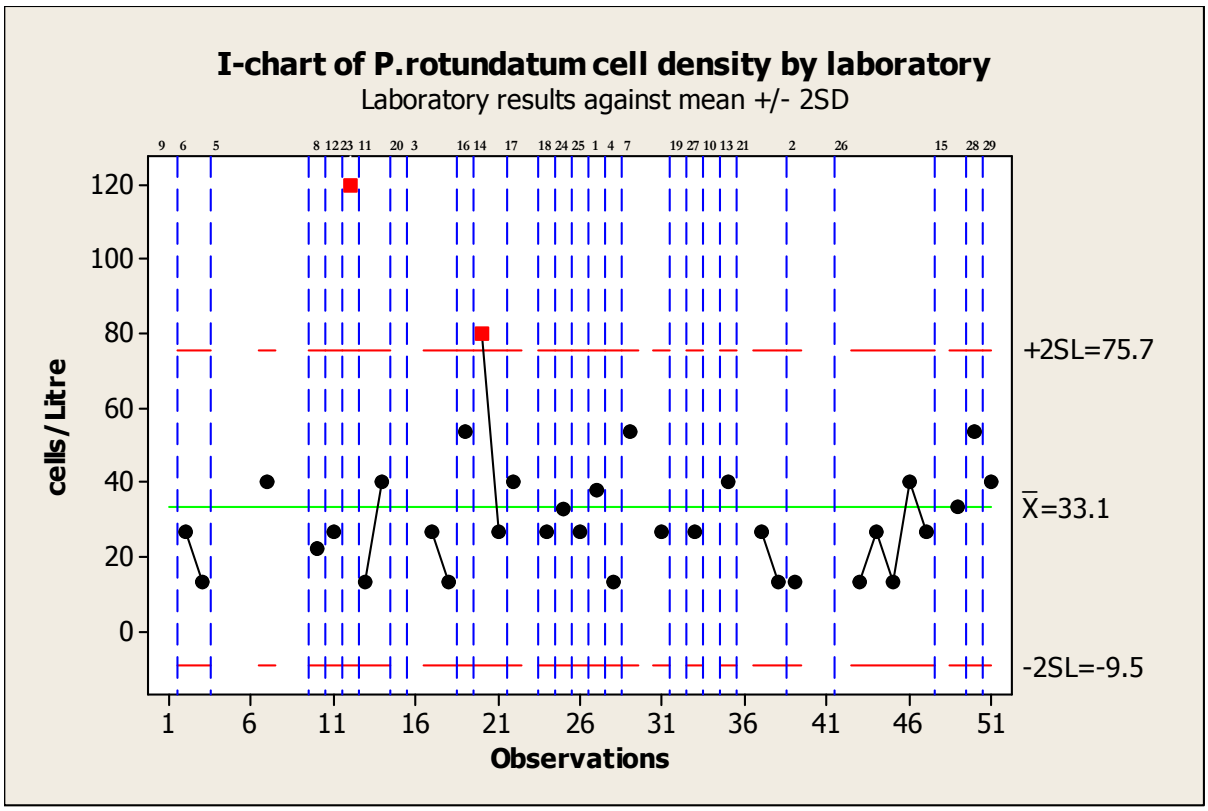


Figure 11.



Figures 12 to 14: Z-scores of analysts for *D.acuminata, L.polyedrum* and *K.selliformis*.

Figure 12.

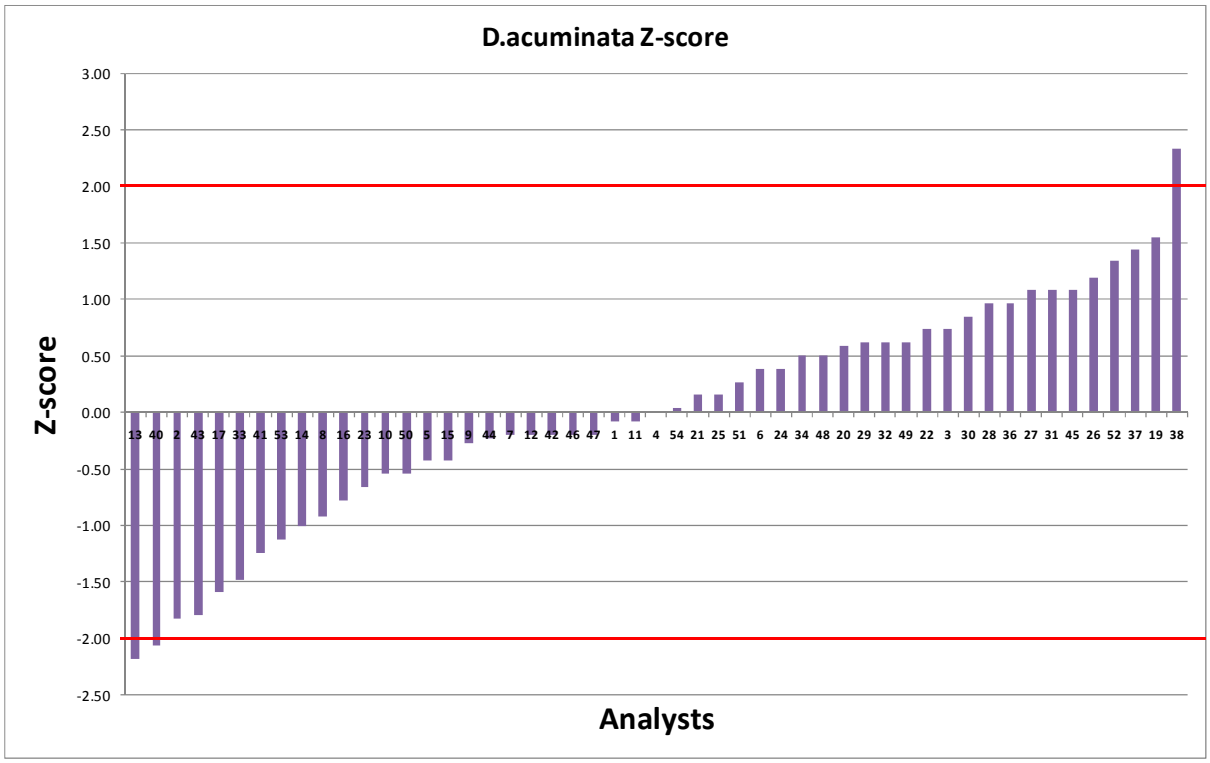
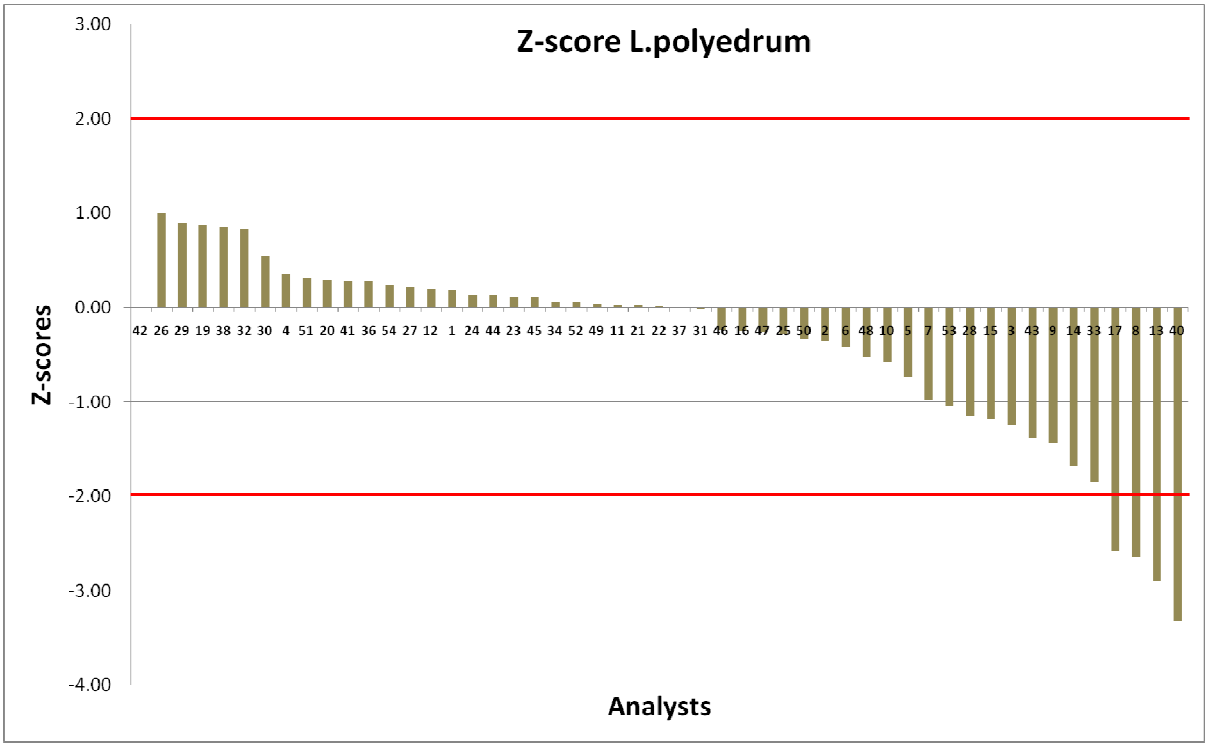
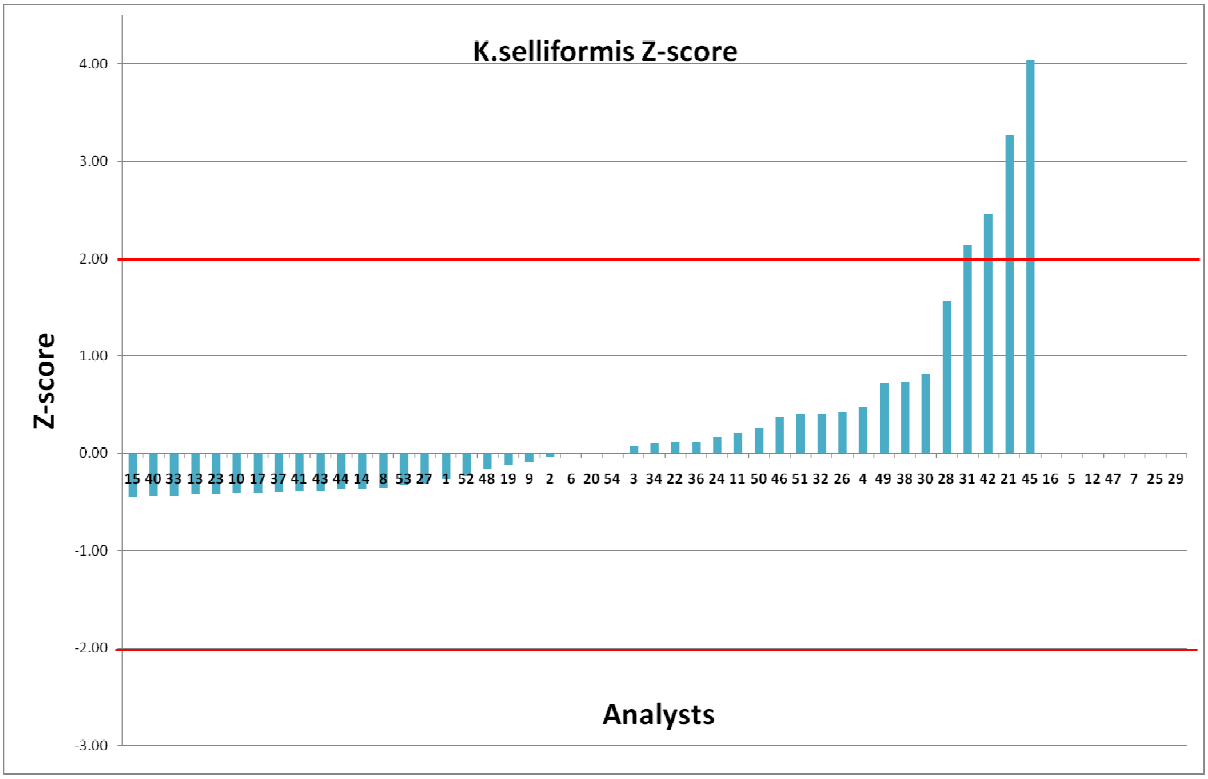


Figure 13.



Figures 12 to 14 represent the Z-scores by analyst code for three out of the five species. There is no Z-scores for *C.*monotis (being non toxic) and *P.rotundatum* (limit of detection). Analysts have to fall within the 2 standard deviations of the mean/median of all the results. In some cases, there are no results for some analysts on particular species, this mean that the analysts did not returned results for these species, and these are also out of specification results.

Figure 14.



4.2 Identification results

4.2.1 Reliability qualitative measure.

Analysts were asked to identify all the organisms in the samples to the highest taxonomic level possible. *D.auminata, P.rotundatum* and *L.polyedrum* were to be identified to species level and the other two organisms (*K.selliformis and C.monotis)* were required to be identified to genus only.

Most analysts identified correctly to species level *D.acuminata* (48 out of 51), *L.polyedrum* (49 out of 51) and to genus level *Karenia* (42 out of 51). *P.rotundatum* identification data is not used here as we cannot ascertain

that all samples contained at least one cell. 17 analysts did not identify the species but the rest identified correctly to species level. *C.monotis* was identified correctly to genus level by 30 analysts, incorrectly by 7 and not identified by 14.

Regarding the identification of *D.acuminata,* three analysts opted for *D.sacculus* but were correct to genus level. The reason is probably bio-geographical. In relation to *L.polyedrum* one analyst did not identified the species and one analyst opted for *Alexandrium* which is incorrect.

Karenia was easy to identify to genus level by most analysts. One analyst opted for 'unarmored dinoflagellate' and 9 analysts did not identify it. At species level, analysts opted for different species (figure 10). This is because the genus *Karenia* is hard to identify beyond genus level using light microscopy.

C.monotis was the hardest to identify, 30 analysts identified it correctly, 15 analysts decided to identify to species level *C.monotis* and the other 15 just to genus level (*Coolia sp.*). Those misidentifying *Coolia* used the names *Alexandrium, Lingulodinium and Glenodinium* instead. 14 analysts did not identified *Coolia.* Three of those did not identified *Coolia* because they precluded that it was *C.monotis* and therefore non-toxic, which they didn't have to identify or count.

Only the correct identification of the organisms to genus level has been used to build this reliability measure for the exercise (Table 4). The reliability measure (Table 5) shows that the sensitivity of this method is high (93%), while the specificity is a bit lower (65%), the reason for this is that the false positive rate is higher (35%) compared to the false negative rate (7%).

The sensitivity is in our method how good we are at identifying toxic algae from non-toxic algae, the specificity in our method is how good we are at identifying algae (other than toxic ones). The efficiency of the test method is measured using the sensitivity and the specificity, that is how good we are overall at correctly identifying algae (whether toxic or not).

The Youden index is a single statistic of the performance of this test. It is a number between -1 and +1. This is calculated simply by adding the sensitivity + the specificity and subtracting 1. If the method was perfect, the sensitivity would be one (that is a 100% sensitive) and the specificity would be also one (that is a 100% efficient), therefore Youden index= 1 + 1 -1= +1, which is the perfect score. In our case the Youden index is +0.58 or 58% for 2012.

The likelihood ratio shows the likelihood of obtaining a false positive or negative response in the method. As the number of false positive responses increase, the likelihood ratio becomes a positive number which increases in value. Equally, if the number of false negative responses increases, the likelihood ratio becomes a negative number. In our case, the likelihood ratio is 0.80 or 80%, so we are more likely to incur in false positive responses.

Table 4: identification results by species and analyst for the reliability study

ANALYST CODE	LAB CODE	species identified correctly (1 or 0) nr= no result			
		C.monotis	L.polyedrum	D.acuminata	K.selliformis
20	9	1	1	1	0
11	6	1	1	1	1
22		1	1	1	1
7	5	1	1	1	0
47		1	1	1	0
16		1	1	1	0
25		1	1	1	0
15		1	1	1	1
12		1	1	1	0
44	8	1	1	1	1
31	12	1	1	1	1
24	23	0	1	1	1
1	11	1	1	1	1
34		1	1	1	1
39	22	nr	nr	nr	nr
50	20	0	1	1	1
6	3	1	1	1	1
46		1	1	1	1
2		1	1	1	0
51	16	1	1	1	1
10	14	1	1	1	1
23		1	1	1	1
30	17	0	1	1	1
49		0	1	1	1
3	18	0	1	1	1
26	24	0	1	1	1
36	25	1	1	1	1
9	1	1	1	1	1
19	4	0	0	1	1
48	7	1	1	1	1
43		0	1	1	1
41		1	1	1	1
5	19	1	1	1	0
38	27	1	1	1	1
42	10	0	0	1	1
32	13	1	1	1	1
28	21	1	1	1	1
18		nr	nr	nr	nr
45		1	1	1	1
21		0	1	1	1
14	2	0	1	1	1
17		0	1	1	1
8		0	1	1	1
13	26	1	1	1	1
37		1	1	1	1
29		0	1	1	0
35		nr	nr	nr	nr
40		1	1	1	1
33		1	1	1	1
27		0	1	1	1
52	15	0	1	1	1
4		0	1	1	1
53	28	0	1	1	1
54	29	1	1	1	1

Table 5: Reliability measure for 2012

51 analysts					
TP= True positives	0	49	51	42	142
TN= True Negatives	33	0	0	0	33
FP= False Positives	18	0	0	0	18
FN= False Negatives	0	2	0	9	11
False Positive rate	0.35				0.35
False Negative rate		0.04	0.00	0.18	0.07
Sensitivity					0.93
Specificity					0.65
Efficiency					0.86
Youden Index					0.58
Likelihood ratio					0.80

4.3 Ocean Teacher online HAB quiz

Table 7 shows the final results as a percentage of correct answers for each question and analyst. 45 analysts submitted a complete online quiz and 6 analysts did not complete the quiz. There were 12 questions in this quiz but question 2 was dropped due to the uncertainty surrounding the answer.

Question 2 of the online quiz asked whether *Pseudo-nitzschia* and *Nitzschia* are the only two genera to produce ASP toxins (within the confines of marine phytoplankton) as red algae (family Rhodomelaceae) can also produce these toxins (Sato et al. 1996). The answer (True/false) divided analysts with 53% saying false and 47% saying true. It was argued by analysts answering 'false' that another genus (*Amphora*) can also produce toxins based on the work by Shimitzu *et al.* 1989 and Maranda *et al.* 1990 on *Amphora coffeaeformis* (C.Agardh) Kützing in Canada. However, Bates *et al.* 1989 demonstrated that cultures of *A.coffeaeformis* did not produce toxins, later Sala *et al.* 1998 reviewed some materials containing *A.coffeaeformis* including the materials from Canada and concluded that the identification of the clone used in Shimitzu and Maranda's work was inconclusive based on a single SEM image as the only material remaining from that investigation. Recently, *A.coffeaeformis* was placed in the *Halamphora* genus (Levkov et al. 2009) further complicating the real identity of the species. Therefore, it was decided that given the uncertainty surrounding the identity of the species and whether it is able to produce toxins or not, that the question be dropped (to avoid a bloodbath!).

Most analysts performed well and over the 80% mark (figure 15), eight analysts with perfect scores and fifteen analysts between 80 and 90% and fourteen analysts outside the 80% mark. Figure 16 shows the % of correct answers by question. Q4 and 12 appear to be the most difficult ones to answer followed by Q3 and 11. The easier question types are the matching, multiple choice and numerical types (figure 17). The most difficult are the true/false and the short answer questions.

Question 4 also divided analysts in their answer. The question asked was: 'Is *Coolia monotis* a toxic alga? (true/false). Here, 58% of analysts answered true and 42% answered false. Holmes *et al.* 1995, reported a strain of *C.monotis* to be toxic to mice through ip injection in 1995 from Australian waters, it said that it produced a toxin analogue of Yessotoxins called Cooliatoxins but this was never confirmed. Rhodes *et al.* 2000, found the extracts from *C.monotis* not to be toxic to mice, later Riobó *et al.* 2003, Penna *et al.* 2005 and Fraga *et al.*2008, reported that *C.monotis* don't produce toxins. However, the genus *Coolia* contains some species that can produce toxins as is the case of *Coolia tropicalis* Faust, therefore if samples contain *Coolia* cells, analysts should try to count them as it would be difficult to identify them to species level on preserved samples.

Q12 was a very technical question on armoured dinoflagellate physiology, important because the orders Gonyaulacales and peridiniales can be separated by the type of division these cells undergo allowing for differentiation at order level, with gonyaulacales dividing by desmoschisis and peridiniales by Eleutheroschisis

In table 6 we compared the answers given to Q4 in the online HAB quiz and the results from the actual samples. Q4 asked whether *C.monotis* was toxic? (true or false) and *C.monotis* was also one of the species spiked in the samples.

This table shows 45 answers from the online HAB quiz to the question and whether the same analysts had correctly identified the species in the samples or not. From 28 analysts that correctly identified the species in the samples 14 of them thought that the species were toxic and the other 14 thought they were non-toxic based on Q4 answers in the quiz. Another 6 analysts identified incorrectly the species in the samples and of those 4 thought it was toxic and 2 non-toxic. The remaining analysts (11) did not identified the species in the samples but 8 thought it was toxic and 3 non-toxic. Table 8 shows the final analysts scores.

Table 6: HAB online quiz Question 4 answer against sample results

Sample id.	Online quiz		Total
	Toxic	non-toxic	
Correctly identified	14	14	28
incorrectly identified	4	2	6
not identified	8	3	11

Table 7: Ocean teacher Hab quiz 2012 results

Analyst code	Lab code	Q1	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Grade
20	9	100	100	100	100	100	100	50	100	100	100	100	95
11	6	100	100	100	100	100	100	100	100	100	100	100	100
22	6	100	0	0	100	100	85	100	100	100	100	100	80
7	5	100	100	100	100	100	100	100	100	100	0	0	82
47	5	100	100	0	100	100	100	100	100	100	0	0	73
16	5	100	100	0	100	100	95	100	100	33	100	100	84
25	5	100	100	0	100	100	100	100	100	100	0	100	82
15	5	100	100	0	100	100	100	100	100	100	0	100	82
12	5	100	100	0	100	100	100	100	100	100	100	100	91
44	8	100	100	0	100	100	95	100	100	100	100	0	81
31	12	100	100	0	0	100	100	100	100	100	0	0	64
24	23	100	100	0	100	93	100	100	100	100	100	0	81
1	11	100	100	100	100	100	100	100	100	100	100	100	100
34	11	100	100	100	100	100	100	100	100	100	100	100	100
50	20	100	100	0	100	100	100	100	100	100	0	0	73
46	20	0	100	100	0	100	95	100	100	100	100	0	72
2	20	100	100	100	100	100	100	100	100	100	100	100	100
51	16	100	100	100	100	100	100	100	100	100	100	100	100
10	14	100	100	100	100	100	100	100	100	100	100	100	100
23	14	100	100	0	100	100	100	100	100	100	100	0	82
30	17	100	100	0	100	87	100	100	100	100	100	100	90
49	17	100	100	100	100	93	100	100	100	100	100	100	99
3	18	0	0	100	100	100	100	100	100	100	100	100	82
26	24	100	100	0	100	100	100	100	100	100	100	100	91
36	25	0	0	100	100	100	100	50	100	100	0	0	59
9	1	100	100	100	100	100	100	100	100	100	100	100	100
19	4	100	100	0	100	100	100	50	100	100	100	100	86
48	7	100	0	0	100	100	100	100	100	100	100	0	73
43	7	100	0	0	100	80	100	100	100	100	0	0	62
41	7	100	0	0	100	100	100	100	100	100	100	100	82
5	19	100	100	0	100	100	100	100	100	100	0	100	82
38	27	100	100	100	100	100	100	100	100	100	100	100	100
42	10	100	0	0	0	100	85	100	100	100	0	0	53
32	13	100	100	100	100	100	100	100	100	100	100	0	91
28	21	0	0	100	100	100	100	100	100	100	100	0	73
45	21	100	100	100	100	100	95	100	100	100	100	100	100
14	2	100	100	0	0	87	100	100	100	33	100	0	65
17	2	100	100	0	100	80	100	100	100	33	100	0	74
37	2	100	100	0	100	93	100	100	100	100	100	100	90
29	2	100	0	0	100	93	95	100	50	100	100	0	67
33	2	100	100	0	100	93	90	50	100	100	0	0	67
52	15	100	100	0	100	93	100	100	100	100	100	0	81
4	15	100	0	100	100	93	100	100	100	100	100	0	81
53	28	100	100	0	0	100	100	100	100	100	0	100	73
54	29	100	100	100	100	100	95	100	100	100	100	100	100
	Total	91	78	42	89	97	98	96	99	96	73	56	83

Figure 15: Individual value plot of HAB quiz results by analysts

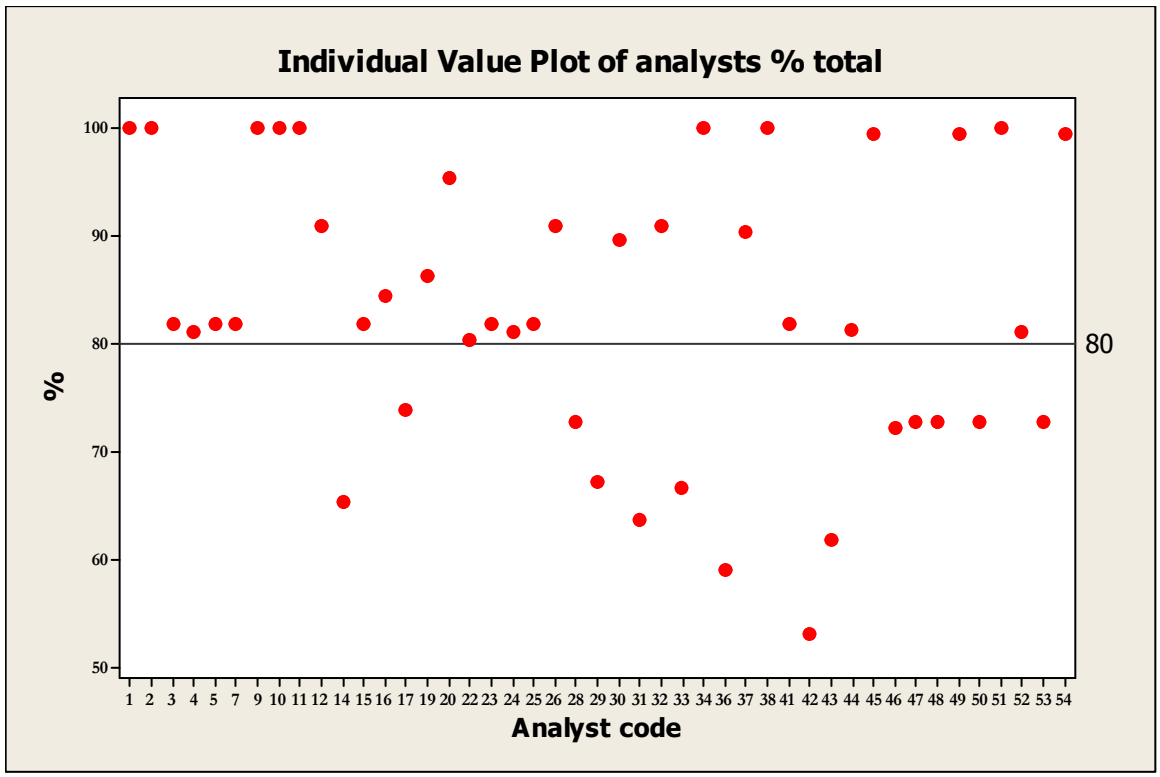


Figure 16: Individual value plot of % correct answers by question

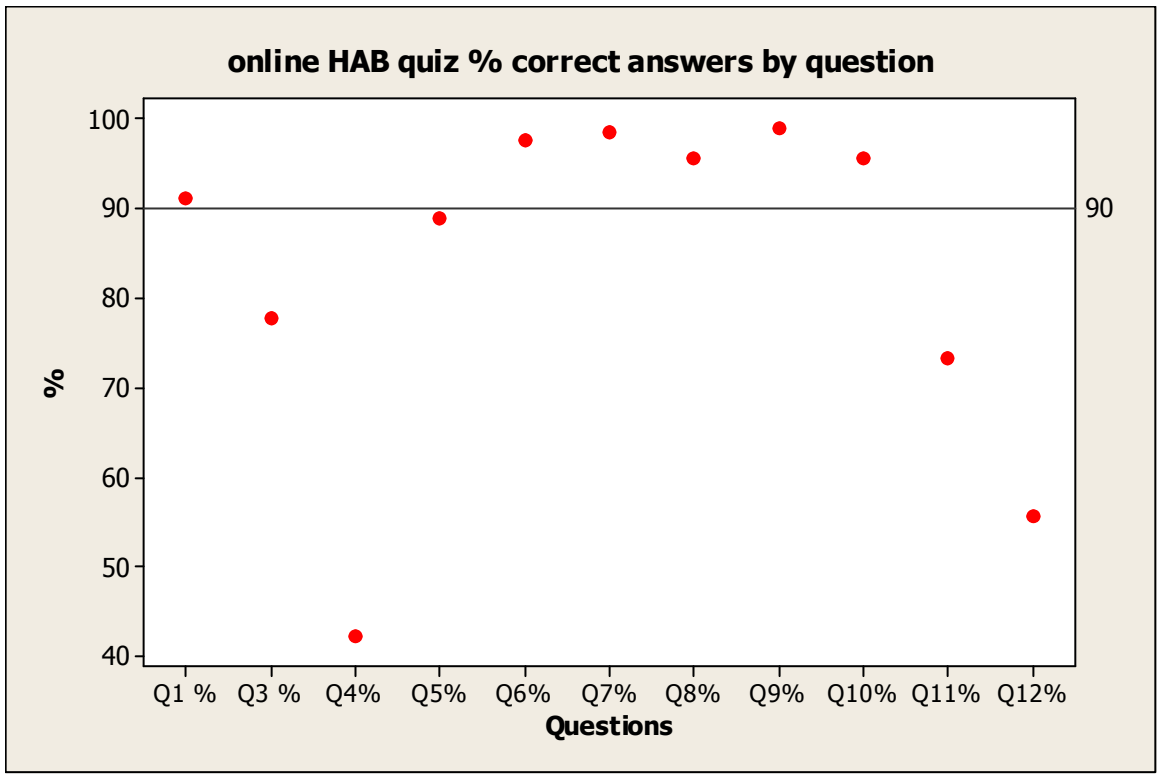


Figure 17: Box plot of % correct answers by question types

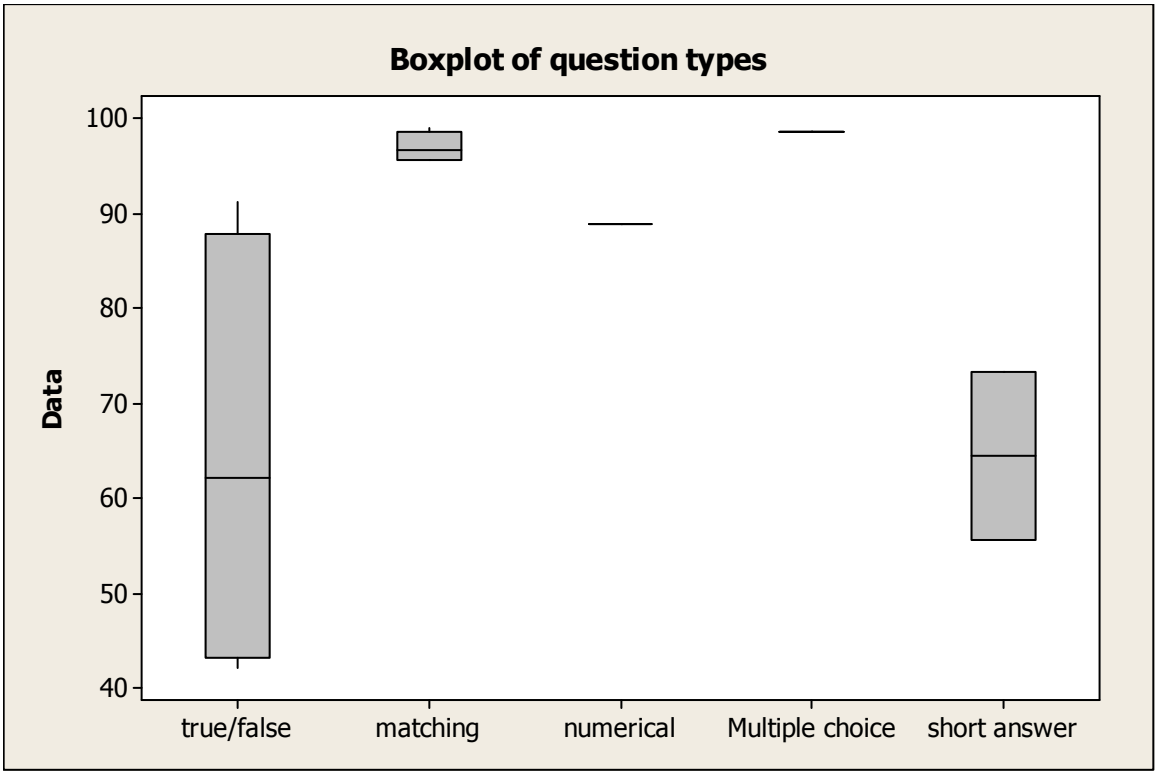


Table 8: Top scores online HAB quiz

Analyst code	Lab code	Grade	Analyst code	Lab code	Grade
11	6	100.0	3	18	81.8
1	11	100.0	41	7	81.8
34	11	100.0	5	19	81.8
2	20	100.0	44	8	81.4
51	16	100.0	24	23	81.2
10	14	100.0	52	15	81.2
9	1	100.0	4	15	81.2
38	27	100.0	22	6	80.5
45	21	99.6	17	2	73.9
54	29	99.6	47	5	72.7
49	17	99.4	50	20	72.7
20	9	95.5	48	7	72.7
12	5	90.9	28	21	72.7
26	24	90.9	53	28	72.7
32	13	90.9	46	20	72.3
37	2	90.3	29	2	67.1
30	17	89.7	33	2	66.7
19	4	86.4	14	2	65.5
16	5	84.4	31	12	63.6
7	5	81.8	43	7	61.8
25	5	81.8	36	25	59.1
15	5	81.8	42	10	53.2
23	14	81.8			

5. Conclusions

Regarding the enumeration part of the exercise, there were five species of interest which analysts had to count. The descriptive and summary statistics of the counts suggest that in most cases the data follows a normal distribution. This is the case for the *D.acuminata* count and also for the *L.polyedrum* and *C.monotis* counts once the outliers (data not shown) have been omitted. The *P.rotundatum* count doesn't follow a normal distribution and it is very much one tailed, a plausible reason for this is that at the limit of detection of the method, that is when densities are very low close to one cell, there is a chance that samples may not contain any cells and also that analysts are more likely to miss them. Also, because of the limited number of cells in the sample, it would be difficult to achieve a normal distribution and a one tailed distribution is therefore more likely. In the case of the *Karenia* cell count, the data shows more variability than in any of the other counts, it is possible that these cells do not preserve as well as armoured dinoflagellates do or that they tend to stick to the walls of the sterilin tubes and therefore the identification and counting upon preservation becomes more difficult. In this case, the data doesn't follow a normal distribution.

Even, with this lack of reproducibility between analysts, most laboratories and analysts are well within the two standard deviations of each other as shown in the I-charts and Z-scores. While, the charts of the *C.monotis* and *P.rotundatum* counts were used for data analysis, the z-scores weren't used for the final scores, in the case of *C.monotis* because it is not considered to be toxic and some analysts did not considered counting them for that reason and in the case of *P.rotundatum* because at the limit of detection we could not ascertain that all samples contained at least one cell and therefore it would be prejudicial to those analysts that did not see any cells in the samples.

Although some analysts had out of specification results for some counts, most analysts performed within the tolerances prescribed for the exercise for all counts. The biggest problems were caused by the *C.monotis* count where five laboratories failed to identify the species in the samples and another five laboratories identified the species incorrectly. The *Karenia* count showed that the species should only be identified to genus level rather than to species level as the results showed a division between laboratories about which species should be. This could also be said about the *C.monotis* identification, given that the genus includes some toxin producing species, a precautionary principle should be used on samples analysed using light microscopy by identifying to genus only rather than species level and also to count them, even if you consider them to be non toxic as it is very difficult to identify to species level under lugol's iodine preserved conditions and therefore separate them from toxin producing species.

The sample data returned also suggests that some laboratories where there are various analysts taking part in the exercise tend to have similar identification results, suggesting perhaps some level of conferring between analysts within the laboratory. This may be good when the identification and enumeration are correct but it can be a problem otherwise. It is better, that analysts try to do their own work at all times.

A qualitative reliability measure for the test method was also calculated to demonstrate how good analysts are at identifying correctly organisms. This measure is updated from year to year to give a frequency over time about the accuracy of the identifications. Also, this measure gives information about how particular laboratories and analysts perform and go about identifying particular species.

For example, this exercise has shown that the identification to species level of some of the organisms was influenced by the geographical area where the laboratory comes from. This geographical bias on species identification is becoming more apparent now that laboratories from other regions of the world have started joining in and participating in this intercomparison.

Also, there is a tendency to over-identify species and use the most commonly found or used species name, as was the case this year with *C.monotis* and *K.selliformis*. Many analysts decided to identify to species level based on light microscopy alone. Most analysts were correct in their identification but this was based more on an assumption generally that if it is *Coolia* will definitely be *C.monotis* and if it is *Karenia* then it should be *K.mikimotoi* rather than a more solid scrutiny of the sample through other techniques.

This reliability measure is also telling us that analysts are more likely to incur in false positive responses than false negatives responses, that is the ability of identifying a non-toxic organism correctly is 65% compared to the ability of correctly identifying a toxic organism (93%). This is good as the ability of identifying correctly toxic algae in samples is from a monitoring perspective the most important aspect of it. This means that the method is quite sensitive, but less specific at identifying everything else. The method is over 86% efficient, which is the ability to discriminate between toxic or non-toxic species.

The performance of the test is given by the Youden index (58%) which is a measure of how sensitive and specific the test method is overall. The likelihood ratio is the possibility of incurring on a false type response. This was calculated as 0.80, given that the ratio is high and positive we can conclude that analysts are more likely to incur on a false positive response than vice versa, this is, more likely to name a non-toxic species as toxic than the other way around.

Regarding the Ocean teacher online Hab quiz, the results have shown that analysts have a good theoretical knowledge on phytoplankton taxonomy with many analysts performing above the 80% mark.

This year, we were able to correlate one of the questions from the online quiz back to the samples. The answers to Question 4 on *C.monotis* on the quiz were used to compare against the identification on the samples, we found that 26 analysts thought that *C.monotis* was toxic (quiz answers) yet 8 of them did not identify the species in the samples and another 4 incorrectly identified the species.

The performance of the quiz was good generally and analysts tended to answer better matching and multiple choice questions (around 90% correct) than true/false (40 to 80%) or short answer ones (60-70%).

6. **Recommendations from workshop 2012**

- Homogeneity and stability test of samples to be carried out next year according to ISO 13528. This is to check that the samples to be used in the intercomparison exercise are adequately homogeneous and stable.

- Formation of an expert group to critically review the design of the test, materials and quiz. The expert group will consist of scientists working in phytoplankton monitoring programmes and have large experience of phytoplankton taxonomy counting and protocols. Their main purpose would be to check that the test is fit for purpose and of the desired standard prior to its publication.

- 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. Our aim is to apply for accreditation in 2013 with the view to be accredited in 2014.

- The Level of difficulty of the test is generally thought to be of a good standard, there is a difference of opinion as to whether the test should be more or less challenging. There is a possibility for the online quiz to be built to different skill levels allowing new entrants to choose their skill level.

- Review pricing of the scheme

- Workshop attendance was good. Participants thought that it was worthwhile to have a longer workshop 2-3 days to allow for training sessions using microscopes and samples. Participants would like to bring samples to the workshop from their geographical areas to be analysed and discussed. This workshop could be used to update taxonomists of any new advances and developments on phytoplankton monitoring.

- Participants could use the workshop to present some of the work they carry out in their laboratories in relation to routine monitoring of phytoplankton, unusual blooms or events that could be of interest to the others.

- To discuss further how to develop and certify reference materials for phytoplankton intercomparisons.

ANNEX 1: Form 1 return slip and checklist

Bequalm Intercomparison PHY-ICN-12-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 _____	YES	NO
Set of Instructions	YES	NO
Enumeration and identification result log sheet **(Form 2)**	YES	NO

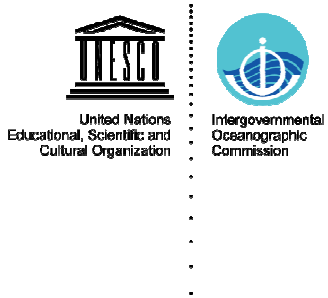
I confirm that I have received the items, as detailed above.

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

SIGNED: _____

DATE: _____

ANNEX 2: Form 2 Enumeration and identification results log sheet



Bequalm Intercomparison PHY-ICN-12-MI1

FORM 2: ENUMERATION AND IDENTIFICATION RESULTS LOGSHEET

Analyst Name:	
Laboratory Code:	
Analyst Code :	

	Organism	Cell	Multiplication	Number
Sample No:				
Settlement date:				
Analysis date:				
Volume Chamber (ml):				

	Organism	Cell	Multiplication	Number
Sample No:				
Settlement date:				
Analysis date:				
Volume Chamber (ml):				

	Organism	Cell	Multiplication	Number
Sample No:				
Settlement date:				
Analysis date:				
Volume Chamber (ml):				

Form 2 Enumeration and identification results log sheet

ANNEX 3: Test instructions

Marine Institute- IOC- BEQUALM-NMBAQC Phytoplankton Proficiency Test PHY-ICN-12-MI1

Instructions for Sample Preparation, Cell counting, calculations & Identification

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

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

1. Introduction

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

The purpose of this exercise is to compare the performance of laboratories engaged in national official/non-official phytoplankton monitoring programmes and other laboratories working in the area of 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 Phytoplankton Ring Test is being conducted to determine the variability within and between laboratories in the abundance and composition of marine phytoplankton species from a field sample which has been spiked with phytoplankton cultured material.

Analysts are asked to identify and count all the toxic and harmful organisms found in the samples. Each analyst will receive an envelope containing four samples in 30ml sterilin tubes preserved in lugol's iodine. Three of the samples will be analysed as part of the exercise and one sample is sent as a spare.

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

2. Preliminary checks and deadlines

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). 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 until the 31st of August 2012 to complete the exercise and return the results to Rafael Salas, Marine Institute, Phytoplankton laboratory, Rinville, Oranmore, Co. Galway, Ireland by post or e-mail (Rafael.salas@marine.ie). The enumeration and identification results log sheet (Form 2) **must be received** by the Marine Institute by **August 31st , 2012**.

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.

3. 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.

4. Equipment

The following are the equipment requirements to complete this exercise:

Sedimentation chambers (25ml volume if possible). 3 X fully assembled

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

5. Sedimentation chambers and sample preparation

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

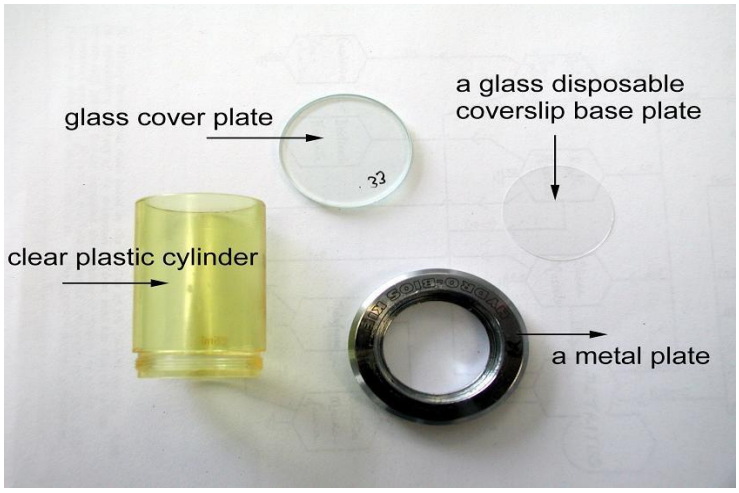


Fig 1: Sedimentation counting chamber

5.1 All sedimentation chambers should be cleaned before start

5.2 Place a 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 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 on **Form 2 enumeration and identification results log sheet.**

5.9 If using a different method to the Utermöhl test method, please send the Standard Operating Procedure for your method with your results. Explain briefly how it works and how samples are homogenized, set up, analysed, counted and how you calculate the final concentration.

6. Counting strategy

Each analyst should carry out a whole chamber cell count (WC) of all the toxic/harmful species identified in the samples where possible. Other counting strategies can also be used where the cell density in the sample is high.

7. Samples

Analysts will have to analyse 3 samples to complete this test. These have been made up from a field sample to which culture material has been added with a number of toxic/harmful species. The cultures come from the Marine Institute Phytoplankton culture collection, and the IOC Science and communication centre for Harmful Algae culture collection in Denmark. All the materials have been preserved using neutral lugol's iodine and then homogenized following the IOC Manual on Harmful Marine Algae technique of 100 times sample inversion to extract sub-samples.

Each analyst must count and identify **all known toxic and harmful phytoplankton species** found in the samples. There is no need to identify or count non-toxic, non-harmful species for this intercomparison.

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.

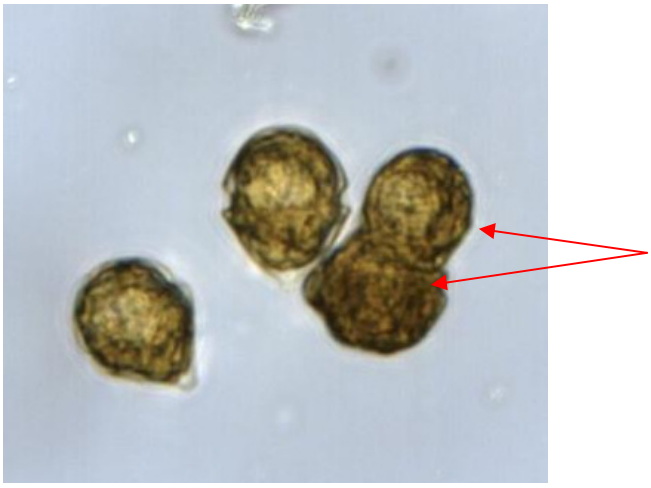


Figure 1: Two Cells fusing

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

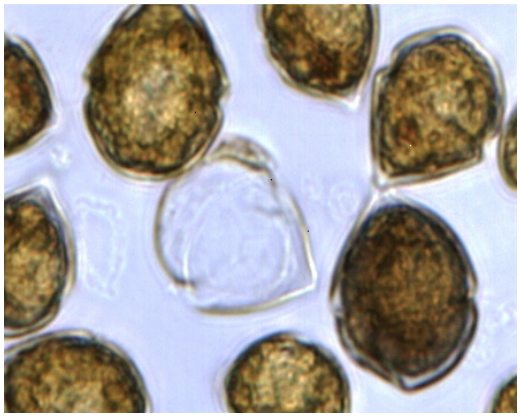


Figure 2: Empty theca

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.

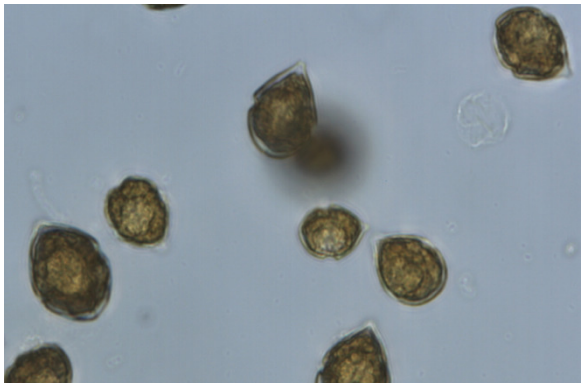


Figure 3: Big versus small cells

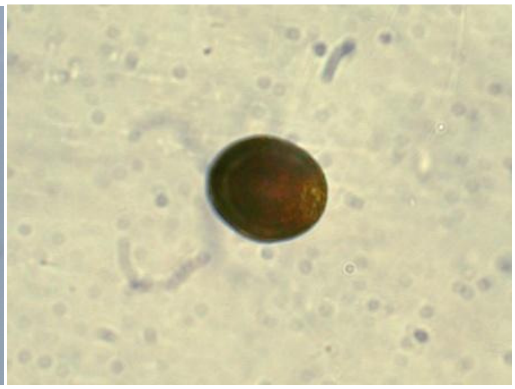


Figure 4: Plasmolised cell

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 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) Identify to the highest taxonomic level possible all toxic/harmful species in the samples

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

These rules are only applicable to this intercomparison exercise.

8. Conversion calculations of cell counts

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

9. Identification

A HAB taxonomic quiz will be developed in the web platform 'Ocean teacher' and it should be ready by September 2012. Participants will be given a username and password to be able to access this facility and complete this part of the test. More information and instructions to complete this part of the exercise will be sent separately.

10. Points to remember

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

2. **If you are sending your results by post, make sure you make a copy before you send them.**

3. Form 2: Enumeration and identification results log sheet, must be received by the Marine Institute, Phytoplankton unit by **August, 31st ,2012.**

ANNEX 4: Workshop agenda

Agenda Bequalm Phytoplankton Intercomparison workshop

Hillerød, Denmark 2-4 December 2012.

Sunday, 2 Dec 2012

Arrival of participants in the afternoon. Sunday dinner at 18:00pm

Monday, 3 Dec 2012

Breakfast 8:00 am

Morning session:

Intercomparison exercise results (RSalas)

Enumeration and identification exercise results.

Ocean teacher online HABs quiz exercise results.

Lunch 12:00-13:30 pm

Afternoon session:

Discussion of exercise and ideas for 2013 (All)

Lecture and microscope demonstration of the Karenia group (J.Larsen)

Presentation on Azadinium genera (R.Salas)

Discussion

Dinner 18:00pm

Tuesday, 4 Dec 2012

Breakfast 08:00 am

Morning session:

Lecture and microscope demonstration of the Diplopsalis group (J.Larsen) and microscopic demonstration using fluorescence microscopy and oil immersion of mixed samples focusing on toxic and potentially toxic species with reference to the IOC taxonomic reference list. (J.Larsen)

Lunch 13:00 pm

Afternoon session: Departure of participants

ANNEX 5: Participating Laboratories

#	Company Name	Address
1	School of Biology University of Thessaloniki, Greece	Thessaloniki, GR-54124 Greece
2	SAMS Research Services, Scotland	Toxic Phytoplankton Monitoring Programme, Scottish Marine Institute Oban, Argyll, Scotland
3	CEFAS, Lowestoft	Lowestoft Laboratory, Pakefield Road, Lowestoft Suffolk, NR33 OHT UK
4	Koeman En Bijkerk bv	Ecological Consultancy and Research Postbox 111 9750AC Haren, The Netherlands
5	Agri Food and Biosciences Institute	Newforge Lane, Belfast BT9 5PX, Northern Ireland,
6	Isle of Man Government Laboratory	Dept of Environment, Food and Agriculture, Ballakermeen Road, Douglas, Isle of Man, IM1 4BR
7	IRTA, Spain	Ctra. de Poble Nou, Km 5,5 E-43540 Sant Carles de la Ràpita (Tarragona) Spain
8	CEFAS, Weymouth	The Nothe, Barrack Road, Weymouth, Dorset DT4 8UB, UK
9	Center De Balear De Biologia Aplicada (CBBA, Spain)	18, Llucmajor St., Palma de mallorca, Mallorca, Spain 07006
10	Marine Scotland	375 Victoria Road, Aberdeen AB11 9DB, UK
11	Laboratorio De Control De Calidad De Los Recursos Pesqueros	Carretera Punta Umbría, 21459, Cartaya (Huelva), Spain
12	Certificaciones Del Peru S.A	Av. Santa Rosa 601, La Perla, Callao, Peru
13	Scottish EPA	Clearwater House, Heriot-Watt Research Park, Edinburgh, EH14 4AP, Scotland
14	Irish EPA	John Moore Road, Castlebar, Co. Mayo
15	WEAQ AB	Doktorsgatan 9 d, SE-26252 Angelholm, Sweden
16	Corben LTD	Loch Melfort, Arduaine, Argyll, PA34 4XQ, UK
17	Institut de Ciències del Mar -CSIC	c/ Passeig Marítim de la Barceloneta, 37-49. E-08003 Barcelona, Spain
18	Sir Alister Hardy Foundation for Ocean Science (SAHFOS)	The Laboratory, Citadel Hill, Plymouth, PL1 2PB, UK
19	Instituto de Fomento Pesquero	Balmaceda Chile, # 252 Puerto Montt. Chile
20	IMARES	Korringaweg 5, PO Box 77, 4400 AB Yerseke, The Netherlands
21	Microalgal Services Australia	308 Tucker Rd, Ormond VIC 3204, Australia
22	APEM Limited	Riverview A17 Embankment Business Park, Heaton Mersey, Stockport, SK4 3GN UK
23	Swedish Meteorological and Hydrological Institute	Sven Källfelts gata 15 , SE - 426 71 Västra Frölunda, Sweden
24	Complete Laboratory Solutions (CLS)	Saotharlann Chonamara Teo, Ros Muc, Co. Galway, Ireland.
25	Marine Institute Bantry laboratory	Gortalassa, Bantry, Ireland
26	Marine Instiute Galway laboratory	Rinville, Oranmore, Galway, Ireland
27	Institut National des sciences et Technologies de la Mer	Centre de Sfax- Tunisie
28	INRH-Laboratoire des Effllorescences Nuisibles	2 rue de Tiznit, INRH-Casablanca, Morocco
29	INTECMAR	Peirao de Vilaxoán s/n. Vilagarcía de Arousa, Pontevedra, 36611, Galicia, Spain

ANNEX 6: 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 2012

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

Statement Issued:
Statement Number: MI-BQM-12-

Summary of results:

Component Name	Subcontracted	Results		identification
		Z-score (+/- 2 Sigma limits)		
Phytoplankton abundance and composition PHY-ICN-12-MI1	Marine Institute	*Lingulodinium polyedrum*		
		Karenia selliformis		
		Dinophysis acuminata		
Overall Result Taxonomic quiz (Pass Mark 70%, over 90% proficient)				
Phytoplankton Taxonomy quiz PHY-ICN-12-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
PhytoplanktonEnumeration 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 spiked material and give a result to within ±2SD or sigma limits of the true value. The true value is the mean/median calculated from a sample population of the total by the participating laboratories 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 identification exercise	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 7: Ocean Teacher HAB Quiz

Question 1

Not yet answered

Marked out of 1.00

Edit question

Are all benthic diatoms raphid? True or False

Select one:

- True
- False

Question 2

Not yet answered

Marked out of 1.00

Edit question

Are *Pseudo-nitzschia* and *Nitzschia*, the only diatom genera that produces ASP toxins? True or False

Select one:

- True
- False

Question 3

Not yet answered

Marked out of 1.00

Edit question

Are all centric diatoms planktonic? True or False

Select one:

- True
- False

Question 4

Not yet answered

Marked out of 1.00

Edit question

Is *Coolia monotis* a toxic algae? True or False

Select one:

- True
- False

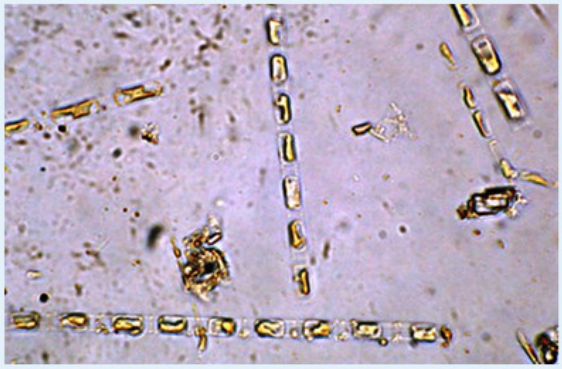
Question 5

Not yet answered

Marked out of 1.00

Edit question

How many cells of the diatom *Skeletonema* sp. can you count in this picture? Indicate number (numeric value) text not allowed.



Answer:

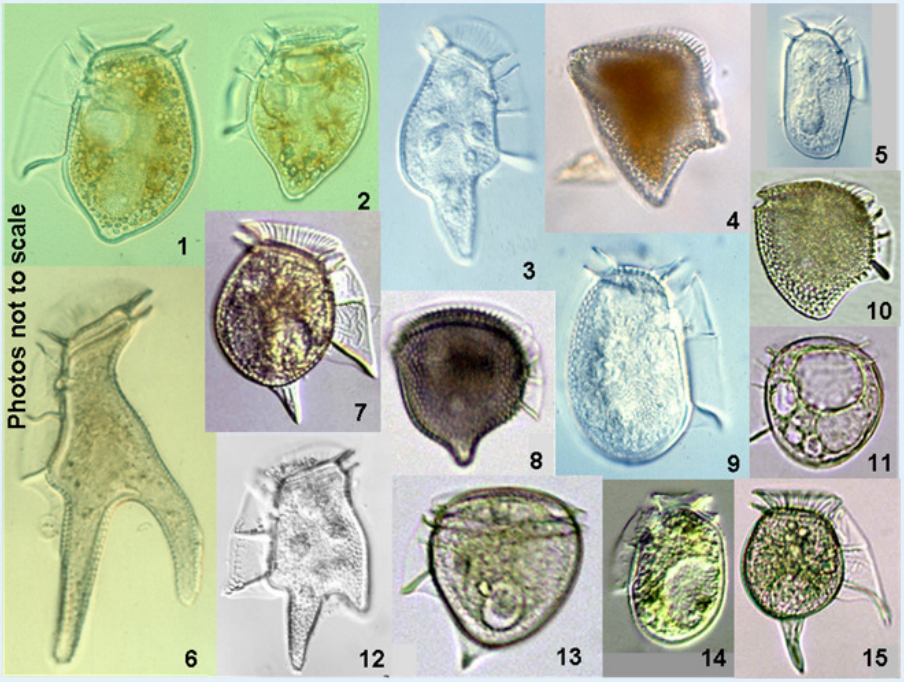
Question 6

Partially correct

Mark 0.73 out of 1.00

Edit question

Several species of *Dinophysis* and *Phalacroma* may cause diarrheic shellfish poisoning (DSP). The photos below (not to scale) show 15 different species of *Dinophysis/Phalacroma*. Identify these species.



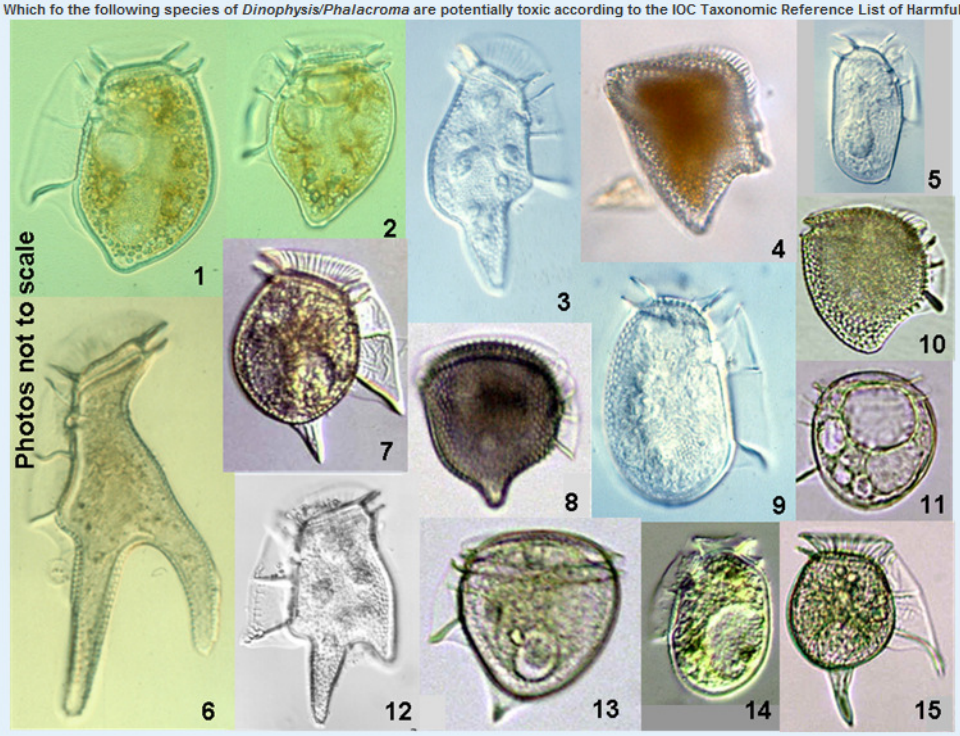
Question 7

Partially correct

Mark 0.75 out of 1.00

Edit question

Which fo the following species of *Dinophysis/Phalacroma* are potentially toxic according to the IOC Taxonomic Reference List of Harmful Microalgae.



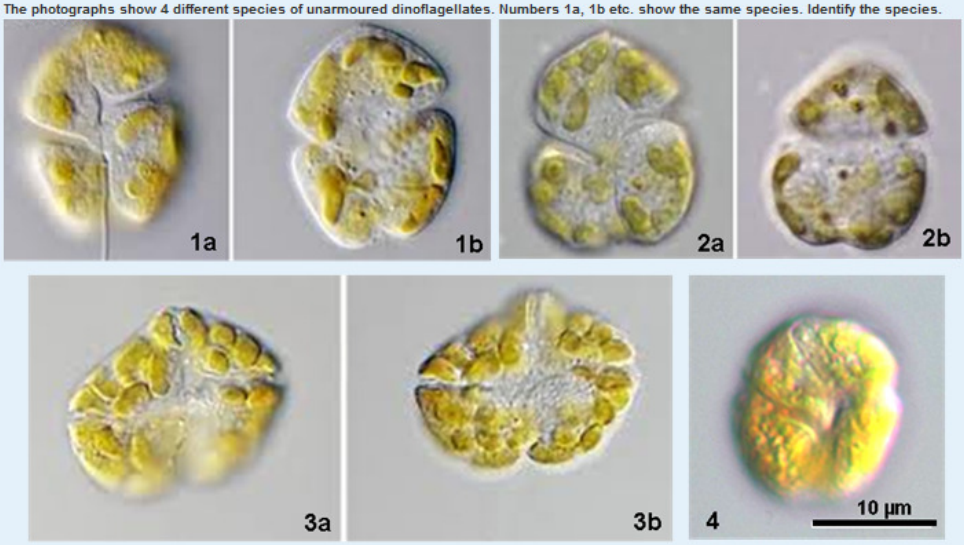
Question 8

Partially correct

Mark 0.50 out of 1.00

Edit question

The photographs show 4 different species of unarmoured dinoflagellates. Numbers 1a, 1b etc. show the same species. Identify the species.



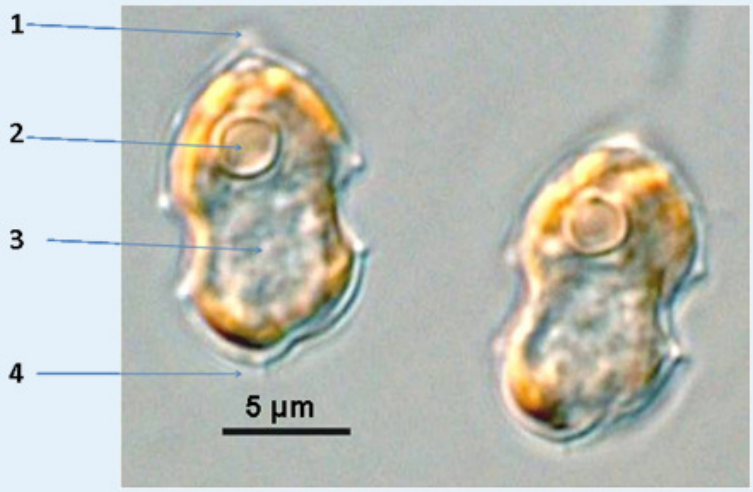
Question 9

Not yet answered

Marked out of 1.00

Edit question

These cells belong to the genus *Azadinium*. Indicate the morphological features (1-4) pointed to by the arrows.



Arrow 4 Choose...

Arrow 1 Choose...

Arrow 2 Choose...

Arrow 3 Choose...

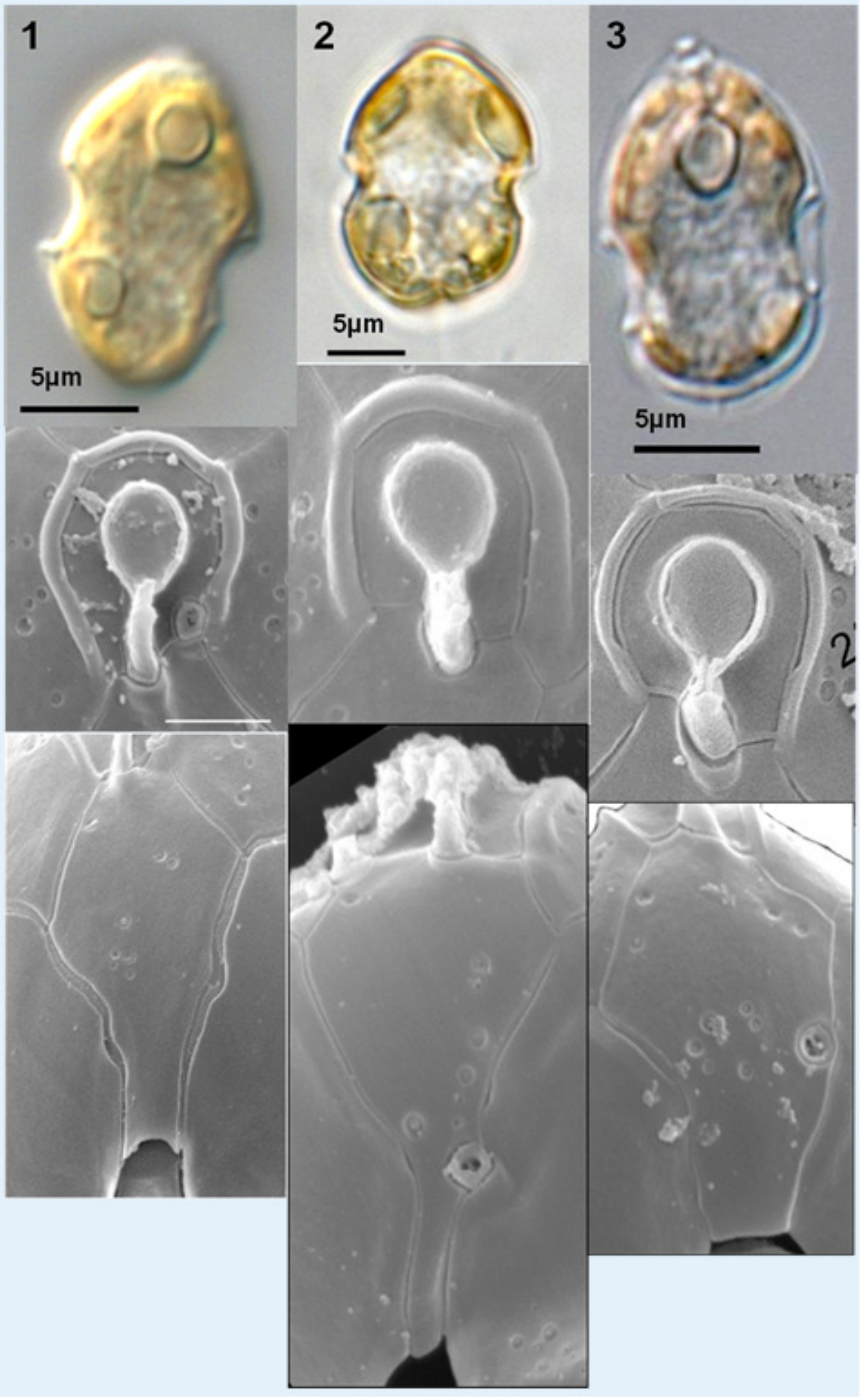
Question 10

Not yet answered

Marked out of 1.00

Edit question

Based on these light microscopy and SEM images of the APC and 1' apical plate details of these armoured dinoflagellates. Identify these three cells of Azadinium to species level?



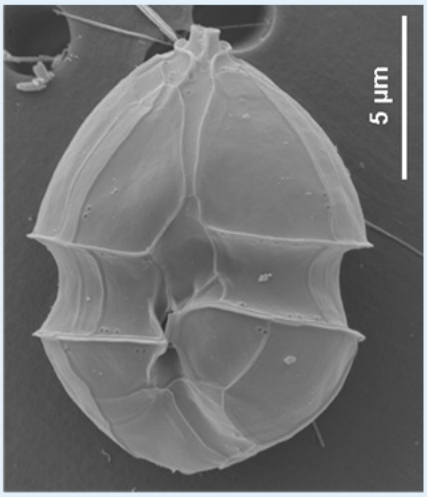
Question **11**

Not yet answered

Marked out of 1.00

Edit question

The kofoidean tabulation of this armoured dinoflagellate is Po, cp, x, 6', 0a, 6'', 6c, 5s, 6''', 2''''. Identify this dinoflagellate to genus.



Answer:

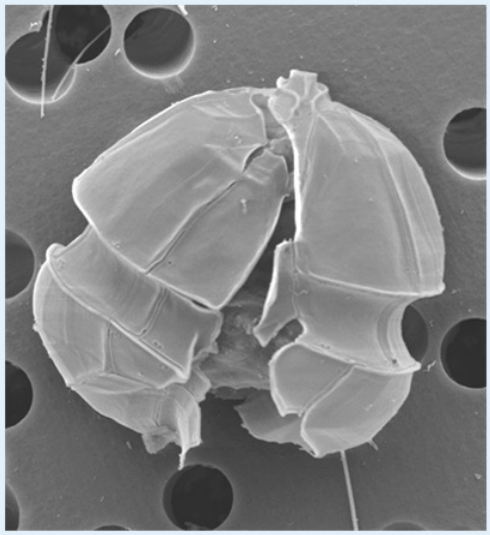
Question **12**

Not yet answered

Marked out of 1.00

Edit question

This armoured dinoflagellate is undergoing binary fission. The theca is shared between the daughter cells. Do you know what's the name of this type of division in armoured dinoflagellates?



Answer: