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 2011 report. PHY-ICN-11-MI1 VR 2.0

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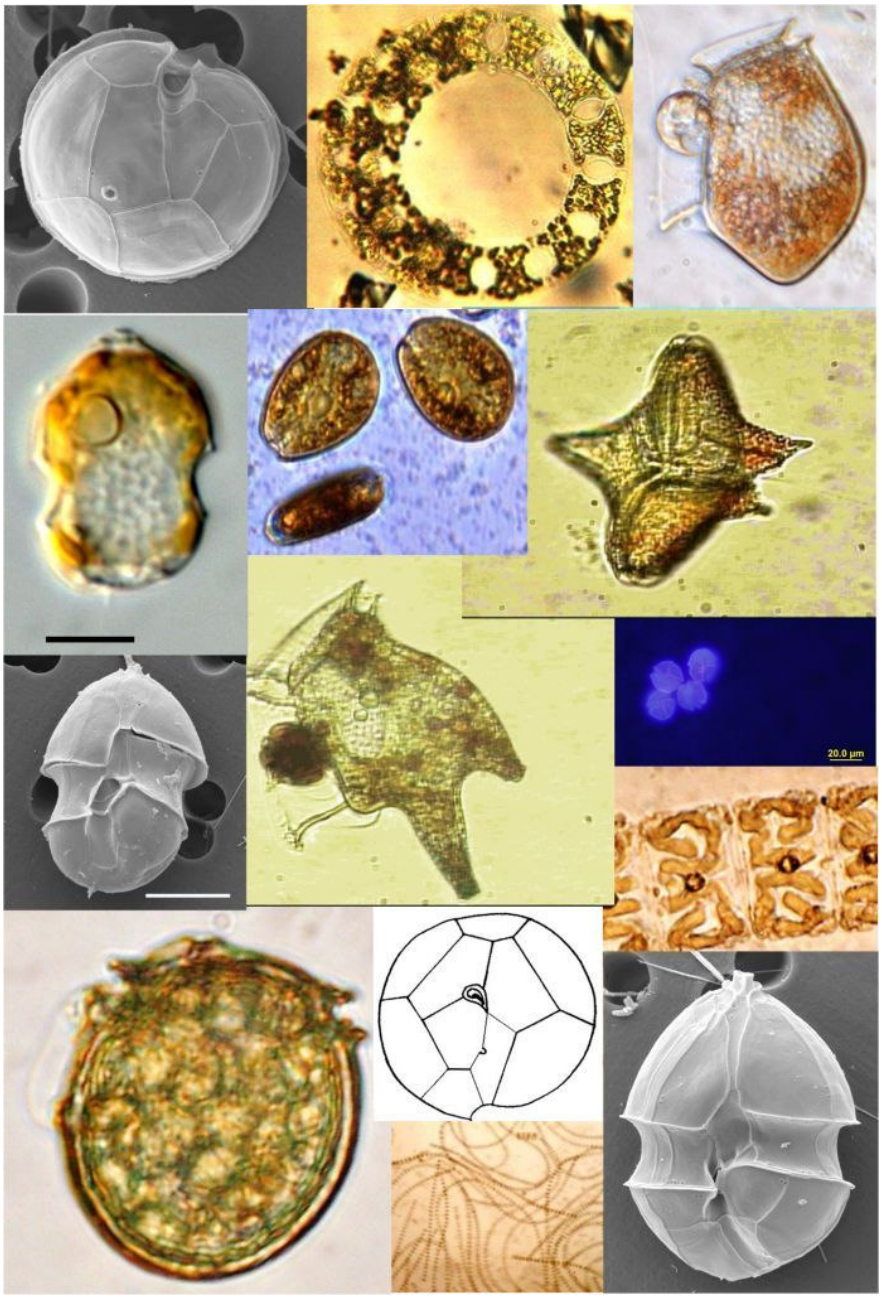

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1. Summary of results

- 30 analysts from 20 laboratories across Europe returned results. There is a lack of reproducibility between laboratories and reference values: If the reference values were validated, most laboratories would be outside the accepted variance of 2 Standard deviations of the mean.

- The descriptive statistics suggests, the data don't follow a normal distribution for most counts, despite this, most Individual charts and Z-scores suggest most analysts perform within the 2 standard deviation of the mean of the other analyst's results.

- Six organisms were preserved and spiked in the samples. Three toxic species and three non-toxic species. Analysts were better overall at identifying the toxic from the non-toxic species.

- Three of the species were large in size (*A.sanguinea, G.pacificus, P.*lima) compared to the other three which were smaller in size (*A.minutum, S.trochoidea, H.minima*). Analysts performed better at identifying the larger species from the smaller ones.

- *Heterocapsa minima* were the most difficult organism to identify: 13 analysts did not find this species in the sample. 4 analysts misidentified it, three of which named it as *Azadinium*.

- *Akashiwo sanguinea* and *Prorocentrum lima* were the easiest organisms to identify. All analysts recorded these species correctly. *Gambierdiscus pacificus* was easy to identify to genus level but most analysts (15 in total) thought it was the species *G.toxicus*.

- *G.pacificus* was not identified by four analysts. These analysts came from laboratories which don't find these species in their waters.

- A reliability qualitative measure calculated for the method indicates that the method is more sensitive (91%) than specific (76%) and its efficiency based in the data is 83%. The false positive rate is higher (29%) than the false negative rates (8%) indicating that we are more likely to mis-identify a non-toxic species than the other way around.

- Most analysts performed above the 90% mark for the 'Ocean Teacher' Bequalm Hab quiz exercise. Questions 4,7,8,9 and 10 were perfectly answered by all analysts. Q12 was the worst answered question. This was the question on the diatom taxonomy of the genus *Pseudo-nitzschia spp.*

2. Introduction

The Phytoplankton Bequalm intercomparison study in 2011 was designed to test the ability of analysts to identify and enumerate correctly marine phytoplankton species in spiked preserved water samples using culture material.

This study was designed to study size range bias; large versus small size of organisms, toxic versus non-toxic, reproducibility between and within laboratories, Z-scores of analysts against the mean and 2 Standard deviation of all the analysts results, a comparison between analysts results and non validated reference values set up by the organizing laboratory and false positive/negative rates for the test method as a reliability measure of how good the test method is at the composition of a sample.

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 involved the use of algal cultures from the Scandinavian Culture Collection of Algae and Protozoa in Copenhagen and also included the elaboration of a marine phytoplankton taxonomy quiz using an online platform called 'Ocean Teacher'. This Hab quiz was designed by Jacob Larsen (IOC).

This year, 34 analysts from 20 laboratories across Europe took part in this exercise. It is the first year we had participants from Greece. There are now three countries in the Mediterranean area taking part in this intercomparison; this includes laboratories from Spain (3), Croatia (1) and Greece (1). In the Atlantic area of influence, there are 9 laboratories across the UK, 2 in the Netherlands, 2 in Spain and 2 in Ireland.

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-11-MI1. PHY standing for phytoplankton, ICN for intercomparison,

11 refers to the year 2011, 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 2011.

3. Materials and Methods

Analysts were instructed where possible to use the light microscopy technique of Utermöhl (Utermöhl 1931, 1958) for the analysis of water samples for abundance and composition of marine phytoplankton. Samples were sent to the analysts in 30ml plastic sterilin tubes, these were preserved using neutral Lugol's iodine solution. A 25ml volume was set as the preferred analysis volume but other sub-sample volumes were allowed (see instructions: Annex 3).

Analysts were given four weeks from sample receipt to analyse and return the results to the Marine Institute phytoplankton laboratory.

3.1 Culture material selection

The organisms spiked in the samples were sourced from the Marine Institute culture collection (CCMI) in Ireland and the Scandinavian Culture Collection of Algae and Protozoa (SCCAP) in Denmark.

Six organisms were selected from a wider group of organisms for the exercise. Each culture was assessed for suitability in respect of shape, size, concentration and culture condition. The selected species for this exercise were: *Akashiwo sanguinea* (Hirasaka) Hansen & Moestrup, *Gambierdiscus pacificus* (Chinain & Faust), *Prorocentrum lima* (Erhenberg), *Alexandrium minutum* (Halim), *Scrippsiella trochoidea* (Stein) Balech & Loeblich III and *Heterocapsa minima* (Pomroy).

This list was decided to study size ranges: small organisms (*A.minutum*, *S. trochoidea*, *H.minima*) and large organisms (*G.pacificus, A.sanguinea, P.lima*) and the ability to produce marine biotoxins: toxic species (*G.pacificus, P.lima* and *A.minutum*) from non-toxic species (*S.trochoidea, A.sanguinea* and *H.minima*). The designed is balanced with three species of each.

3.2 Cell concentrations

The cell concentration of the organisms was not considered critical for this particular study. Generally the cell concentrations were low and ranged from approximately 500 cells for *A.sanguinea* to 20 cells for *H.minima* in each 30ml sample. The idea was that all analysts would use the same counting strategy, in this case a whole chamber count for all species to avoid variability due to different counting methods. Preliminary cell counts to establish the cell concentration of each species was carried out using a Sedgewick-Rafter (Pyser-SGI, Kent, UK) cell counting chamber 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 the analysts. Each analyst would have to analyse three samples randomly selected from the sample population.

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^{TM}, Kent, UK), autoclaved (Systec V100, Wettenberg , Germany) and preserved using Lugol's iodine solution (Clin-tech, Dublin, Ireland).

A master mix of each organism was prepared separately using 500ml screw top Schott glass bottles (Duran®, Mainz, Germany), containing the filtered seawater. These 500ml bottles were considered to be the best to aliquot 200 x 1ml samples in total, thus avoiding concentration effects due to the master mix volume being too small or too large, If the volume is too small, quenching effects occur and if it is too large, manual homogenization of the mixture becomes an issue. Each bottle 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 30ml plastic sterilin tubes (Sardstedt, Nümbrech, Germany).

The final volume of each sample was 30 ml approximately, which gives enough volume to fill a 25ml sedimentation chamber to the top. As the samples would contain a 1ml aliquot of each of the 6 organisms, the volume of sterile filtered seawater aliquot was 24ml + 1ml x 6 of each species, to make the 30ml sample. The 24ml of filtered seawater were measured 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, so approximately 24.6g were equivalent to 24ml volume.

200 samples were produced for this study. 102 samples were sent to 34 analysts at 3 samples per analyst, and a further 15 samples were used to obtain reference values by the organizing laboratory. So, 117 samples of the total sample population were analysed.

3.5 Sample randomization

All the samples were allocated randomly to the participants with Minitab statistical software vr15.0 randomization tool.

3.6 Bequalm HAB quiz

This year the HAB quiz was organized and set up by Jacob Larsen (IOC UNESCO, Centre for Science and Communication on Harmful Algae) in Denmark.

The exercise was uploaded into a web platform called 'Ocean teacher' that participants have to access in order to participate. The analysts had to go to the following web address http://classroom.oceanteacher.org/ scroll down and login. This login process allows the website to gather the required information about the participant and it goes through a typical registration protocol, where you sign in with a username and password. These can be used later to access the test and attempt it. 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 and quiz content from here once they were given permission by the manager of the course (Jacob Larsen).

The test itself consisted of 13 questions (see Annex 7). There were three questions related to nanoflagellates, seven questions on armoured dinoflagellates, four of those based on

Alexandrium, two questions on diatoms and one general question on biotoxin symptoms in humans. Most questions had either a drop down menu where you choose from a list or you had to choose one of a number of options by ticking a box. One question was open ended and needed text input from the analysts. This was the first time to use this platform for the Bequalm exercise.

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 of samples, samples in working order 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 Europe, in order for all the laboratories to have the same arrival time.

3.8 Statistical analysis

Statistical analysis was carried out on Minitab vr15.0 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 and 2 standard deviations of the analysts' results and also against a set of reference values set by the organizing laboratory. A whiskers and box plot was used to compare the data set between the laboratories and the organizing laboratory.

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

The sample composition 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 *P.lima, G.pacificus and A.minutum* were considered to be true positives, the correct identification of *A.sanguinea, S.trochoidea and*

H.minima, 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

30 analysts from 20 laboratories returned enumeration results on three replicate samples containing seawater spiked with 6 cultured organisms.
The mean of the three replicates for each analyst has been used to generate z-scores, I charts and descriptive statistics of the data. The data was manipulated using Microsoft Excel 7 and the graphs were generated using Minitab vr.15.0 and Excel also.

4.1.2 reference data.

The organizing laboratory had generated reference data values for each species from 15 replicate counts. This data has been used to compare values with the analysts but have not been used to generate z-scores or I-charts. It has only been used for comparative purposes only.

4.1.3 Analysts results

4.1.3.1 Descriptive statistics

Table 1 shows the mean results returned by the analysts for this intercomparison. The * symbol signifies that no counts were returned by an analyst in a particular species, this has been treated statistically as a non-result, and ‘nr’ means ‘no results received’. 30 analysts out of 33 that took part in the intercomparison returned results to the organizing laboratory. The results are expressed in cells per litre.

The descriptive statistics of the mean results for each organism were represented using the Anderson-Darling normality plot. This test doesn’t assume that the data is normal. The P-value reported is the probability of the data being normally distributed. If the P-value is less than 0.05,

then the data is not normally distributed. Figures 1 to 6 are an example of the type of summary statistics that can be drawn from the data. The output contains several important descriptive statistics of the data.

Table 2: Analysts results

ANALYST CODE	LAB CODE	SAMPLE CODES			Average of 3 samples in Cells/L					
					A.sanguinea	G.pacificus	P.lima	A.minutum	S.trochoidea	H.minima
31	C	189	4	2	7853	2800	1120	2933	4773	*
20		57	77	95	9907	1933	1413	*	*	7453
27		166	146	135	nr	nr	nr	nr	nr	nr
32	G	97	47	219	nr	nr	nr	nr	nr	nr
17		209	91	155	8000	2543	1067	3066	2567	*
33		132	202	190	8591	2276	1310	2653	2120	*
5		13	246	39	7481	2575	1431	2253	3085	*
7	R	235	139	9	4773	1960	1027	2773	3427	507
30		5	78	239	5480	*	1147	5453	3733	*
11	B	1	170	46	9413	3027	1453	2760	4320	920
8	U	23	96	164	7394	1848	1152	*	3576	*
13	H	208	244	75	nr	nr	nr	nr	nr	nr
23	M	206	194	150	10747	2800	2173	1720	2493	93
22		188	201	242	8013	1987	1307	1893	1693	54
2		176	73	142	9467	2053	1253	827	4533	187
19	I	20	76	38	6947	2033	1127	1513	1733	*
10		25	151	83	7400	2333	1467	1987	3093	147
28	L	19	213	8	7903	*	1293	293	2267	293
25	P	168	179	51	7080	3160	1147	2721	4452	989
16	N	43	105	222	7653	2720	1413	*	2027	3360
24		17	40	127	12093	2400	1400	1907	4867	*
4		196	108	159	8600	4680	1067	1533	2800	53
21		173	61	175	11800	2040	1387	2747	2627	253
18	D	240	156	102	11333	3240	1693	7507	*	440
26	K	107	130	49	9507	1880	1160	3160	4547	1200
15		199	211	41	8120	2107	1240	3053	2800	653
12	O	29	121	42	10667	3167	1500	1167	4667	*
3	E	215	134	119	6650	*	1917	3117	2683	*
29	A	245	145	24	5929	3095	1462	2744	4387	510
1	F	243	36	174	3560	2507	800	1227	4120	520
9	S	11	60	147	8547	2440	1347	2693	4320	267
6	Q	68	163	120	6320	1760	1307	1040	3093	*
14	T	157	70	204	7867	3187	1400	1947	3160	*

nr= no results

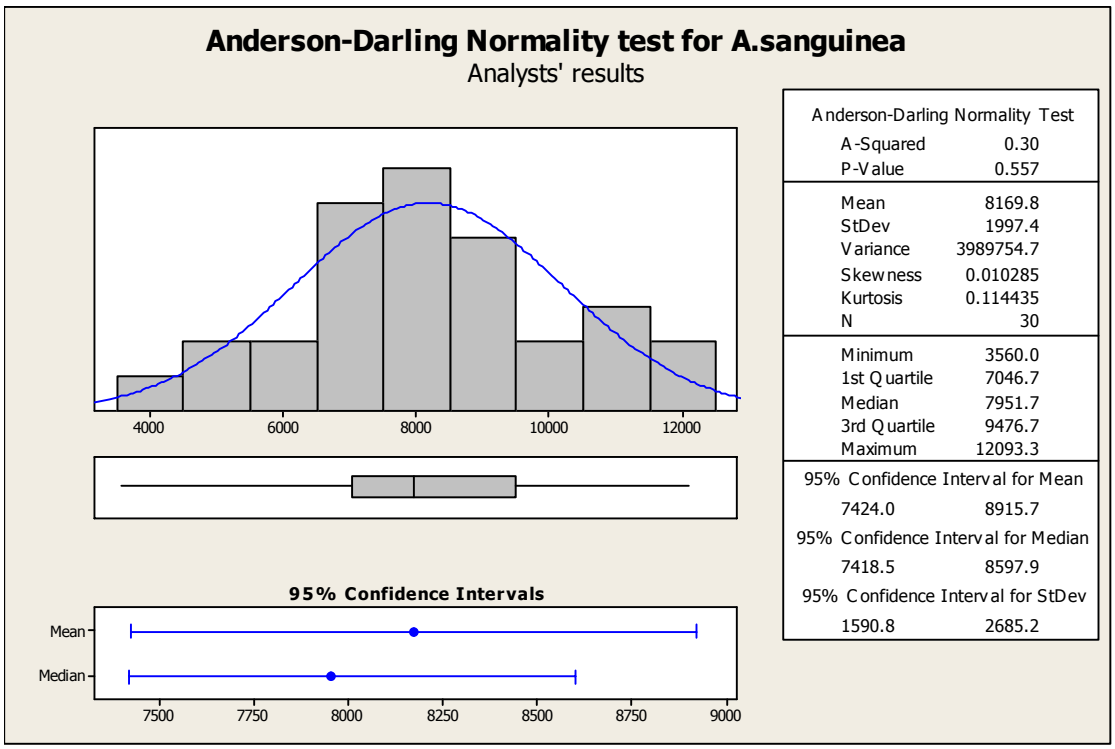
*= no cell count returned for particular organisms.

(Figures 1 to 6)

 # Please note: Analysts 23, 21, 1 and 9 identified incorrectly H.minima but the cell count stand for enumeration purposes.

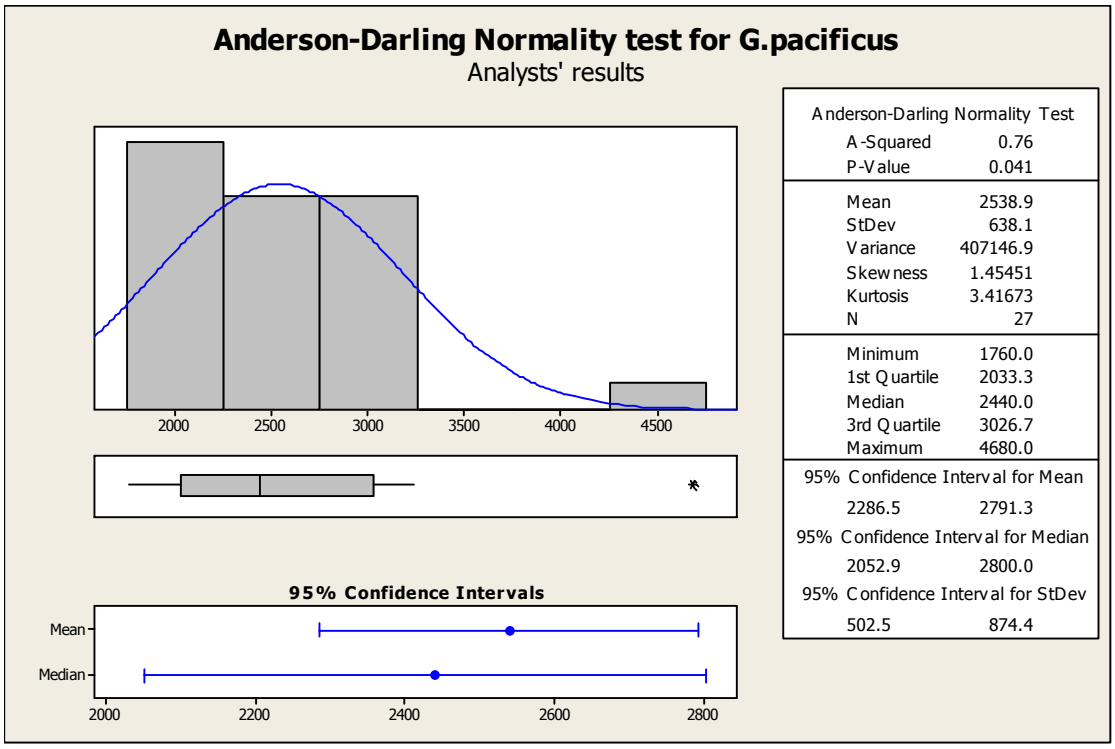
Figure 1 to 6: Anderson-Darling normality test (Analysts' results) for *A.sanguinea, G.pacificus, P.lima, A.minutum, S.trochoidea* and *H.minima*.

Figure 1



Anderson-Darling Normality Test	
A-Squared	0.30
P-Value	0.557
Mean	8169.8
StDev	1997.4
Variance	3989754.7
Skewness	0.010285
Kurtosis	0.114435
N	30
Minimum	3560.0
1st Quartile	7046.7
Median	7951.7
3rd Quartile	9476.7
Maximum	12093.3
95% Confidence Interval for Mean	
7424.0	8915.7
95% Confidence Interval for Median	
7418.5	8597.9
95% Confidence Interval for StDev	
1590.8	2685.2

Figure 2



Anderson-Darling Normality Test	
A-Squared	0.76
P-Value	0.041
Mean	2538.9
StDev	638.1
Variance	407146.9
Skewness	1.45451
Kurtosis	3.41673
N	27
Minimum	1760.0
1st Quartile	2033.3
Median	2440.0
3rd Quartile	3026.7
Maximum	4680.0
95% Confidence Interval for Mean	
2286.5	2791.3
95% Confidence Interval for Median	
2052.9	2800.0
95% Confidence Interval for StDev	
502.5	874.4

Figure 3

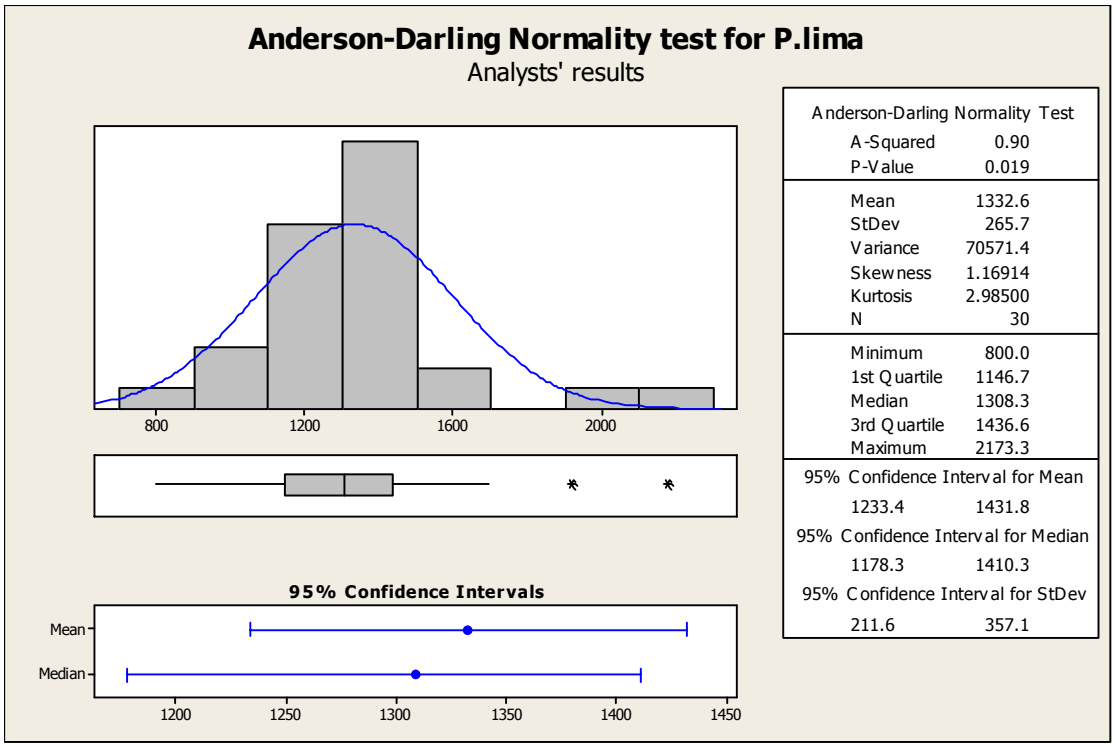


Figure 4

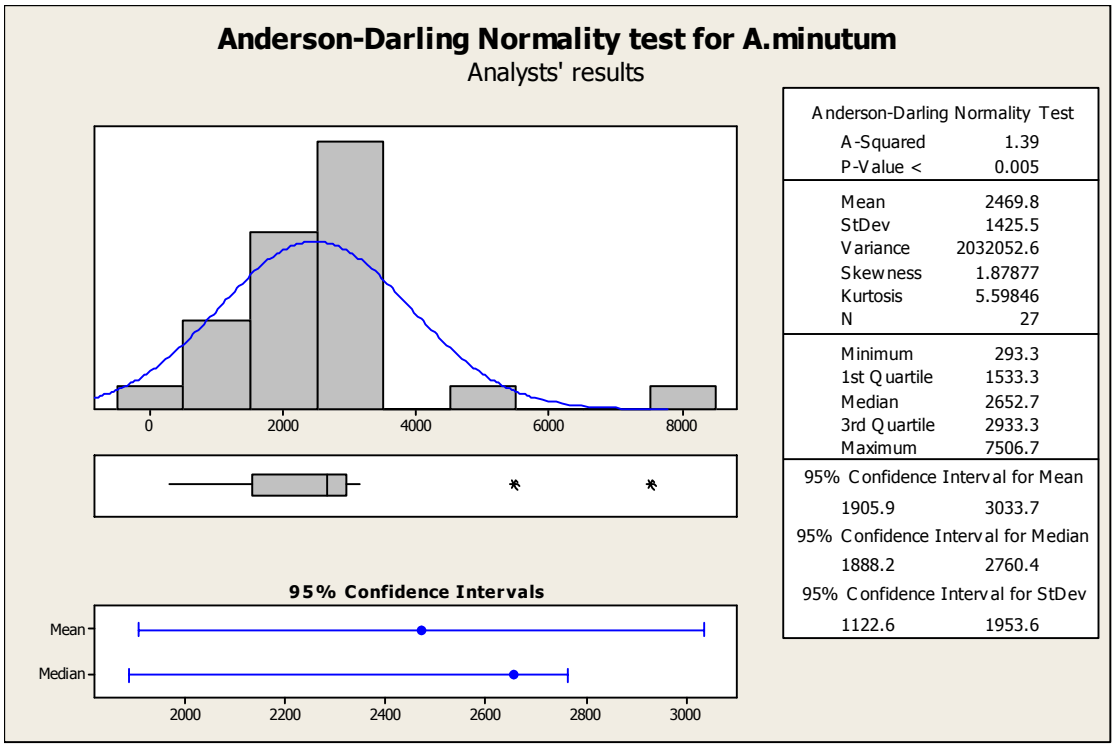


Figure 5

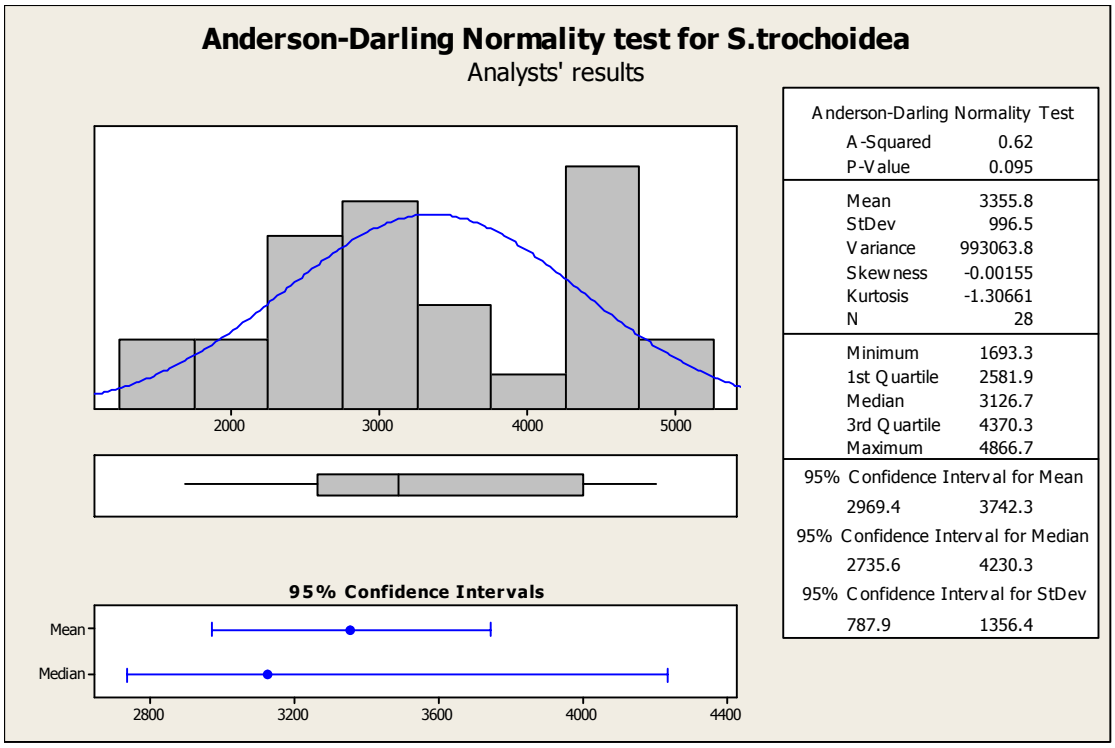
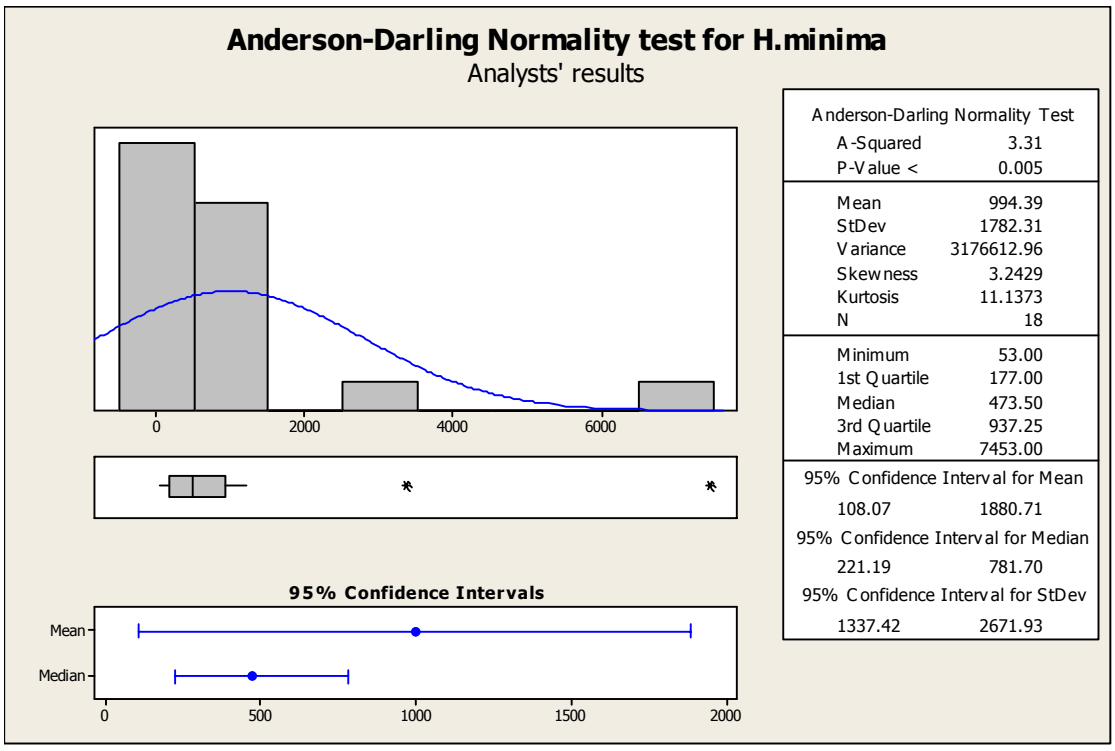


Figure 6



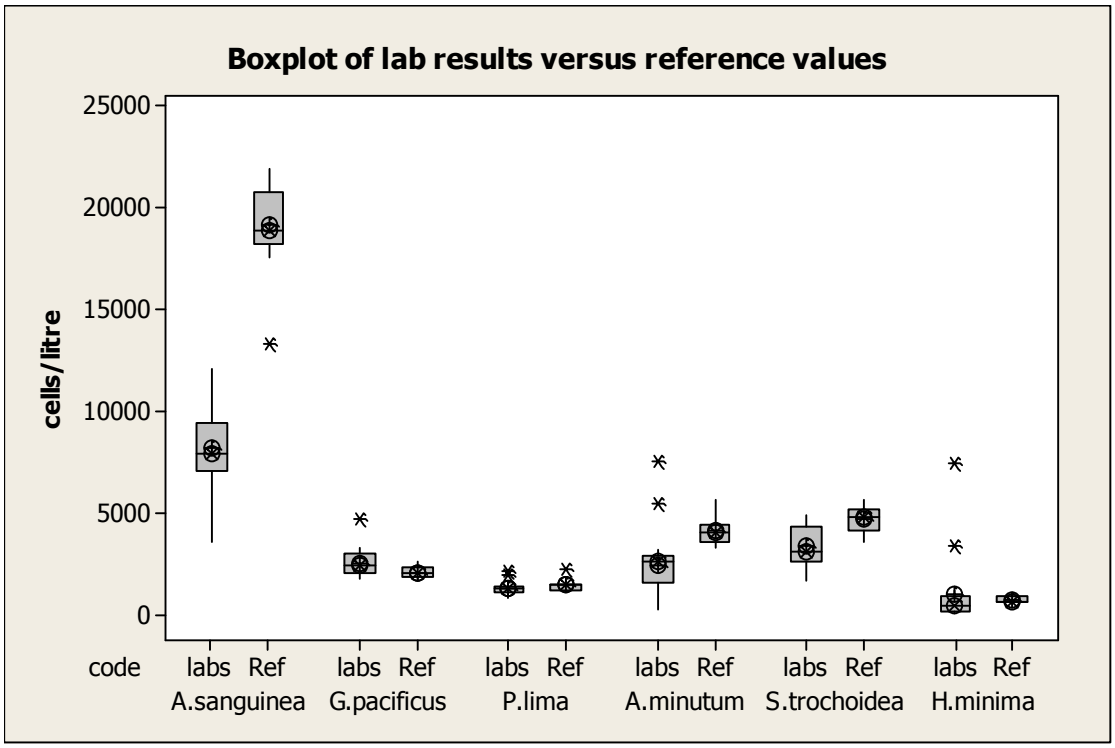
Only the *A.sanguinea* and the *S.trochoidea* data appears to follow a normal distribution, the other four graphs suggest that the data is not normal.

4.1.3.2 reference data comparison to analysts' data

The reference data in this exercise was used for comparative purposes only between the analyst's data and the reference laboratory. This was done to test the hypothesis of a scenario where the analyst data was compared to validated true values. This is only hypothetical as the reference values are not truly validated, so any inferences and conclusions from the following set of data should be interpreted carefully.

The box plot (figure 7) compared analysts' data against the reference data. This suggests that the data is not comparable. The mean value for *A.sanguinea* is double the value found by the participants. This is also the case for *A.minutum* and *S.trochoidea* cell counts.

Figure 7: Box plot of analysts' data versus reference values



Generally, the standard deviation for the reference counts was half that of the analysts. This type of precision is normal as there were more replicate counts for the reference values. This data is

not shown here but can be extracted from the box plot size and shape. If these values had been validated many analysts would have been outside the specification for most counts. Of course, there is no validity to this claim as the reference counts have not been validated and therefore it could be case that the reference counts could be less accurate than the analysts' counts.

4.1.3.3 Individual charts and Z-scores

The individual charts (figures 8 to13) show the individual mean values for each species by laboratory code. Analyst 1 was outside the 2 standard deviation for the *A.sanguinea* cell count. Analyst 4 was out in the *G.pacificus* cell count while other three analysts (30, 28 and 3) did not returned results for this species. Three analysts (1, 3 and 23) were out in the *P.lima* cell count. Analysts 18 and 30 were out in the *A.minutum* cell count and three analysts did not returned counts (20, 8 and 16). Analyst 18 was outside the limits for the *S.trochoidea* cell counts and one analyst (20) did not returned results. Analyst 18 was outside the limits for *H.minima* counts and eleven analysts did not returned results (31, 17, 33, 5, 30, 8, 24, 12, 3, 6 and 14).

Figures 8 to 13: I-charts of individual results split by Laboratory code for *A.sanguinea, G.pacificus, P.lima, A.minutum, S.trochoidea* and *H.minima*.

Figure 8

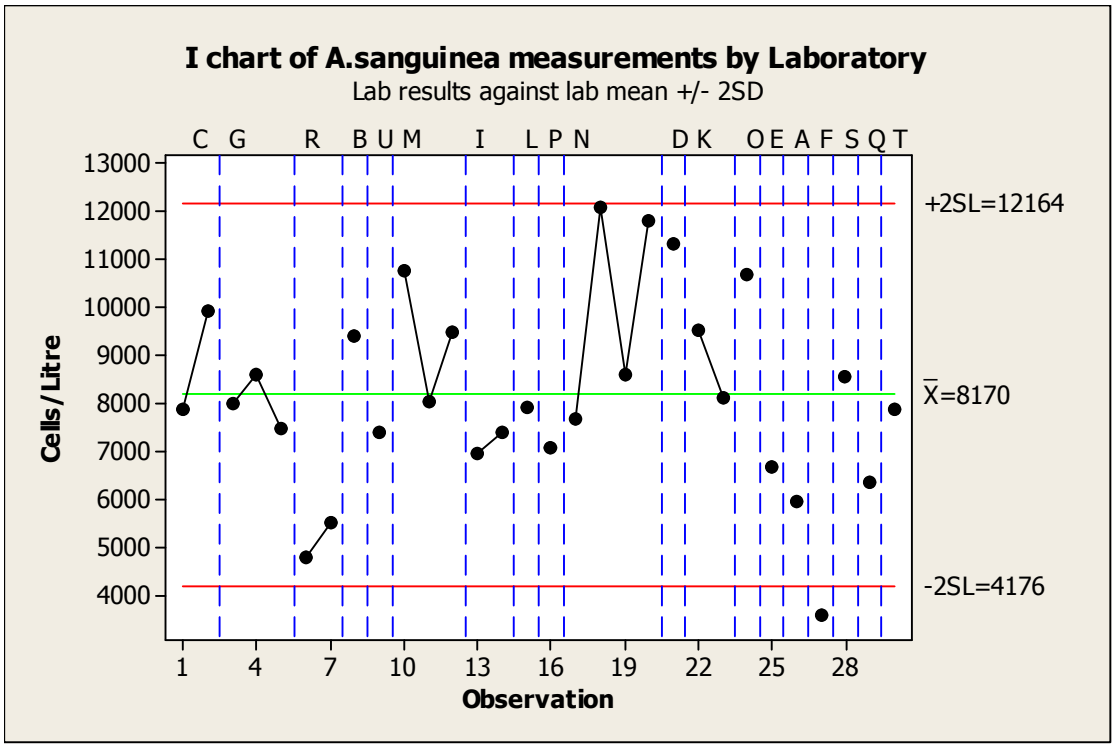


Figure 9

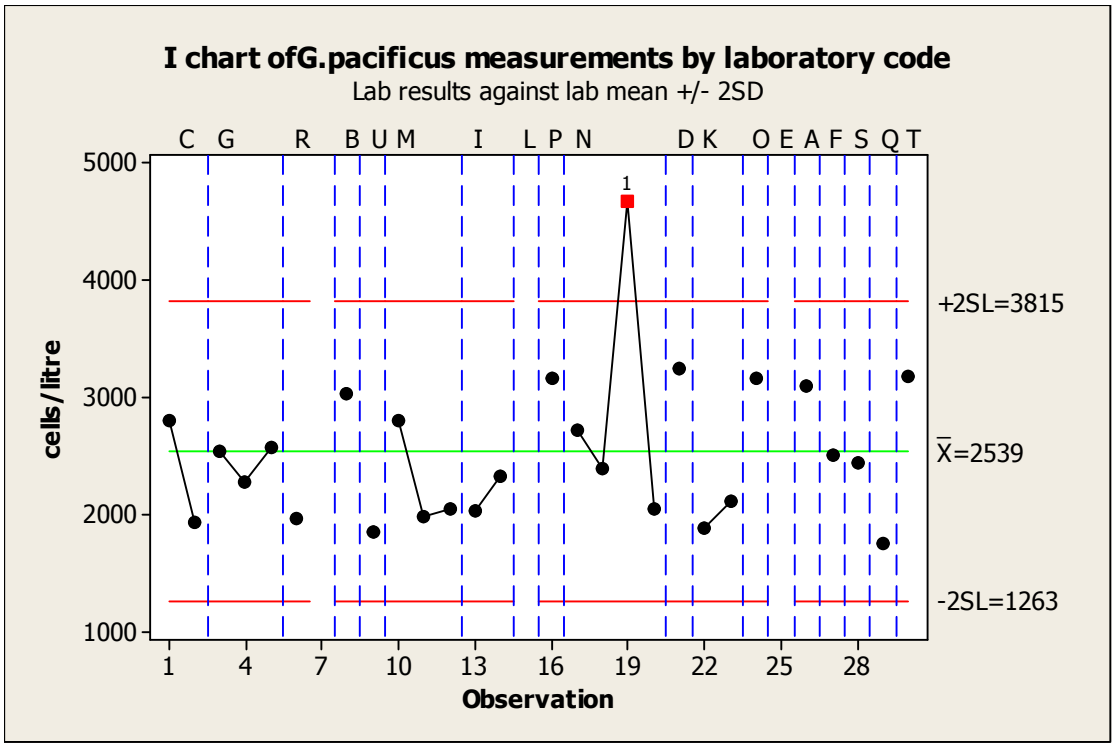


Figure 10

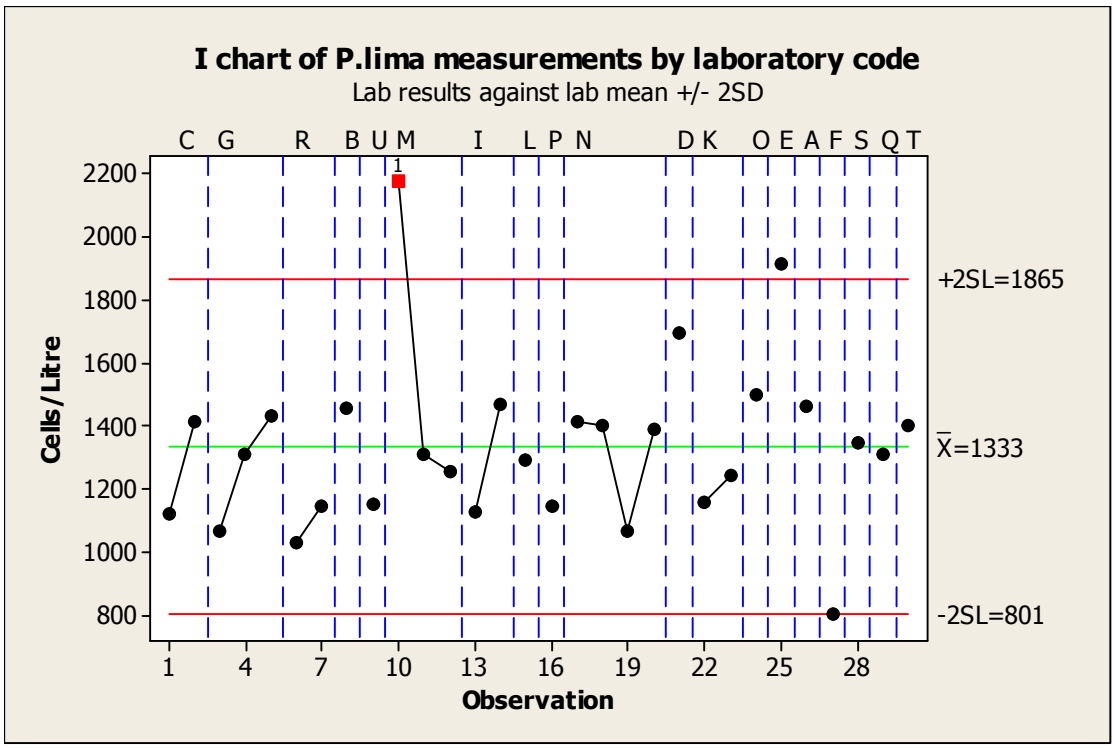


Figure 11

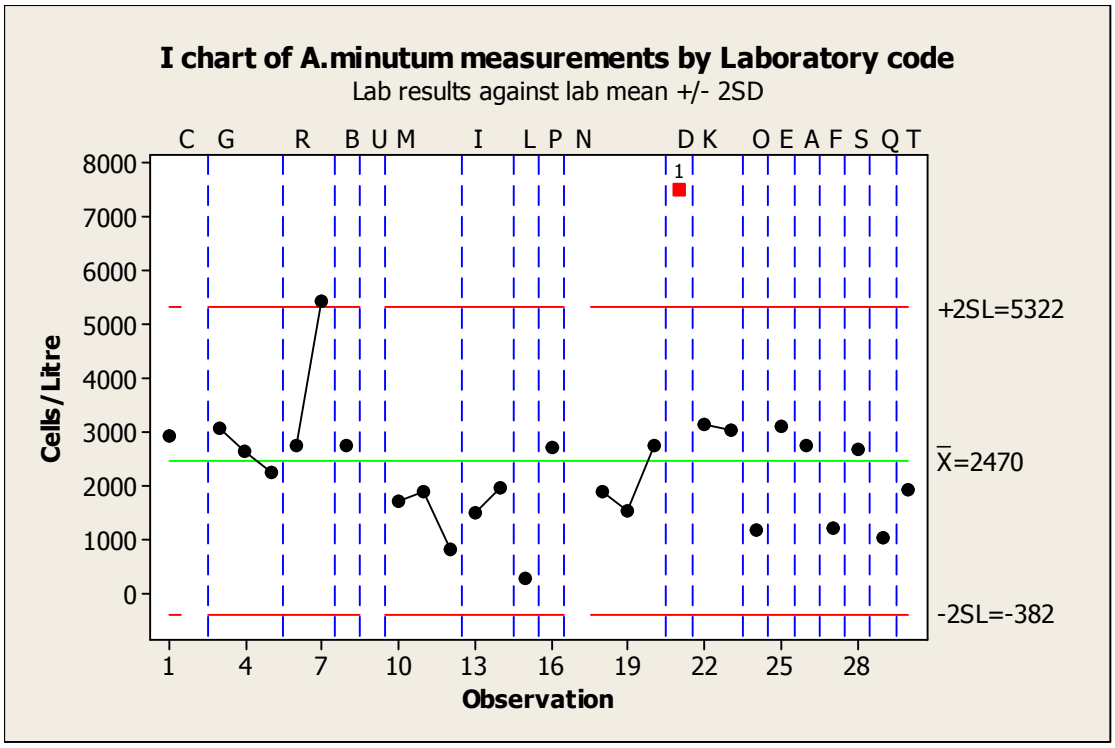


Figure 12

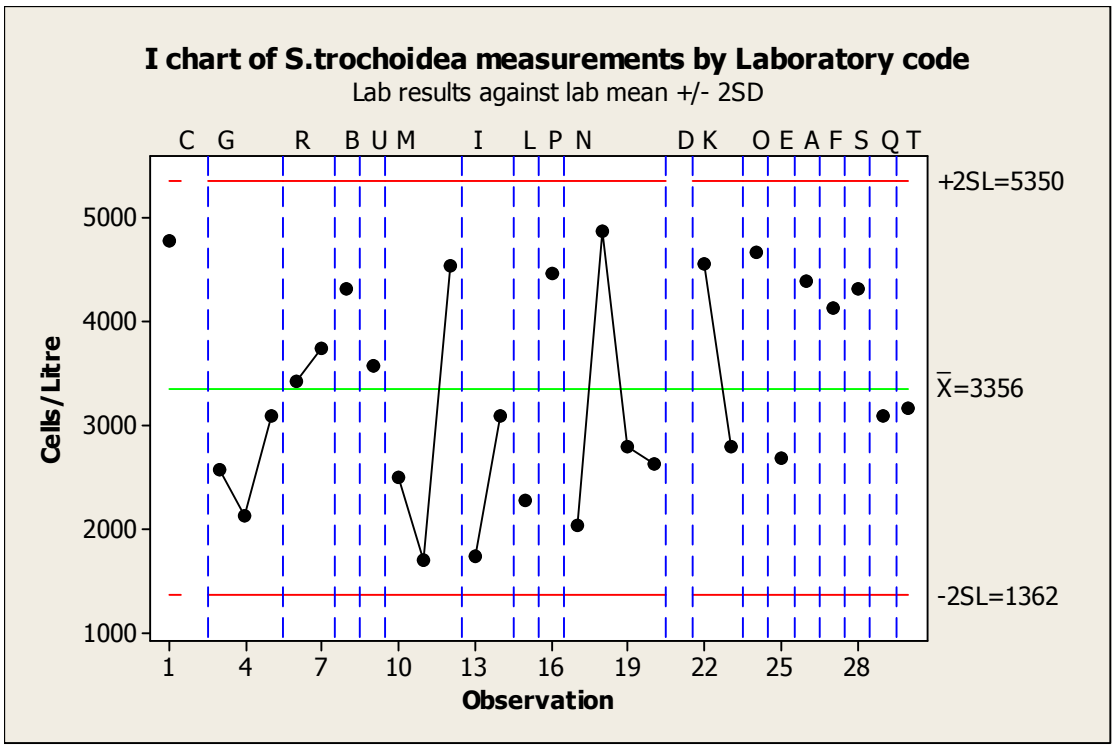
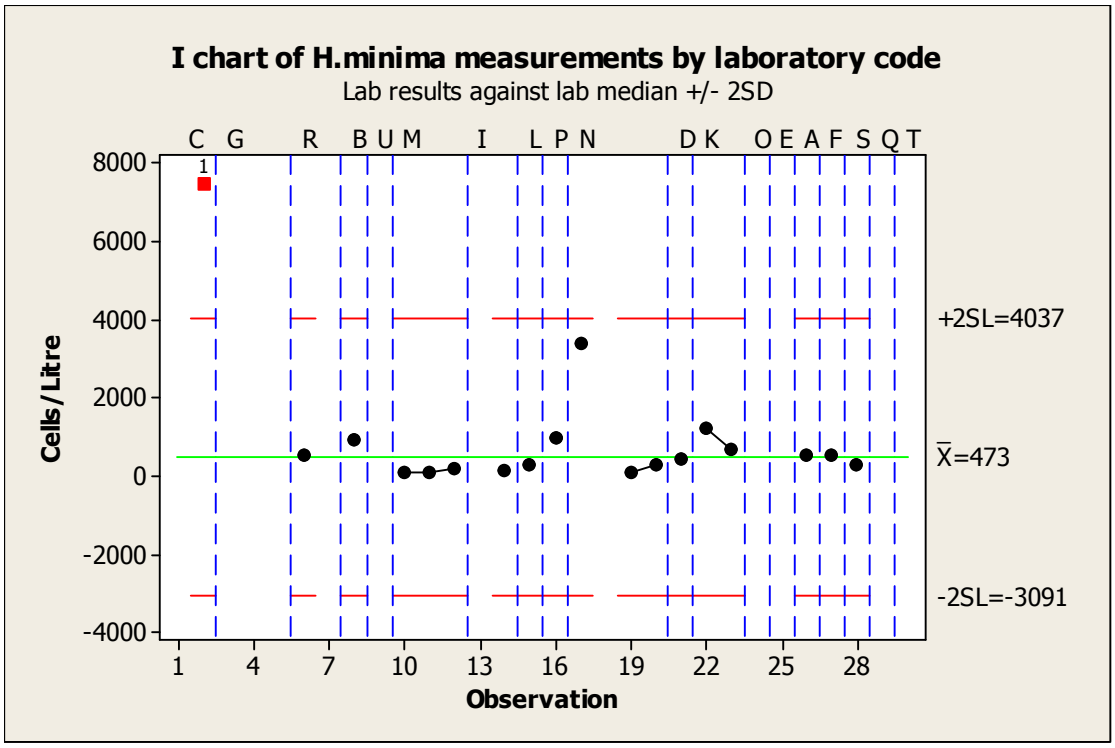


Figure 13



It is important to note that the median and not the mean of the results were used here for *H.minima* to establish the limits for this count. The reason for using the median was that the distribution of *H.minima* was clearly skewed and the mean is nearer the tail of the distribution (see Anderson Darling normality test for *H.minima*) a count by analyst 20 would have brought the mean quite high compare to the rest. It is clear from table 1 that analyst 20 most likely counted *A.minutum* and *S.trochoidea* cells as *H.minima*.

Figures 14 to 19 represent the Z-scores by analyst code for each species. Analysts had to fall within the 2 standard deviations of the mean 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.

Figures 14 to 19: Z-scores by analyst code for *A.sanguinea, G.pacificus, P.lima, A.minutum, S.trochoidea* and *H.minima*.

Figure 14

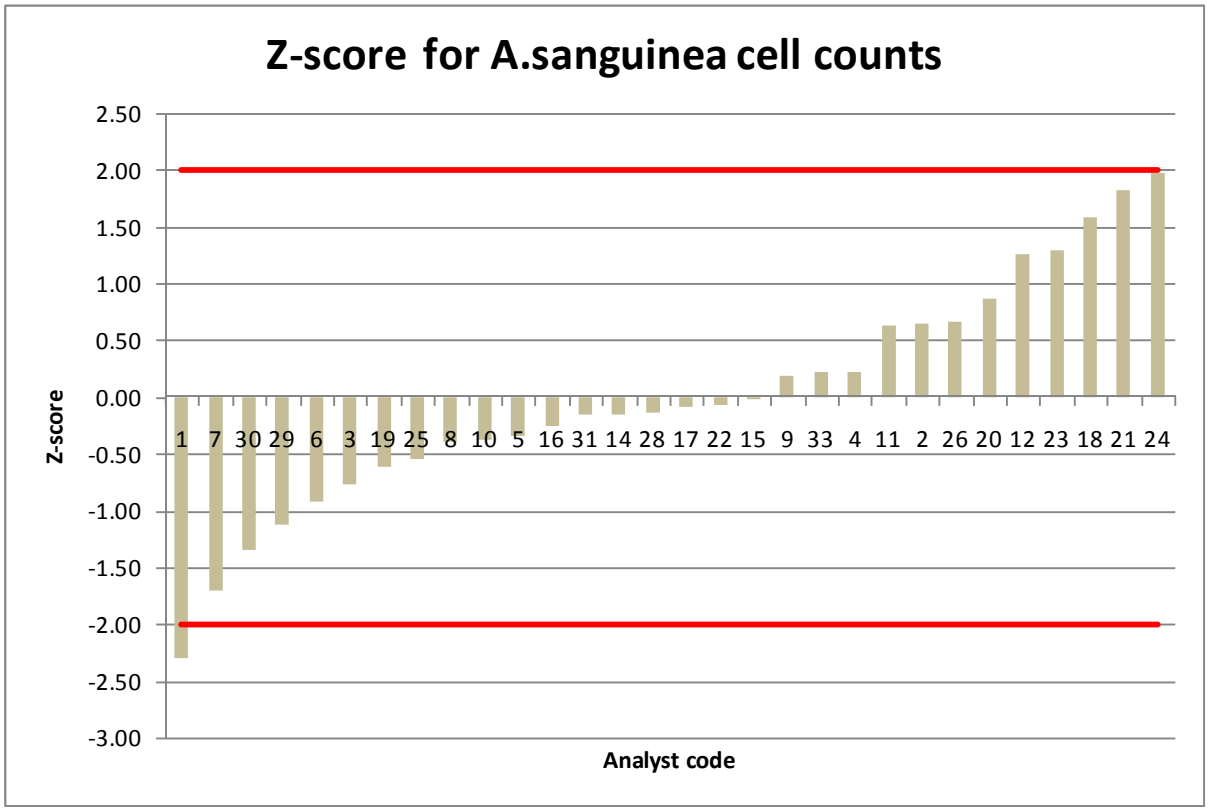


Figure 15

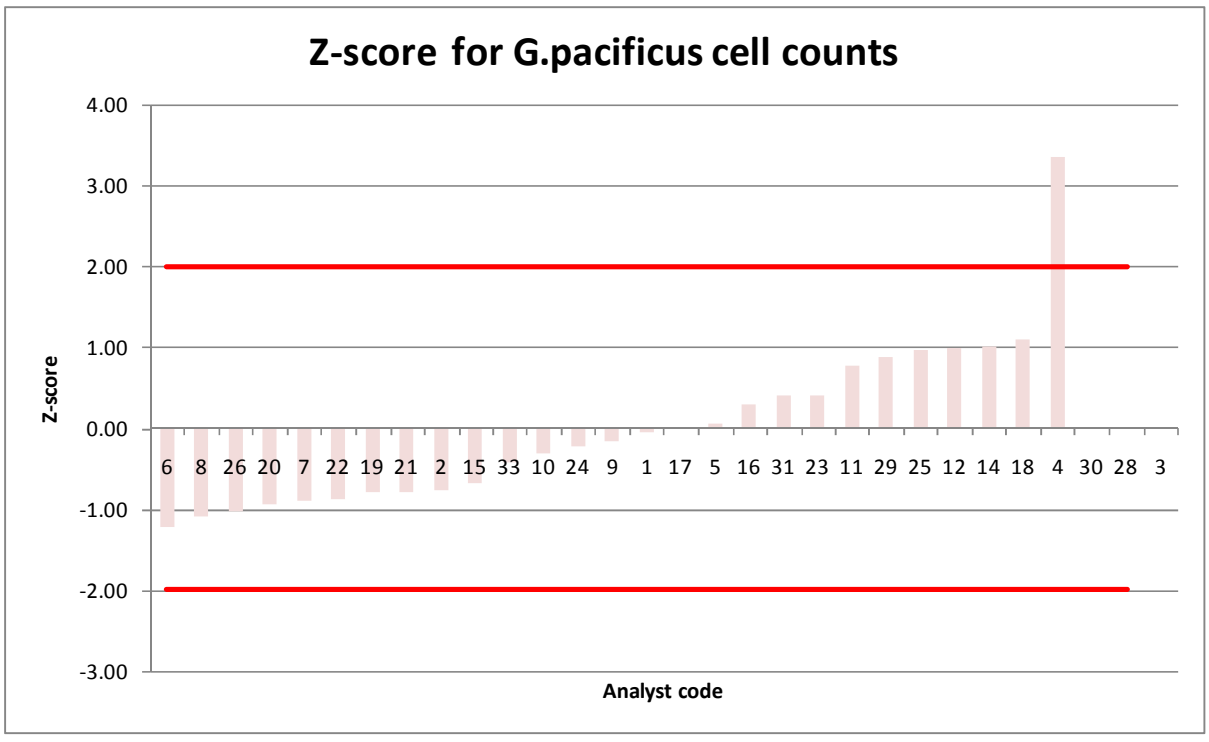


Figure 16

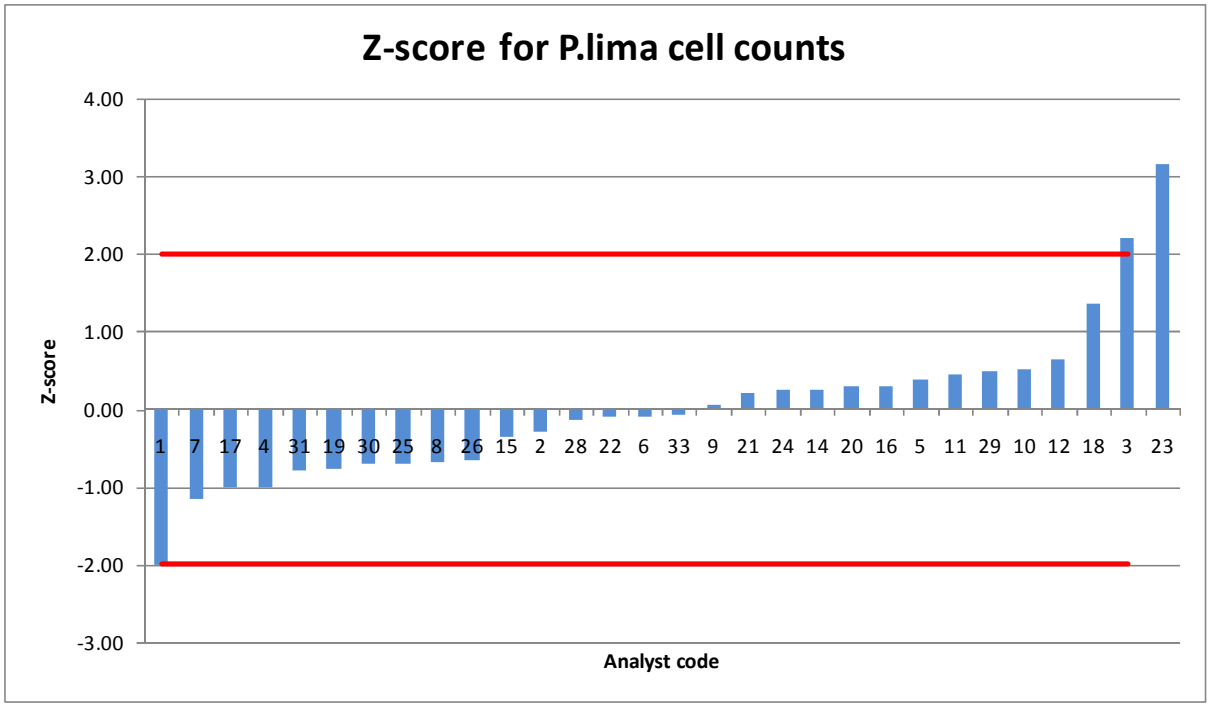


Figure 17

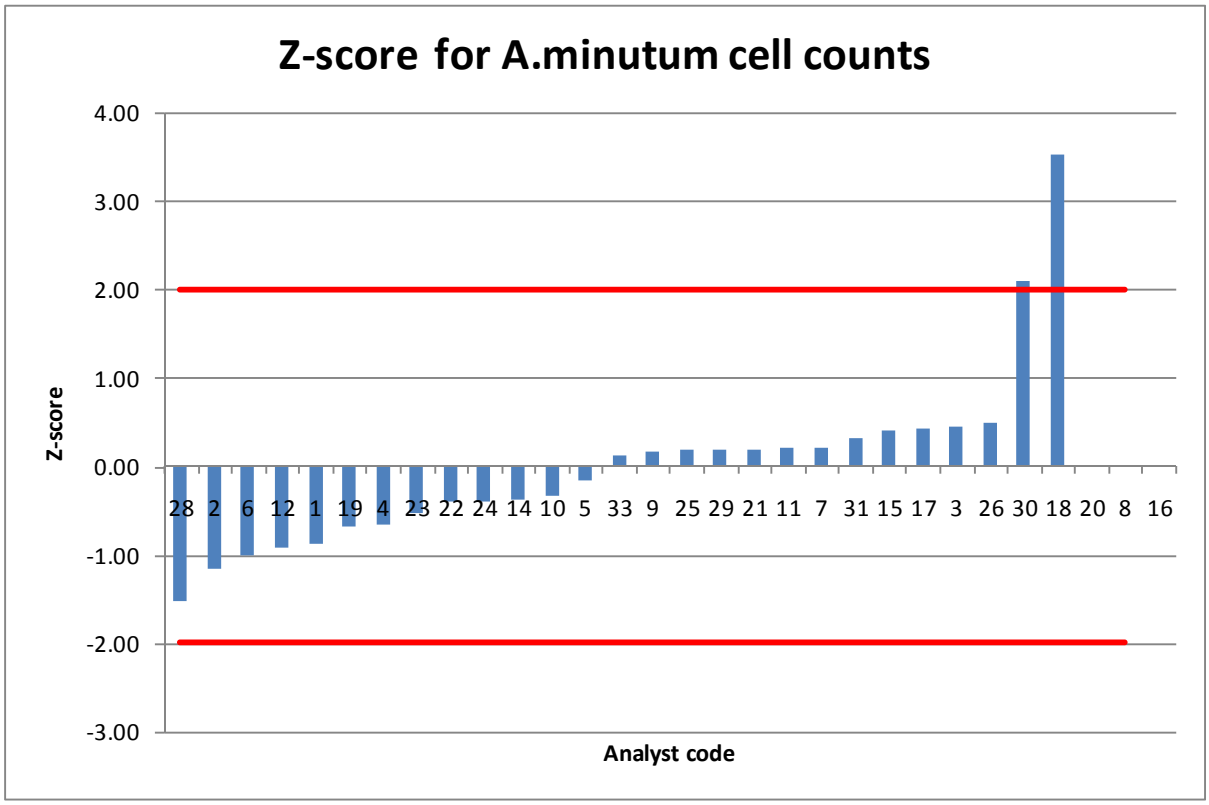


Figure 18

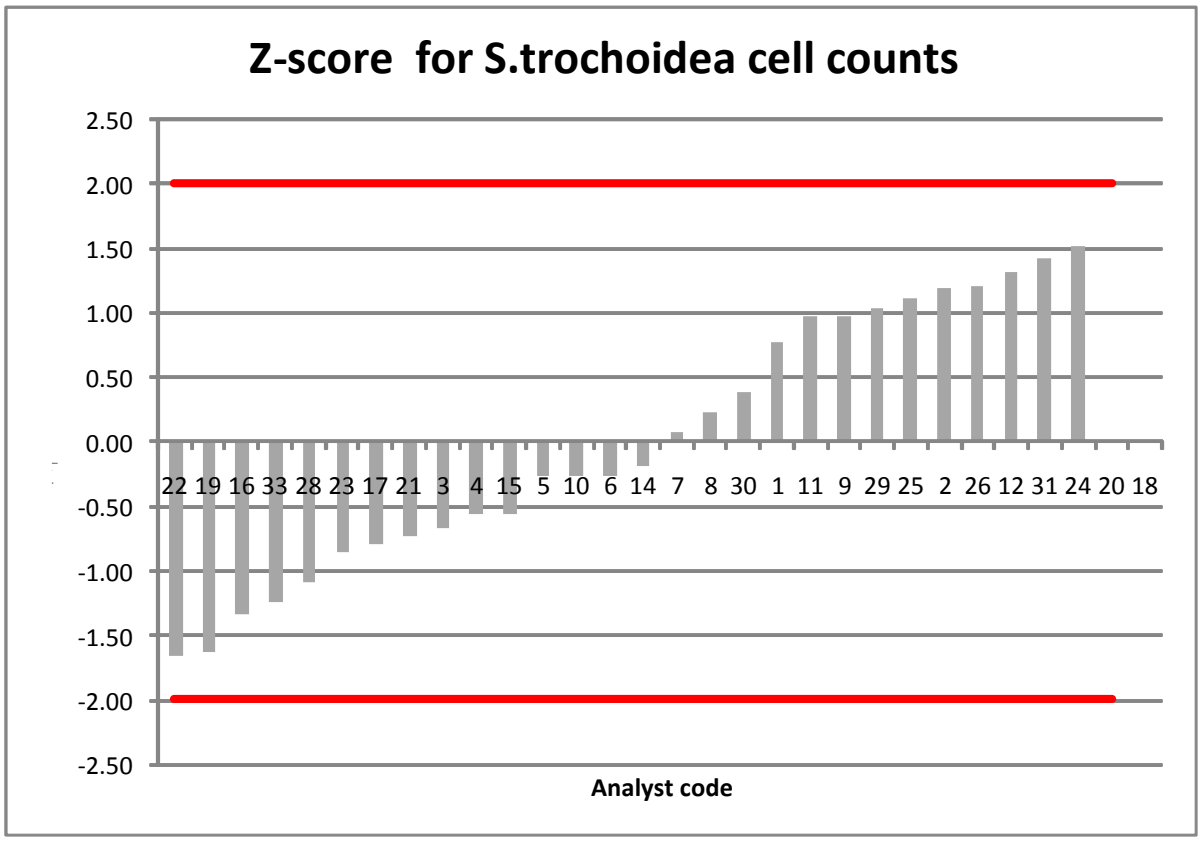
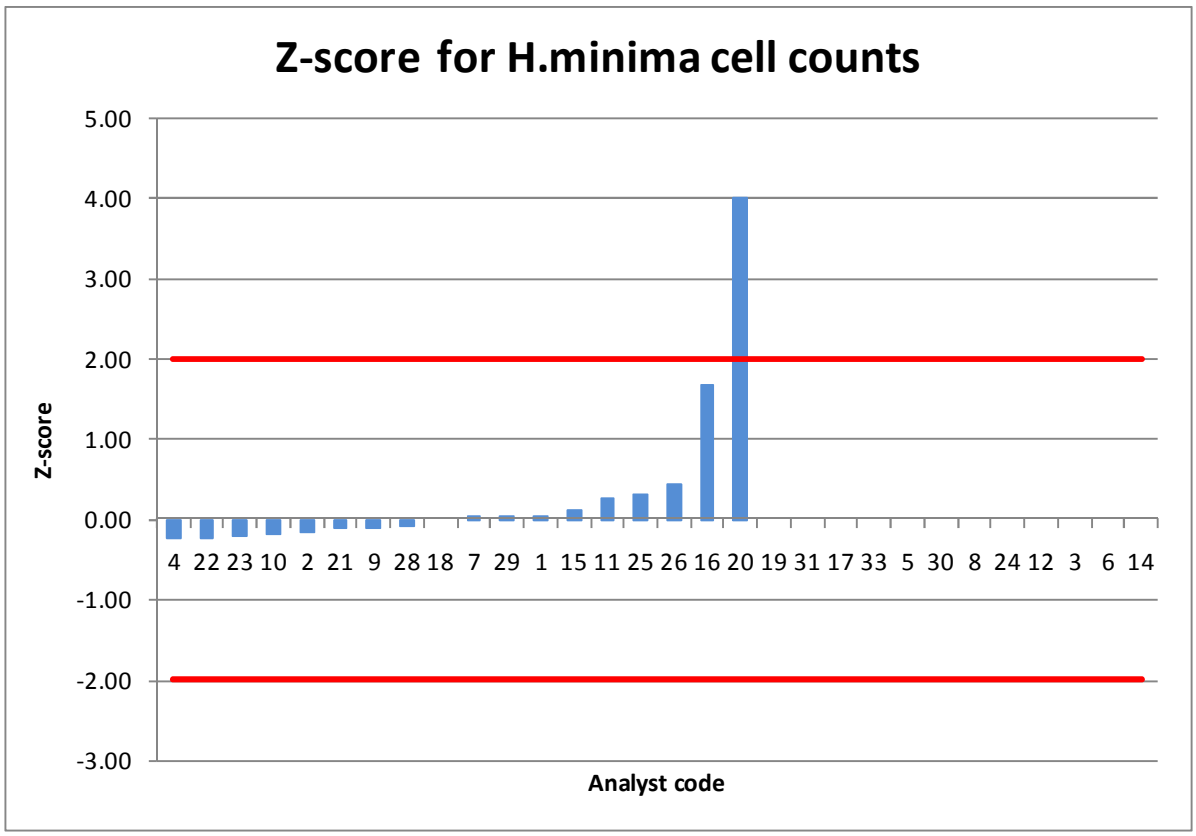


Figure 19

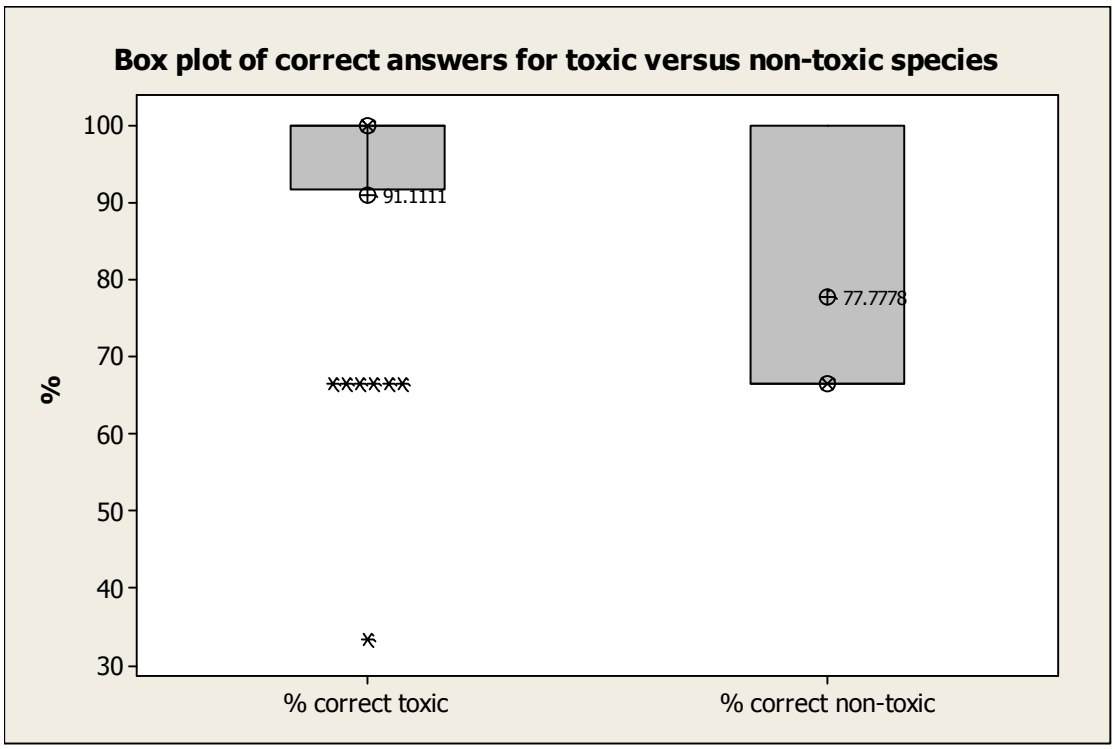


4.2 Identification results

4.2.1 Toxic versus non-toxic species

The samples were spiked with three toxic and three non-toxic organisms. The correct number of responses was compared between these two groups. The box plot (figure 20) of correct toxic versus correct non-toxic suggests that analysts were better at recognizing the toxic organisms from the non-toxic ones. The mean of % toxic correct results was 91.1% compared to 77.8% for non-toxic correct results.

Figure 20: Box plot of correct answers for toxic against non-toxic species



The case profile plots (figures 21 and 22) represent the comparison of % correct answers between toxic and non-toxic species by analysts and laboratories. The % of correct answers by analysts is high and it follows the trend of better recognition of toxic than non toxic algae. Only analyst 20 in figure 21 breaks this trend. Figure 22 shows the mean results by laboratory, as some laboratories are represented by one analyst only, the results are not directly comparable across the board between all the laboratories.

Figure 21: Case profile plot by analyst of correct answers between toxic and non-toxic species

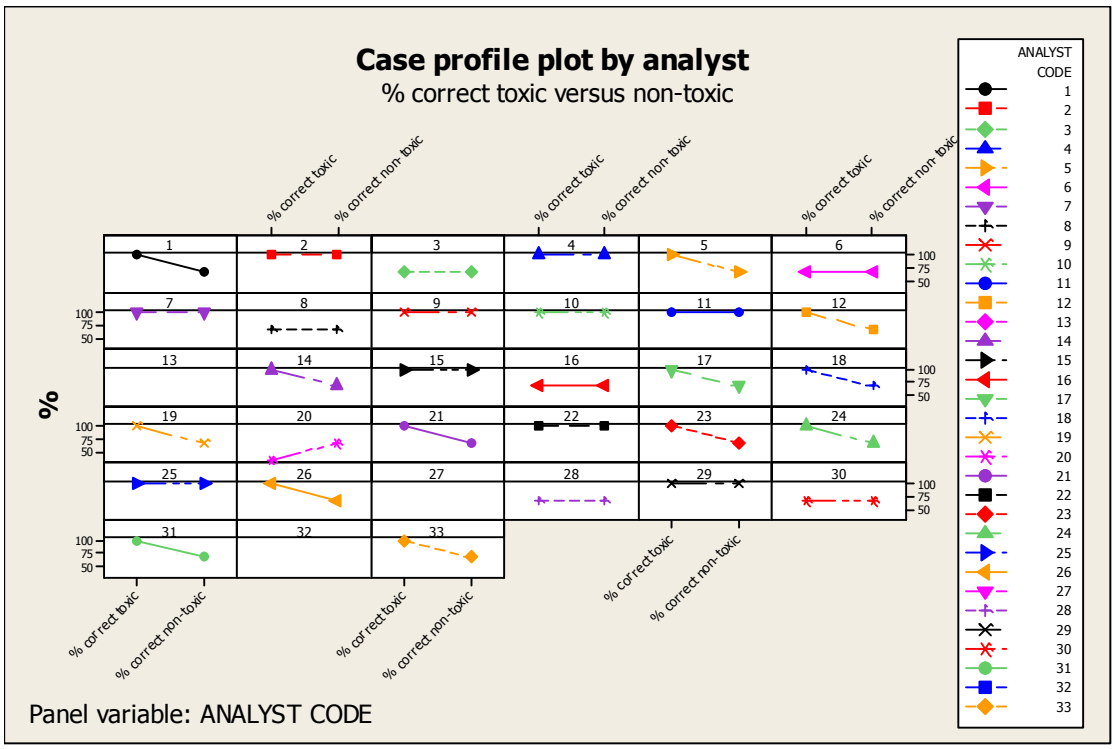
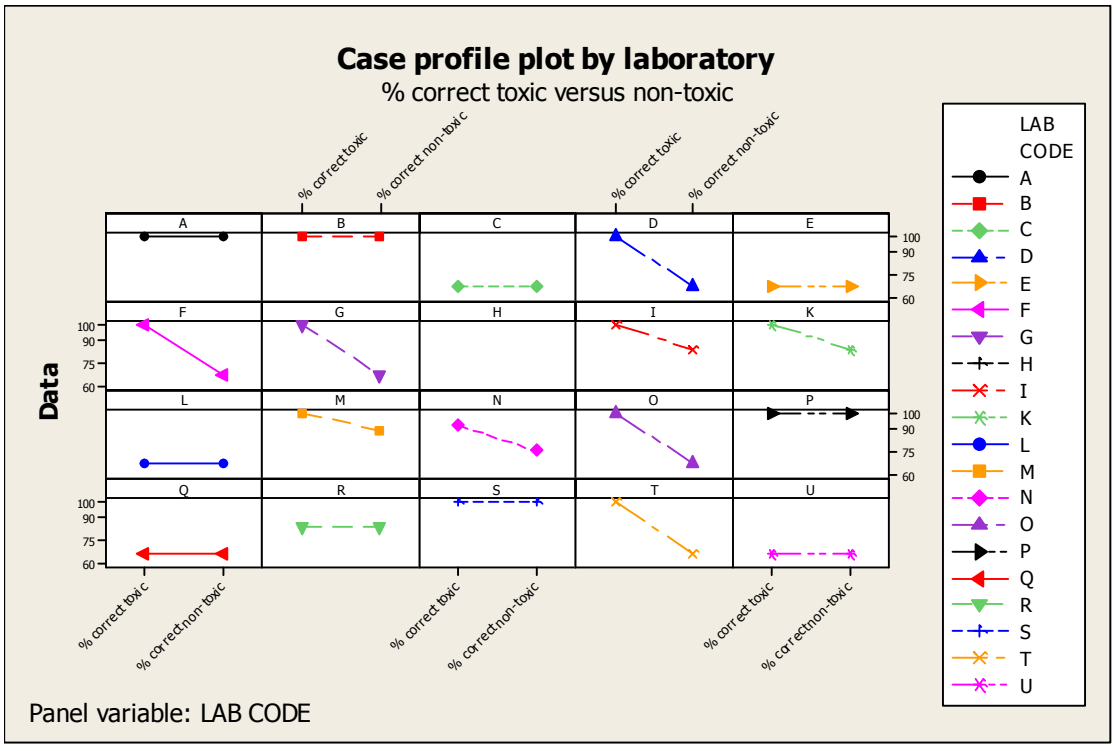


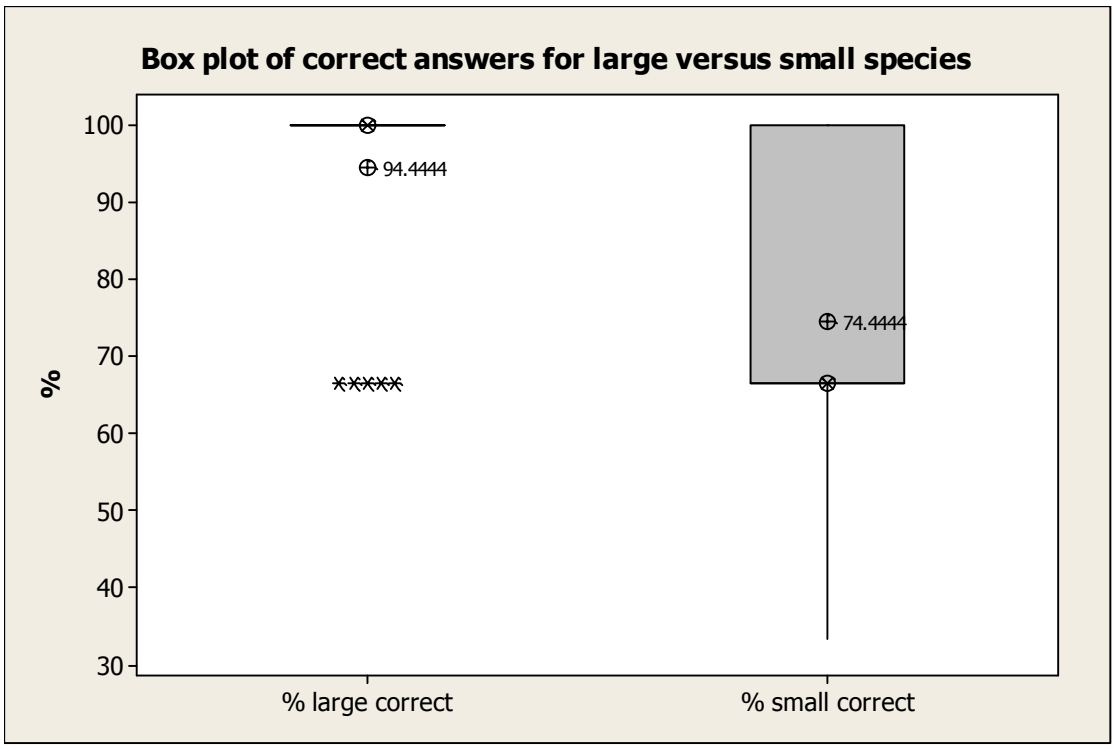
Figure 22: Case profile plot by Laboratory of correct answers between toxic and non-toxic species



4.2.2 Large size versus small size species

A comparison was made between large and small size organisms in the samples. The box plot of the % correct answers for large and small size species (figure 23) shows that analysts were better at correctly identifying large cells from smaller cells. This was 94.4% correct large cells against 74.4% correct small cells. It is clear that analysts are better at identifying larger size cells in the samples.

Figure 23: Box plot of correct answers for large against small species



The case profile plots (figure 24 and 25) between analysts and laboratories show that is easier to identify correctly larger organisms from smaller ones.

Figure 24: Case profile plot by analysts of correct answers between large and small species

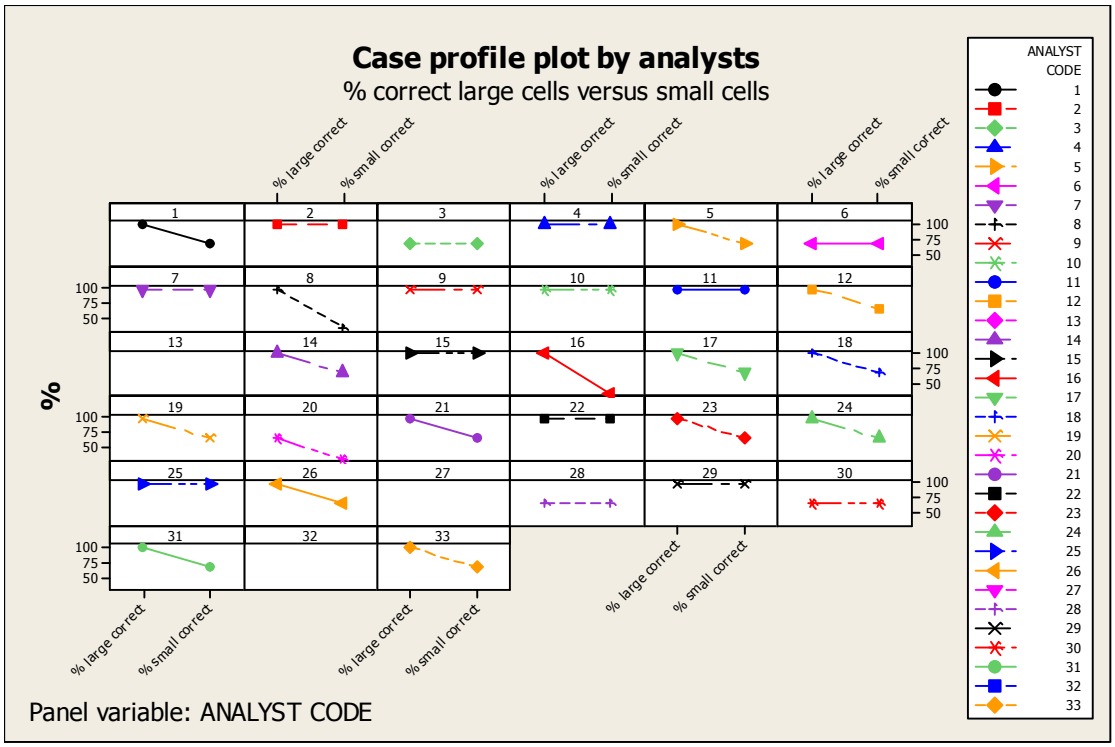
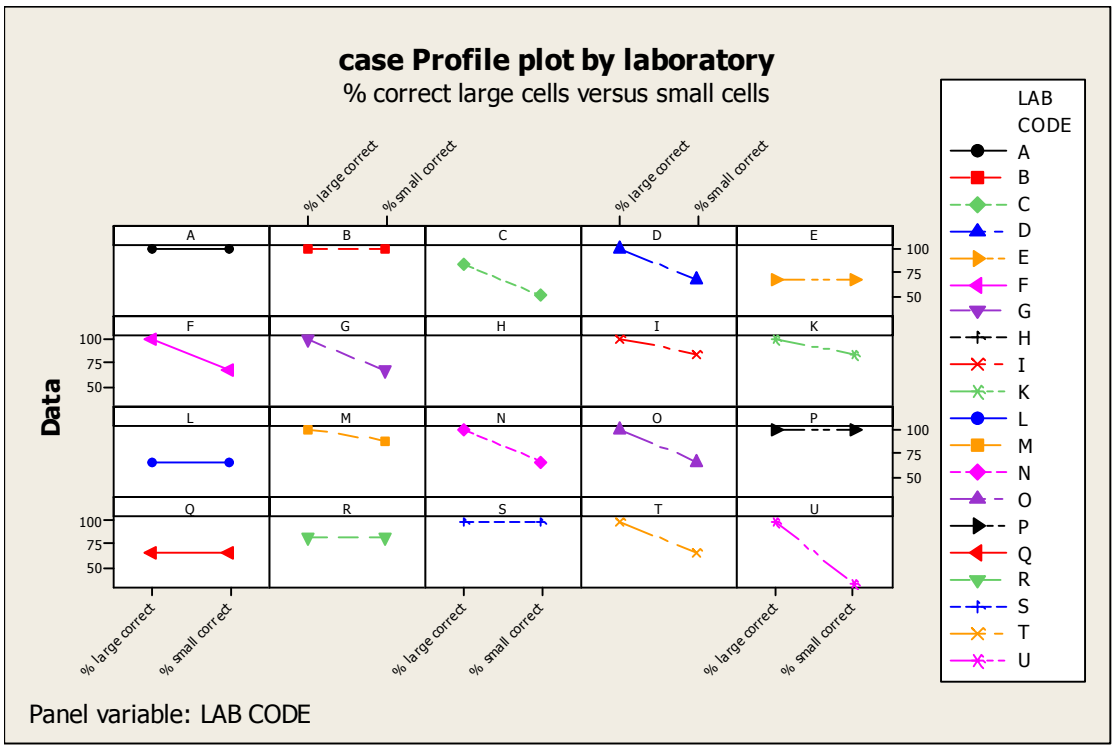


Figure 25: Case profile plot by laboratory of correct answers between large and small species



4.2.3 Reliability qualitative measure.

The results indicate so far that analysts are better at identifying toxic from non-toxic algae and better again at the identification of larger cells from smaller ones.

Analysts were asked to identify all the organisms in the samples to the highest taxonomic level possible. *P.lima* and *A.sanguinea* were to be identified to species level and the other four organisms (*G.pacificus, S.trochoidea, A.minutum and H.minima)* were required to be identified to genus only. All analysts identified correctly to species level the organisms *A.sanguinea* and *P.lima*.

Regarding the identification of *Gambierdiscus pacificus* seven analysts identified to genus level only, four analysts did not identified *Gambierdiscus* and one misidentified it as *Pyrophacus*, the rest (19 analysts) decided to go to species. Fifteen analysts identified this organism as *Gambierdiscus toxicus*, one as *G.belizeaneum* and three analysts were correct (*G.pacificus*).

The *Alexandrium* genus is hard to identify to species level unless analysts can do calcofluor staining or see the thecal plates of empty cells at high magnifications, therefore, identification to genus level was sufficient for the exercise. Twenty seven analysts out of thirty identified *Alexandrium* in their samples. Three analysts did not identify *Alexandrium* at all. Eleven analysts identified to genus level correctly and sixteen analysts decided to identify to species level. Fourteen were correct on naming this organism as *A.minutum* while two others identified as *A.tamarense.*

On *S.trochoidea*, two analysts did not identify these species in the samples, the rest (28 analysts) identified correctly, seventeen stayed at genus level and eleven identified correctly to species. *H.minima* was identified correctly to species level only by four analysts, ten others identified correctly to genus level and thirteen analysts did not identified these species at all. Three analysts also misidentified the species, two of those as *Azadinium* and one other as *Gymnodinium*.

Only the correct identification of the organisms to genus level has been used to build this reliability measure for the exercise (Table 3). The reliability measure (Table 4) shows that the sensitivity of this method is high (91%), while the specificity is a bit lower (76%), the reason for this is that the false positive rate is higher (29%) compared to the false negative rate (8%).

Table 3: identification results by species and analyst for the reliability study

ANALYST CODE	species identified correctly (1) nr= no results					
	A.sanguinea	G.pacificus	P.lima	A.minutum	S.trochoidea	H.minima
31	1	1	1	1	1	0
20	1	0	1	0	0	1
27	nr	nr	nr	nr	nr	nr
32	nr	nr	nr	nr	nr	nr
17	1	1	1	1	1	0
33	1	1	1	1	1	0
5	1	1	1	1	1	0
7	1	1	1	1	1	1
30	1	0	1	1	1	0
11	1	1	1	1	1	1
8	1	1	1	0	1	0
13	nr	nr	nr	nr	nr	nr
23	1	1	1	1	1	0
22	1	1	1	1	1	1
2	1	1	1	1	1	1
19	1	1	1	1	1	0
10	1	1	1	1	1	1
28	1	0	1	1	1	0
25	1	1	1	1	1	1
16	1	1	1	0	1	0
24	1	1	1	1	1	0
4	1	1	1	1	1	1
21	1	1	1	1	1	0
18	1	1	1	1	0	1
26	1	1	1	1	1	0
15	1	1	1	1	1	1
12	1	1	1	1	1	0
3	1	0	1	1	1	0
29	1	1	1	1	1	1
1	1	1	1	1	1	0
9	1	1	1	1	1	1
6	1	0	1	1	1	0
14	1	1	1	1	1	0

Table 4: Reliability measure for 2011

30 analysts							
TP= True positives	0	25	30	27	0	0	82
TN= True Negatives	30	0	0	0	28	10	68
FP= False Positives	0	0	0	0	2	20	22
FN= False Negatives	0	5	0	3	0	0	8
False Positive rate	0.00				0.07	0.67	0.29
False Negative rate		0.17	0.00	0.10			0.08
Sensitivity							0.91
Specificity							0.76
Efficiency							0.83
Youden Index							0.67
Likelihood ratio							0.73

The sensitive 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 a100% 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 +0.61 or 61% for 2011.

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 0.73 or 73%, so we are more likely to incur in false positive responses.

4.3 Ocean Teacher Hab quiz

Table 5 shows all the results as a percentage of correct answers for each question and analyst. 25 analysts returned a complete online quiz. Most analysts performed very well and over the 90% mark (figure 26). Six analysts were just below the 90% mark and one analyst just outside the 80%.

Table 5: Ocean teacher Hab quiz 2011 results

Analysts codes	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Total
31	100	100	100	100	100	100	100	100	100	100	66	0	**89**
7	100	100	100	100	100	100	100	100	100	100	100	100	**100**
30	75	100	100	100	100	100	100	100	100	100	66	0	**87**
11	100	100	100	100	100	100	100	100	100	100	66	100	**97**
8	100	100	100	100	100	100	100	100	100	100	100	100	**100**
23	87	100	100	100	100	100	100	100	100	100	100	0	**91**
22	100	100	100	100	83	100	100	100	100	100	100	0	**90**
2	100	100	100	100	100	100	100	100	100	100	100	100	**100**
19	100	100	100	100	100	100	100	100	100	100	66	100	**97**
10	100	100	100	100	100	100	100	100	100	100	66	0	**89**
28	100	100	100	100	100	100	100	100	100	100	66	100	**97**
25	100	100	100	100	83	100	100	100	100	100	100	0	**90**
16	100	100	100	100	100	100	100	100	100	100	66	100	**97**
24	100	34	100	100	100	100	100	100	100	100	100	0	**86**
4	100	100	100	100	49	100	100	100	100	100	100	0	**87**
21	100	100	100	100	100	100	100	100	100	100	100	0	**92**
18	100	100	100	100	100	100	100	100	100	100	100	0	**92**
15	75	100	0	100	100	0	100	100	100	100	66	100	**78**
12	100	100	100	100	100	100	100	100	100	100	100	0	**92**
3	100	100	100	100	100	100	100	100	100	100	66	100	**97**
29	100	100	100	100	100	100	100	100	100	100	100	100	**100**
1	100	100	100	100	100	100	100	100	100	100	100	0	**92**
9	100	100	100	100	100	100	100	100	100	100	100	0	**92**
6	100	100	100	100	100	100	100	100	100	100	49	0	**87**
14	100	100	100	100	100	100	100	100	100	100	100	100	**100**
	98	**97**	**96**	**100**	**97**	**96**	**100**	**100**	**100**	**100**	**86**	**44**	

The box plot (figure 27) shows the % of correct answers by question. Questions 11 and 12 which related to the diatom *Pseudo-nitzschia spp.* Appeared to be the most difficult to answer for the analysts.

Figure 26: Individual value plot of online Hab quiz results by analysts

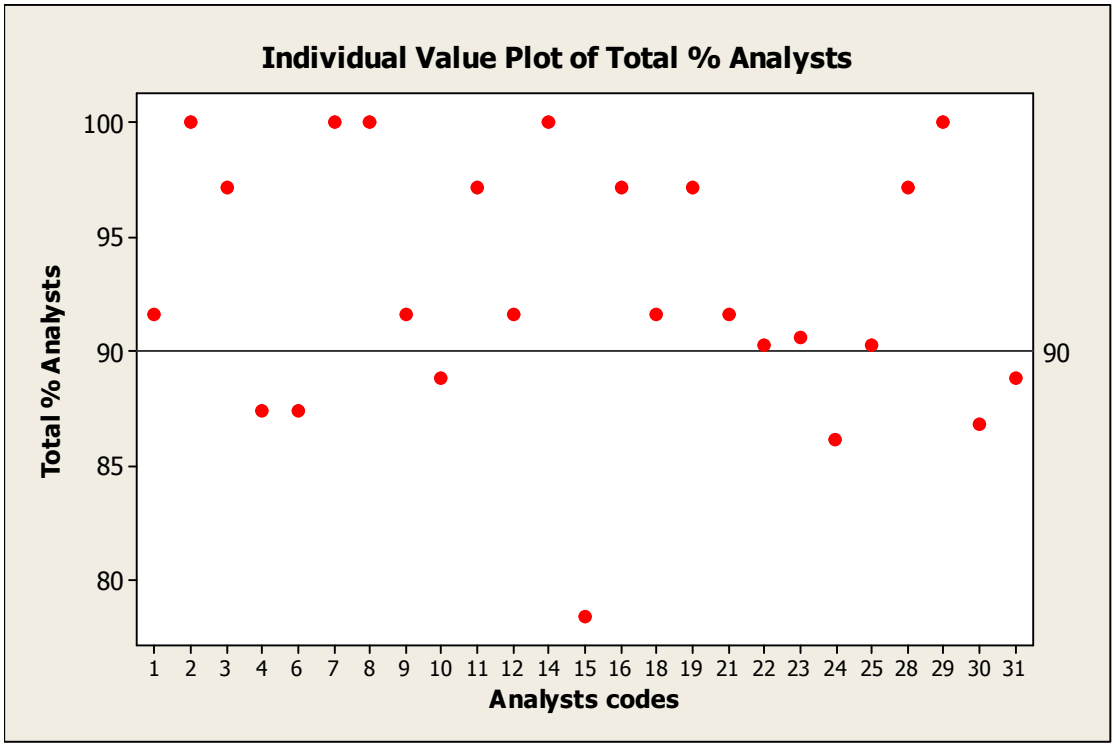
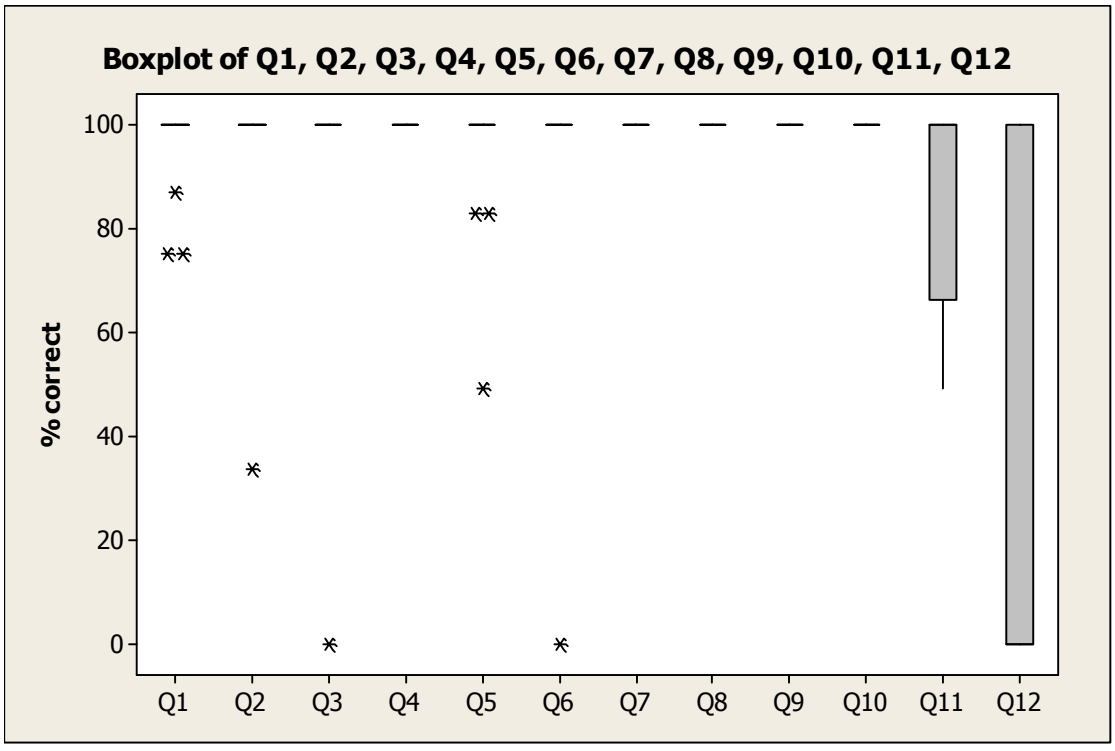
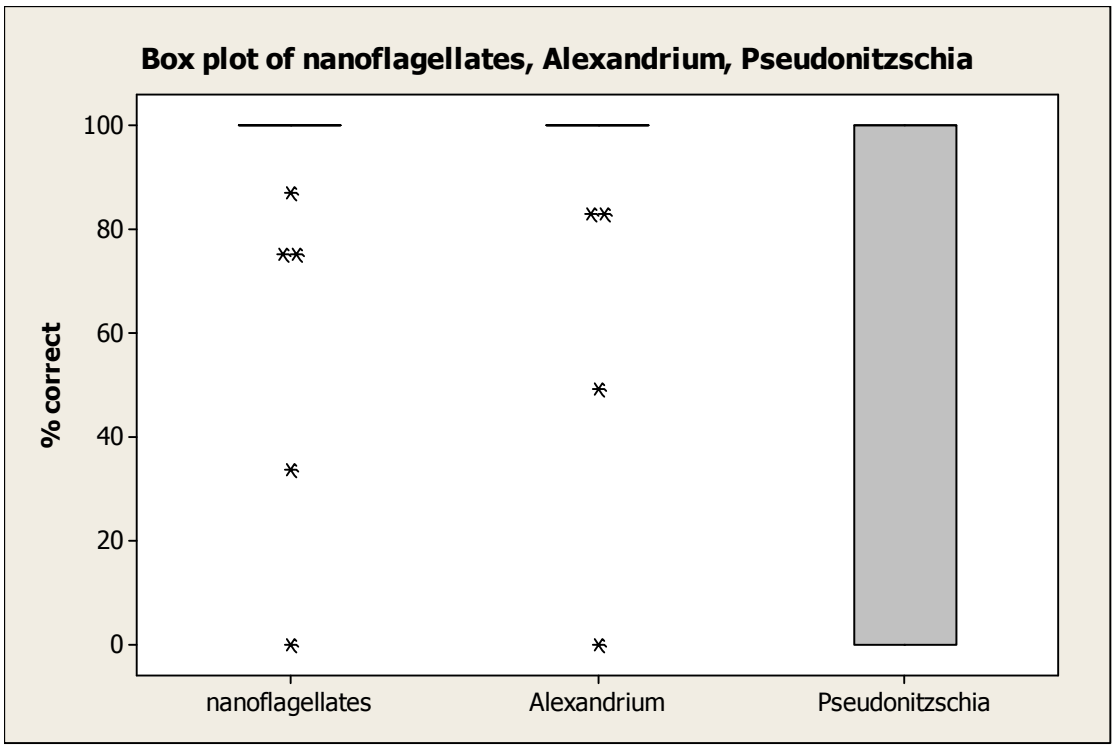


Figure 27: Box plot of % correct answers by question



The questions in this exercise can be pooled into three main groups: Questions 1 to 4 were questions on nanoflagellates, while questions 6 to 10 were on the genus *Alexandrium* and questions 11 and 12 were questions on the genus *Pseudo-nitzschia*. The box plot (figure 28) shows that the questions on the diatom *Pseudo-nitzschia* appear to be the most difficult ones for the analysts while the questions on *Alexandrium* and on nanoflagellates were generally answered correctly by most analysts.

Figure 28: Box plot of % correct answers by group of questions: nanoflagellates, *Alexandrium, Pseudo-nitzschia*.



The descriptive statistics of this exercise show that questions 4, 6,7,8,9 and 10 were the easiest to answer with perfect scores and question 12 the worst with a mean for all analysts of 44% (see table 6). When the results are pooled into group of questions in specific topics (nanoflagellates, *Alexandrium*, *Pseudo-nitzschia*) as a cumulative percentage, we find that analysts were better at answering questions relating to nanoflagellates and *Alexandrium* (21 analysts with perfect scores in each group) and found it more difficult to answer the questions on *Pseudo-nitzschia* (only 5 analysts got it perfectly right) (table 7).

Table 6: Descriptive statistics Hab quiz 2011 results

Box plot of Q1, Q2, Q3, Q4, Q5, Q6, Q7, Q8, Q9, Q10, Q11, Q12

Descriptive Statistics: Q1, Q2, Q3, Q4, Q5, Q6, Q7, Q8, Q9, Q10, Q11, Q12

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3
Q1	25	0	97.51	1.43	7.16	75.32	100.00	100.00	100.00
Q2	25	0	97.35	2.65	13.25	33.77	100.00	100.00	100.00
Q3	25	0	96.00	4.00	20.00	0.00	100.00	100.00	100.00
Q4	25	0	100.00	0.000000	0.000000	100.00	100.00	100.00	100.00
Q5	25	0	96.62	2.18	10.90	49.35	100.00	100.00	100.00
Q6	25	0	96.00	4.00	20.00	0.00	100.00	100.00	100.00
Q7	25	0	100.00	0.000000	0.000000	100.00	100.00	100.00	100.00
Q8	25	0	100.00	0.000000	0.000000	100.00	100.00	100.00	100.00
Q9	25	0	100.00	0.000000	0.000000	100.00	100.00	100.00	100.00
Q10	25	0	100.00	0.000000	0.000000	100.00	100.00	100.00	100.00
Q11	25	0	85.82	3.61	18.03	49.35	66.23	100.00	100.00
Q12	25	0	44.0	10.1	50.7	0.0	0.0	0.0	100.0

Variable	Maximum
Q1	100.00
Q2	100.00
Q3	100.00
Q4	100.00
Q5	100.00
Q6	100.00
Q7	100.00
Q8	100.00
Q9	100.00
Q10	100.00
Q11	100.00
Q12	100.0

Table 7: Descriptive statistics Hab quiz 2011 by groups

Tally for Discrete Variables: Total nanoflagellates, Total Alexandrium, Total Pseudo-nitzschia

Total nanoflagellates	Count	CumPct
58	1	4.00
78	1	8.00
92	1	12.00
96	1	16.00
100	21	100.00
N=	25	

Total Alexandrium	Count	CumPct
75	1	4.00
87	1	8.00
96	2	16.00
100	21	100.00
N=	25	

Total Pseudo-nitzschia	Count	CumPct
25	1	4.00
33	3	16.00
50	10	56.00
83	6	80.00
100	5	100.00
N=	25	

5. Conclusions

There is a lack of reproducibility between laboratory results and reference values set up by the organizing laboratory, as these values are not validated we cannot conclude that analysts' results are more or less accurate than those achieved by the reference laboratory. It is suggested, that the fact that the organizing laboratory is not blinded to the experiment design might have a bearing at least on the precision of the counts, as the standard deviation of the reference laboratory is for most counts half that of the analysts. Also, the analysts' counts for most species do not appear to follow a normal distribution while those by the reference laboratory for the most part do.

It has been suggested that transport might be a source of bias and that sample lost could be an issue for the lack of reproducibility between the laboratories and the organizing laboratory. However, there are also participants within the organizing laboratory and the samples for these do not travel, and the differences with the reference values still persist, so we can conclude that transport might not be the problem here. Time is not an issue as the reference counts have been carried out more or less at the same time as those by the analysts.

It is still possible that some samples may contain significantly different cell densities due to the techniques used, but these would have been minimized by homogenization, replication and sample randomization. The likelihood of one analyst receiving three samples with low densities compared to the others is very small. It is, therefore suggested here that the variability found in the samples by the analysts compared to the organizing laboratory is due to the fact that the organizing laboratory was not blinded to the experiment.

As these reference counts are not really validated, we cannot conclude that the results found by the organizing laboratory are more or less accurate that those found by the analysts. A suggestion is made that if the results had been validated most laboratories would have been outside the 2 standard deviation of the mean. This poses serious questions about the use in the future of certified reference materials.

Also, it poses questions as to how we arrive at these reference cell densities. Perhaps, reference counts should also be blinded to the organizing laboratory. Either way, there is a need first to prove whether not blinding the experiment to the reference count is causing these effects and also whether certifying reference materials would show these differences between laboratories.

Regarding the identification of the species spiked in the samples, we can conclude that in general analysts are better at identifying toxic species (91.1% correct) from non-toxic (77.8% correct) and better at identifying larger cells (94.4%) from smaller ones (74.4%). These results are influenced in some part by the fact that the small armoured dinoflagellate *H.minima* was the most difficult species to identify in the sample due to its size and also due to its density which was very low.

This exercise demonstrated by proxy that if we had spiked *Azadinium spinosum* (AZA producer) instead, as it is of similar size and shape (in fact 3 analysts had identified it as *Azadinium*) we would have found similar results. This shows the difficulty for monitoring programmes around the world to positively identify *Azadinium* by light microscopy alone when found in low densities in samples containing other species. 11 analysts from a field of 30 did not find the species in the sample.

A qualitative reliability measure for the test method was also calculated to demonstrate how good analysts are at identifying correctly organisms. This measure can be 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 three analysts, which were unable to identify G*ambierdiscus pacificus* in the samples came from geographical areas where this organism is not commonly found. Given that changes are occurring in our oceans, it is possible that species which are only found in tropical or sub-tropical areas may start appearing at other latitudes; therefore laboratory training should take this into consideration.

We have also found, that there is a tendency to over-identify species and use the most commonly found or used species name, as is the case with small dinoflagellate organisms like *Scrippsiella trochoidea* and *Alexandrium minutum*. Most 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 that any small armoured dinoflagellate which resembles a '*Scrippsiella'* type cell would generally be described as *S.trochoidea* even though it could either be *the genus Ensiculifera* or *pentapharsodinium* and a small *Alexandrium* would be most likely named *A.minutum* rather than *tamutum* or *tailorii* which are also very similar.

This was also the case for *G.pacificus*. The most common name used by analysts was *G.toxicus*; the reason for this was probably because it is the better known species of this genus.

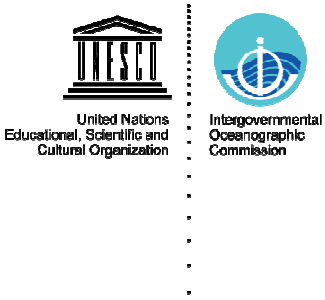
This reliability measure is also telling us that analysts are more likely to incur in false positive responses than false negatives responses, that is the ability of identifying a non-toxic organism correctly is 76% compared to the ability of correctly identifying a toxic organism (91%). This is good 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 80% efficient, which is the ability to discriminate between toxic or non-toxic species.

The performance of the test is given by the Youden index (61%) 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 0.73, 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 grasp of theoretical taxonomy of microalgal groups with most analysts performing above the 90% proficiency mark. The worst answered questions were two questions on the taxonomic features of the diatom *Pseudo-nitzschia spp.* and the best answered questions were on the genus *Alexandrium*.

This platform used for the exercise was found to be a very useful way to design taxonomy quizzes in the future.

ANNEX 1: Form 1 return slip and checklist



Bequalm Intercomparison PHY-ICN-11-MI1
FORM 1: RETURN SLIP AND CHECKLIST

se ensure to complete the table below upon receipt of samples, and fax or scan and e immediately to the Marine Institute. + 353 91 387237 or rafael.salas@marine.ie	
yst Name:	
ratory Name:	
yst Code Assigned :	
act Tel. No. / e-mail	

CHECKLIST OF ITEMS RECEIVED	**(Please circle the relevant answer)**	
ble numbers _____	YES	NO
f Instructions	YES	NO
neration and identification result log sheet **(Form 2)**	YES	NO

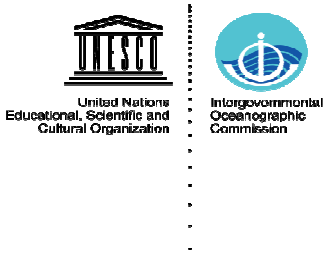
I confirm that I have received the items, as detailed above.

(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-11-MI1

FORM 2: ENUMERATION AND IDENTIFICATION RESULTS LOGSHEET

Analyst Name:	
Laboratory Code:	
Analyst Code :	

	Organism	Cell count	Multiplication factor	Number cells/L
Sample No:				
Settlement date:				
Analysis date:				
Volume Chamber (ml):				

	Organism	Cell count	Multiplication factor	Number cells/L
Sample No:				
Settlement date:				
Analysis date:				
Volume Chamber (ml):				

	Organism	Cell count	Multiplication factor	Number cells/L
Sample No:				
Settlement date:				
Analysis date:				
Volume Chamber (ml):				

Form 2 ENUMERATION AND IDENTIFICATION RESULTS LOGSHEET

ANNEX 3: Test instructions

Marine Institute BEQUALM Phytoplankton Proficiency Test PHY-ICN-11-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 Check and deadlines**
3. **Test Method**
4. **Equipment**
5. **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 annually since 2005.

The purpose of this exercise is to compare the performance of laboratories engaged in national official/non-official phytoplankton monitoring programmes and other labs 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 labs in the enumeration and identification of marine phytoplankton species from a number of samples spiked with phytoplankton cultured material.

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 should 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). Once you are happy that you have received everything you need to complete this exercise and samples are in working order, please complete form 1: Return slip and checklist form and send it by Fax or scan it and send it via e-mail to the Marine Institute, Galway. Fax No +353 91 387237 or 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 you have received the samples, each analyst has 4 weeks to complete the exercise and return the results to Rafael Salas, Marine Institute, Phytoplankton lab, Rinville,

Oranmore, Co. Galway, Ireland. The enumeration and identification results log sheet (Form 2) **must be received** by the Marine Institute by **June 30th, 2011**.

Please note: Results received after the June 30th 2011 date will not be included in the final report.

3. Test Method

The Utermöhl cell counting method is the standard method used in the Marine Institute Phytoplankton programme in Ireland. Our method uses 25ml sedimentation chambers and our lab is accredited to ISO 17025 standard for this method.

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

4. Equipment

Those labs using the Utermöhl method will need to complete the exercise:

- 3 Utermöhl cell counting chambers
- Base plates and glass covers.
- Inverted Microscope equipped with long distance working lenses and condenser of Numerical Aperture (NA) of 0.3 or similar and objectives up to 40x

5. Sample Preparation

Sedimentation counting chambers consist of a clear plastic cylinder, a metal plate, a glass disposable cover-slip base plate and a glass cover plate (Fig 1). 3 sedimentation chambers will be required.

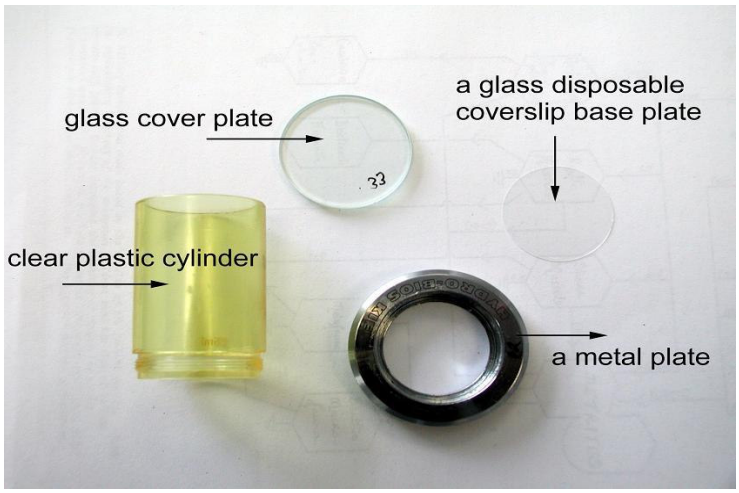


Fig 1: Sedimentation counting chamber

5.1 Place a clean disposable cover slip base plate inside a cleaned metal plate.

5.2 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.

5.3 **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.

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

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

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

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

5.5 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 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 species identified in the samples.

7. Samples

This Intercomparison exercise comprises 3 samples. These have been spiked with cell culture material kept in the Marine Institute Phytoplankton culture collection and in the IOC 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.

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.

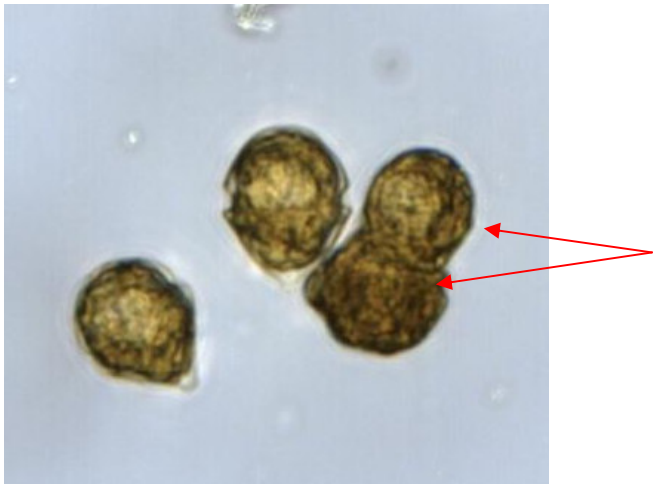


Figure 1: Two Cells fusing

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

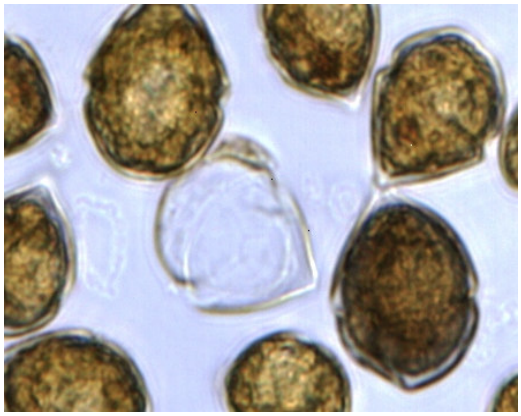


Figure 2: Empty theca

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.

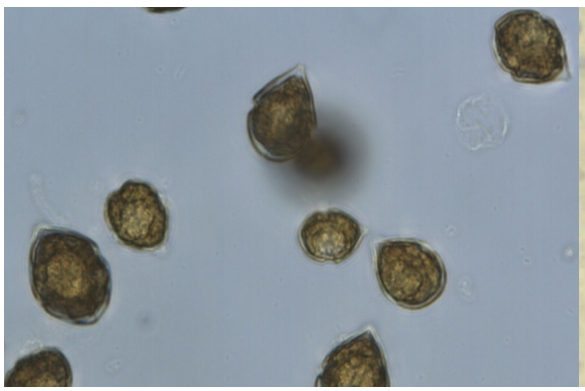


Figure 3: Big versus small cells

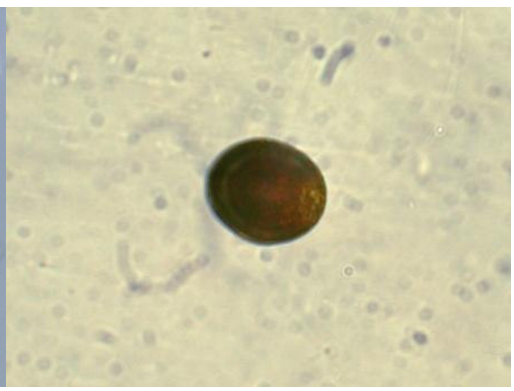


Figure 4: Plasmolised cell

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 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 to avoid bias due to cell counting cultured material.

8. Cell counts Conversion calculations

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 taxonomic quiz will be developed in the web platform 'Ocean teacher' in the next few months and it should be ready by September 2011. 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 in due course.

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 **June 30th, 2011.**

ANNEX 4: Workshop agenda

BEQUALM / National Marine Biological Analytical Quality Control Scheme
Phytoplankton ring test PHY-ICN-11-MI1
Workshop
Tuesday, 8th September 2011
CEFAS, The Center for Environment, Fisheries and Aquaculture Science.
Weymouth Laboratory, The Nothe, Weymouth, Dorset, UK.

Agenda

09:30 **Arrival to CEFAS Laboratories**

09:45 **Lab tour of CEFAS Weymouth facilities**
Dr. Wendy Higman

10:00 **Intercalibration exercise BEQUALM 2011**
Identification exercise results/Enumeration exercise results
Statistical analysis/Conclusions
Rafael Gallardo. Marine Institute. Ireland

11:30 **Coffee Break**

12:00 **"Benthic dinoflagellates in the Mediterranean Sea: past, present & future" by Dr. Katerina Aligizaki.**

13:00 **Lunch**

14:00 **Alex and his evil twin: Alexandrium tamarense in Scottish waters. Keith Davidson SAMS, 2011 (presented by Sarah Swan).**

14:30 **Open Discussion: Future developments Bequalm ICN 2012.**
All participants

15:30 **Closing meeting**

Agenda Intercomparison 2011 workshop

ANNEX 5: Participating Laboratories

BEQUALM 2011 Participating Laboratories

Lab Addresses

Fisheries and Aquatic Ecosystems Branch Newforge Lane Belfast BT9 5PX United Kingdom	CEFAS Barrack Road, The Nothe Weymouth Dorset DT4 8UB United Kingdom
Marine Scotland Science 375, Victoria Road Aberdeen, Aberdeenshire AB11 9DB United Kingdom	Northern Ireland Environmental Agency 17 Antrim Road Lisburn BT29 4QY N.Ireland United Kingdom
Scottish Association for Marine Science Dunstaffnage Marine Laboratory Oban, Argyll PA37 1QA United Kingdom	CEFAS Pakefield Rd Lowestoft NR33 0HT United Kingdom
Laboratorio de Control de Calidad de los recursos pesqueros Ctra. PUNTA UMBRÍA - CARTAYA km 12 Cartaya, Huelva, 21459 Spain	IRTA Carretera del Poblenou km 5,5 Sant Carles de la Ràpita 43540 Spain
Isle of Man Government Laboratory Ballakermeen Road Douglas Isle of Man, IM1 4BR United Kingdom	INTECMAR Peirao de Vilaxoán s/n. Vilagarcía de Arousa Pontevedra 36611 Spain
Marine Institute Phytoplankton unit Rinville, Oranmore, Co.Galway Ireland	Marine Institute Phytoplankton unit Gortalassa, Bantry, Co.Cork Ireland
Scottish Environment Protection Agency Clearwater House, Heriot Watt Research Park Avenue North, Riccarton EH14 4AP Edinburgh, United Kingdom	IMARES Korringaweg 5 Yerseke 4401 NT Netherlands
Apem Ltd A17 Embankment Business Park Heaton Mersey, Stockport Cheshire, SK4 3GN United Kingdom	Koeman en Bijkerk bv Oosterweg 127 Haren 9751PE Netherlands
IZOR Setaliste I. Mestrovica 63 P.O. Box 500 Split, 21000 Croatia	Department of Botany, School of Biology Biology Building, 9th floor, Lab 9.27 Thessaloniki 54124 Greece
Centro Balear de Biologia Aplicada 18, LLUCMAJOR STREET PALMA DE MALLORCA 7006 Spain	LVCC Palmones. c/Trasmallo s/n (Palmones) Los Barrios Cadiz 11379 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 2011

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

Statement Issued:
Statement Number: MI-BQM-11-

Summary of results:

Component Name	Subcontracted	Results Z-score (+/- 2 Sigma limits)		identification
Phytoplankton abundance and composition PHY-ICN-11-MI1	Marine Institute	*Akasshiwo sanguinea*		
		Gambierdiscus pacificus		
		Prorocentrum lima		
		Alexandrium minutum		
		Scripssiella trochoidea		
		Heterocapsa minima		
Overall Result Taxonomic quiz (Pass Mark 70%, over 90% proficient)				
Phytoplankton Taxonomy quiz PHY-ICN-11-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

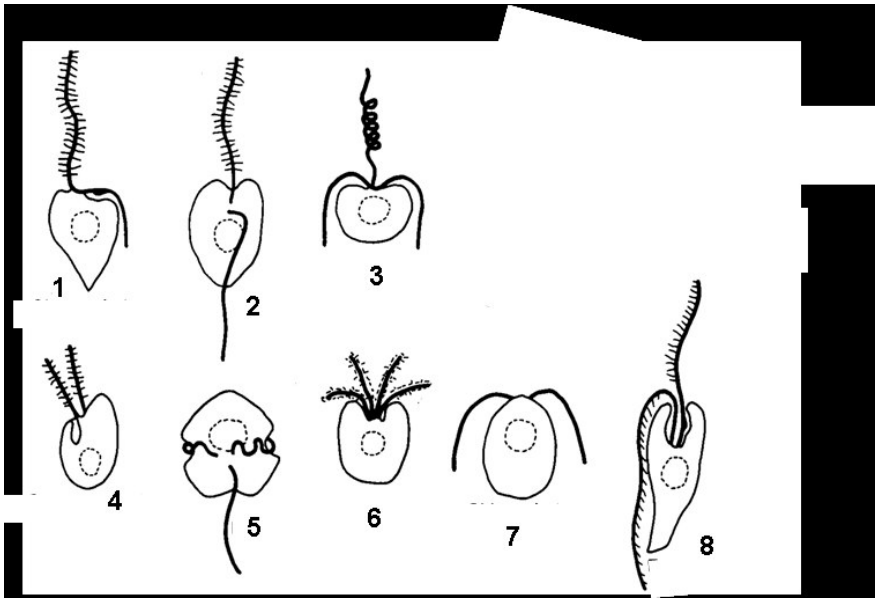
Description of Scheme components and associated performance standards

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 a 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 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, and 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

1. 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 LM. Below are shown schematic line-drawings of the most important groups of marine flagellates - assign them to groups



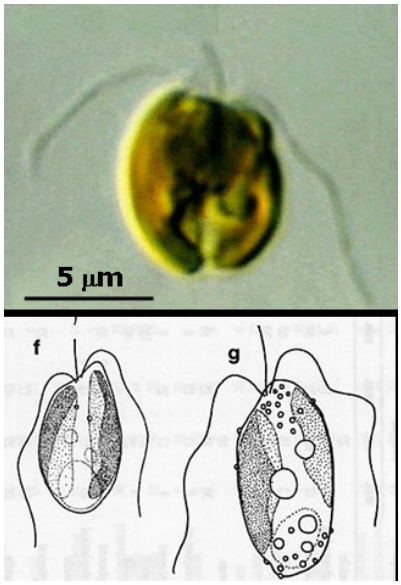
2. Nanoflagellates (<20 µm) are common in the marine environment. In the question 'Marine flagellates 1', you have identified the most important groups of flagellates. Which of the following groups comprise toxic members in the marine environment?

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

Select one or more:

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

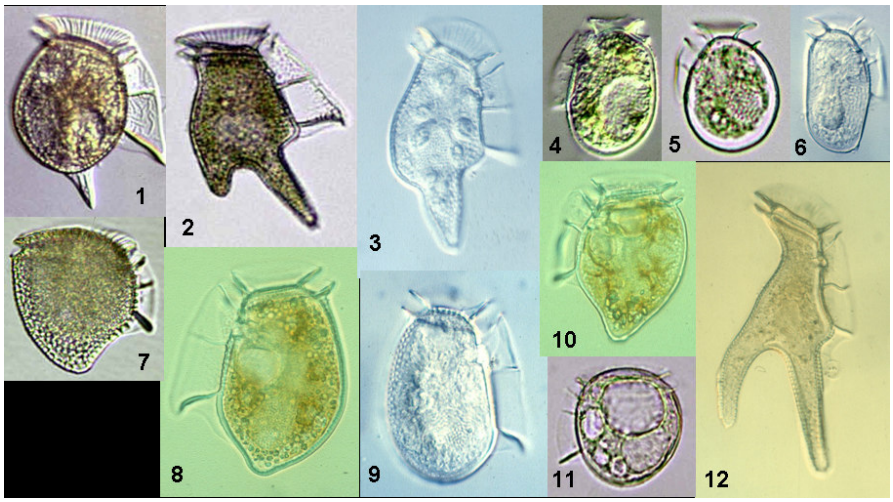
3 Species of *Prymnesium* carry organic scales on the cell surface, and the micro-morphology of these scales which can be observed only by electron microscopy, is used for species identification. What is the correct identification of the species shown below?



Select one or more:

- a. Unidentified flagellate
- b. Prymnesium parvum
- c. Prymnesium sp.

4. Several species of *Dinophysis* and *Phalacroma* may cause diarrhoeic shellfish poisoning (DSP). Currently twelve species belonging in these genera are known or suspected to be toxin producers. The photos below (not to scale) show all the potentially toxic species. Identify these species.



es 7 se...

es 4 se...

es 8 se...

es 3 se...

es 6 se...

es 1 se...

es 9 se...

es 1 se...

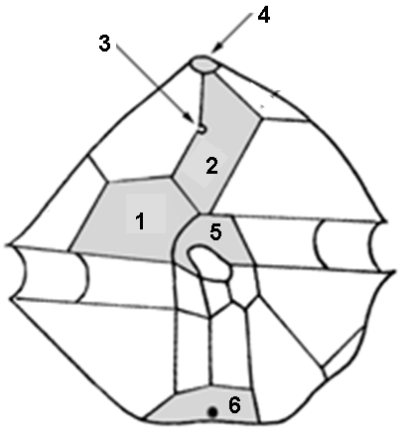
es 1 se...

es 5 se...

es 1 se...

es 2 se...

5. The numbers indicate plates and features important for species identification in *Alexandrium* - name these plates and features



ber 6 indicate se...

ber 1 indicate se...

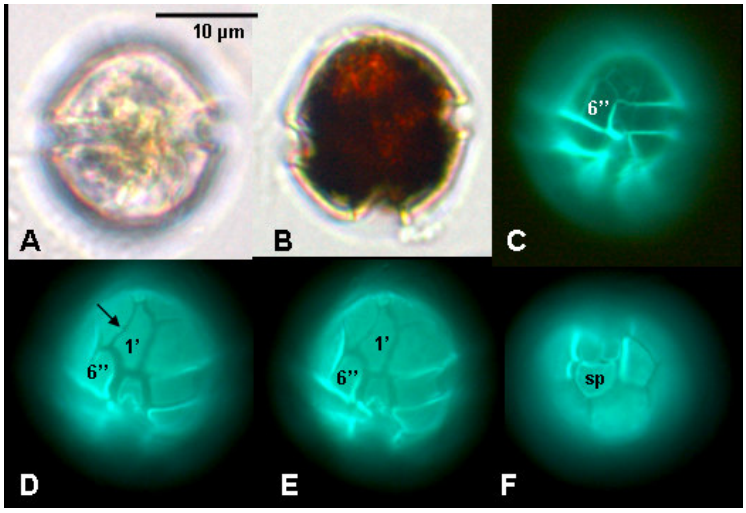
ber 4 indicate se...

ber 5 indicate se...

ber 3 indicate se...

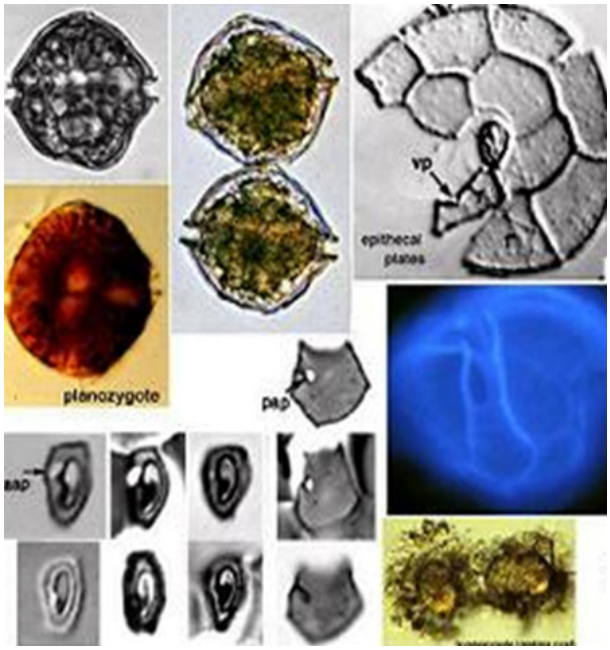
ber 2 indicate se...

6. Identify the species shown (please, note that the answer has to given as genus and species name written in full and spelled correctly for the programme to recognize the correct answer)



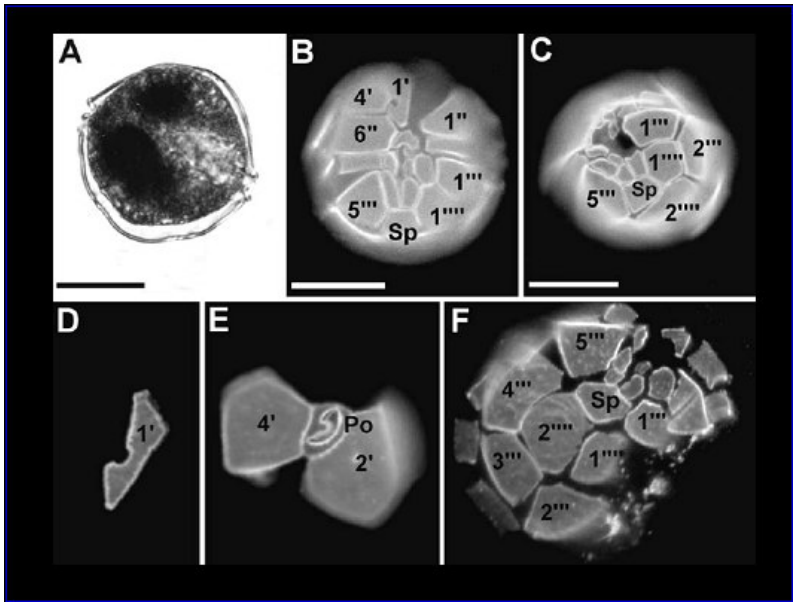
Answer:

7. Identify the species shown (please, note that the answer has to given as genus and species name written in full and spelled correctly for the programme to recognize the correct answer)



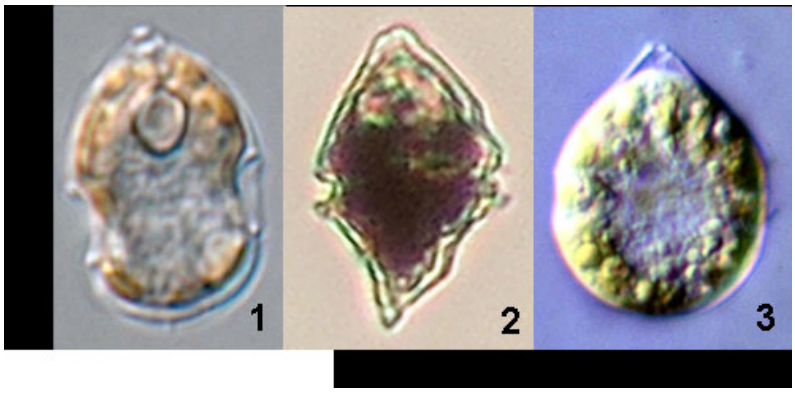
Answer:

8. Identify the species shown (please, note that the answer has to given as genus and species name written in full and spelled correctly for the programme to recognize the correct answer)



Answer:

9. *Azadinium spinosum* is now known to be the producer of azaspiracids which may cause azaspiracid shellfish poisoning (AZP). Speices of *Azadinium* are very small and may be confused with other small armoured species of dinoflagellates. Identify the species shown in Figs 1-3.



fy number se...

fy number se...

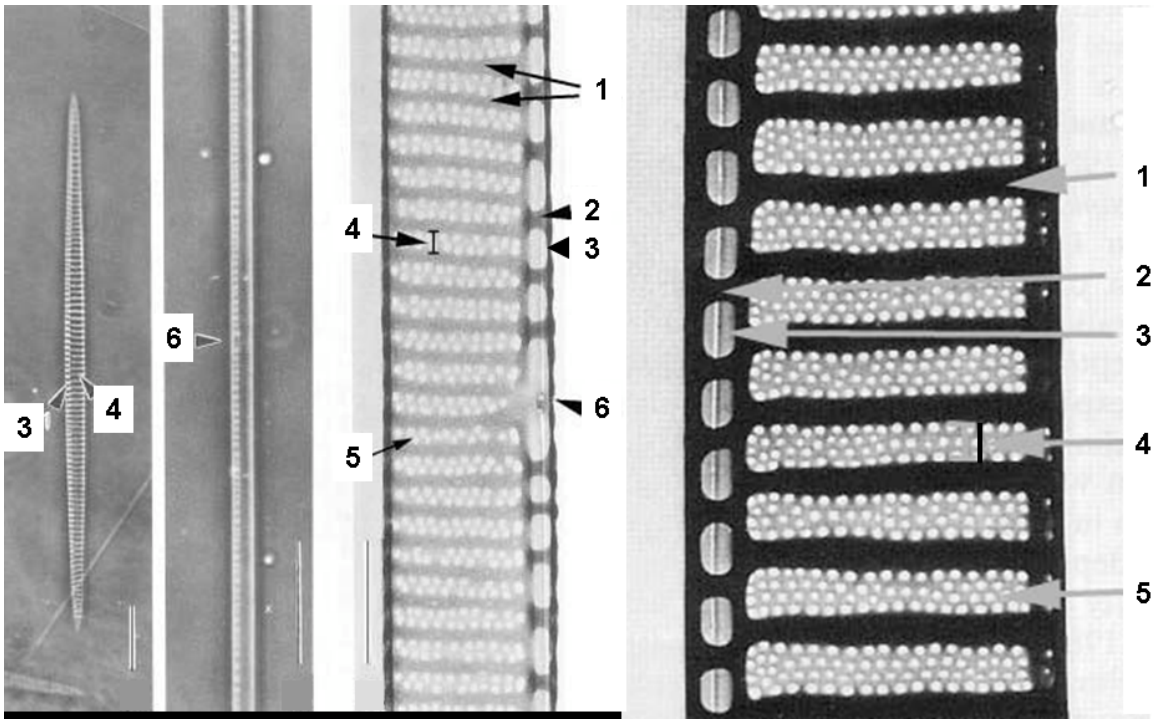
fy number se...

10. *Prorocentrum micans* is a toxic species

Select one:

- True
- False

11. Name the features indicated by the arrows



v head 3 points t se...

v 5 points to se...

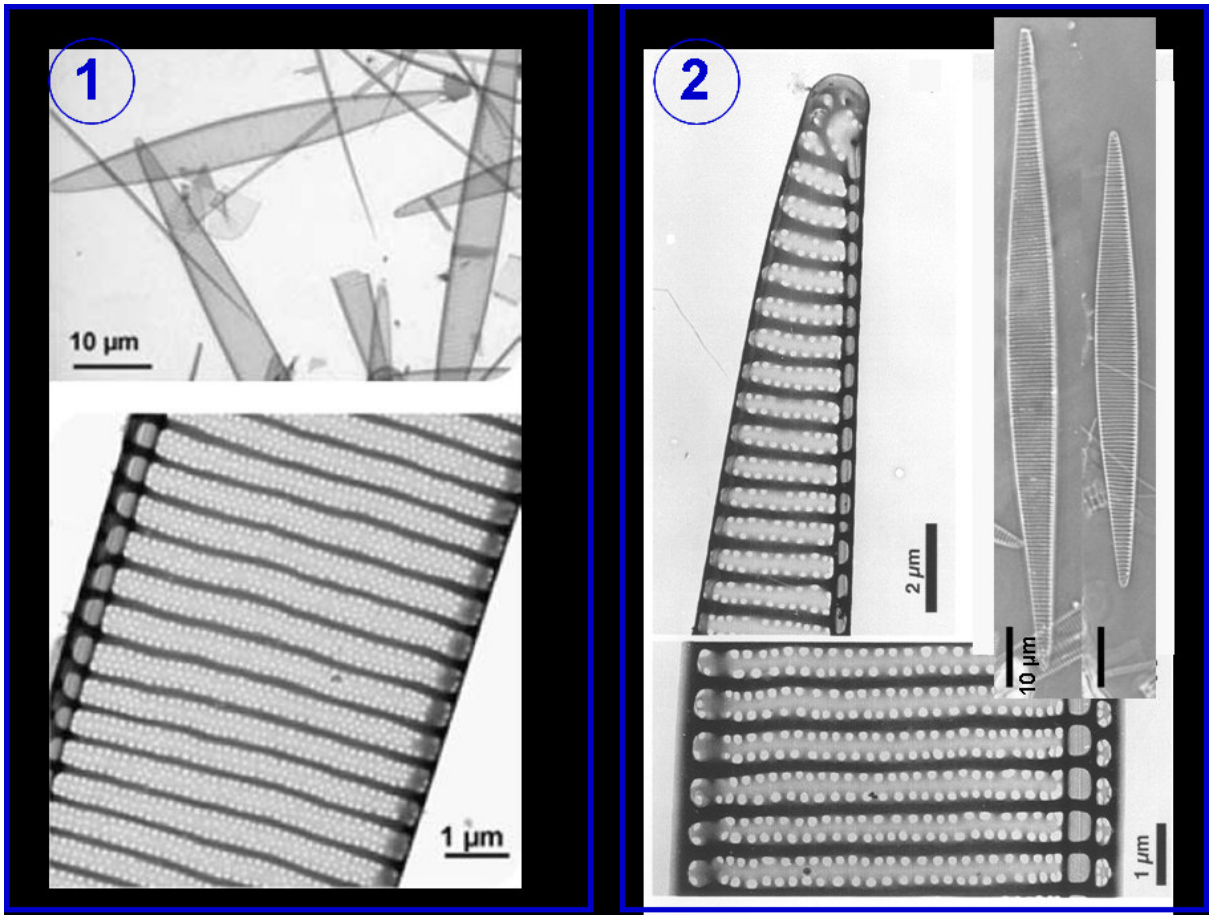
v head 2 points t se...

v 4 points to se...

v 1 points to se...

v 6 points to se...

12. Identify these two species of *Pseudo-nitzschia* (please, note that the answer has to follow the format: 1. P. xxxx - 2. P. xxxx for the programme to recognize correct answers)



Answer:

13. Give a brief description the most important symptoms caused in humans by 1) paralytic shellfish poisoning (PSP); 2) diarrhoeic shellfish poisoning (DSP); 3) amnesic shellfish poisoning (ASP)