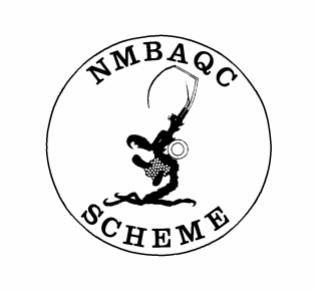
Marine Institute, Ireland



PHYTOPLANKTON ENUMERATION AND IDENTIFICATION ANALYSIS

Ring Test PHY-ICN-08-MI1 Exercise Report, June 2008

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PHY-ICN-08-MI1 VR1.0

Phytoplankton Enumeration and Identification Proficiency Test

Marine Institute (MI) and Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM):

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1. Summary

At the beginning of Feb 2008, all the materials to be able to complete the Intercomparison exercise PHY-ICN-08-MI1 were sent to all participants who had registered through the Bequalm website to this new round of Phytoplankton analysis. The materials included spiked samples, a Taxonomic quiz, a set of instructions, and forms to write results in and to confirm that materials had arrived in perfect conditions.

Analysts were given until the end of February 08 (a month) to return enumeration and identification results to the Marine Institute (MI) Phytoplankton laboratory.

Once all results were received, the Marine Institute Phytoplankton unit collated all the data and organized a workshop where all participants were invited to attend and where the results of the intercomparison were presented. This workshop has a training component and every year guest speakers are invited to give lectures in their area of expertise with a special focus in phytoplankton taxonomy.

Also, the Marine Institute is responsible for producing a report of the exercise which is later published in the Bequalm website and also sent to all participating analysts. This year for the first time we are producing certificates for all participants (see annex V)

This year 29 analysts in 13 labs from across Ireland, Uk and Spain have taken part in this exercise. There has been a good mixture of National Phytoplankton monitoring programmes, environmental agencies, and private consultancy companies taken part in the exercise.

2. Introduction

Biological effects measurements are increasingly being incorporated into national and international environmental monitoring programmes to supplement chemical measurements. The Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM) project, funded by the European Union through the Standards, Measurements and Testing programme of the European Commission, was initiated in 1998. This was in direct response to the requirements of OSPAR to establish a European infrastructure for biological effects QA/QC, in order that laboratories contributing to national and international marine monitoring programmes can attain defined quality standards.

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 in the European North Atlantic area (see

bequalm website www.bequalm.org). Most of the labs taking part in this scheme at present come from the UK and Ireland. This year for the first time a national monitoring programme in Spain, Galician region has taken part. The Marine Institute is accredited to ISO 17025 for Toxic Marine phytoplankton identification and enumeration since 2004, and it recognizes that regular Quality Control assessments are crucial to ensure a high quality output of Phytoplankton data.

In January 2008 an invitation to register for the phytoplankton assemblage component of the community analysis Bequalm scheme was issued to laboratories involved in phytoplankton analysis via the BEQUALM website. This included a timetable showing the dates samples and exercises would be sent to analysts and expected result dates.

3. Participants

In total, twenty nine analysts from thirteen laboratories participated in the exercise PHY-ICN-08-MI1. This code is in accordance to defined protocols in the Marine Institute for the purposes of Quality traceability and auditing. The laboratories taking part were located in Ireland, Northern Ireland, Scotland, England, the Isle of Man and Spain. A complete list of the participating laboratories is given in Appendix I.

4. Materials and Methodology

4.1 Phytoplankton samples and taxonomic quiz

The intercomparison exercise is comprised of two parts:

(a) **Phytoplankton Samples** – A Lugol's preserved sample spiked with the armoured dinoflagellate ***Prorocentrum micans*** was used for the enumeration exercise. A 100ml stock solution was prepared with an approximate concentration of 800 cells per ml. This stock solution was homogenized and divided into 100 X 1ml samples accurately pippeted out with a 1ml eppendorf pipette into sterilin tubes containing 25ml of filtered sterile seawater with Lugol's. Each time that a 1ml aliquot was pippeted out the stock solution was homogenized manually 100 times, following the guidelines set out in the IOC Manual of Harmful Marine Microalgae. Each sample was given a number starting with sample number 1 to number 100. A set of two randomly selected sample bottles was prepared for each analyst. Once prepared, each set of samples was couriered to the analyst. There was a total of 29 analysts at 2 samples each equals 58 samples sent out, the remaining samples were used to calculate the true value for the exercise. 30 samples were

analysed by Senior Lab analyst (Phytoplankton Galway Lab) and the Mean value of these counts was used to calculate the true value of the enumeration exercise and the 3 sigma limits of the population.

(b) **identification exercise** – This year, the participants were given a taxonomic quiz as the identification exercise. The taxonomic quiz consisted of 8 questions. Each question had a set of marks. The total mark for the exercise was 300. Each question was designed to test different identification skills of the participant. Participants were asked not only to identify the pictures but also to identify and name taxonomic characteristics that were typical of what they were identifying. The emphasis, was on toxic species, but some questions were asked on other species that although don't produce toxins, they can be harmful to fish and other marine life. Also, some questions probed analysts to differenciate between toxic and non-toxic species. Question 1 was designed to test analysts on *Dinophysis spp.* As this is such an important toxic Phytoplankton group. This question not only asked to identify the species from photographs but also to name certain taxonomic features with the aid of arrows. Questions 2, 3 and 4 were on armoured Dinoflagellates. Q2 & Q3 on *Alexandrium versus Gonyaulax*, and plate structure of *Alexandrium spp.* Q4 on identifying typical armoured Dinoflagellates to species level. Q5, 6 & 7 were on diatoms. Q5 on identifying some harmful and non-harmful diatoms, the first part on *chaetoceros spp* from the phaeoceros group. Q6 on identifying the odd one out. Q7 was specific to *Pseudonitzschia spp.* As this is another important group of diatoms. Q8 was specific to naked Dinoflagellates and participants were asked to name the genus from a diagrammatic representation supplied.

4.2 Instructions for counting and identification

Detailed instructions were sent for the exercise PHY-ICN-08-MI1. These instructions are attached in Appendix II.

Participants were contacted via e-mail and a number of forms were sent in pdf format. It was essential that all participants had all the materials before starting the exercise. Participants were asked to confirm receipt of samples, sample numbers, sample integrity and receipt of all the forms via fax or e-mail to complete the exercise. FORM 1Checklist to Fax bequalm 08 MI1.pdf

(Annex III) had to be sent via fax/e-mail by all participants to the Marine Institute Phytoplankton lab as confirmation of receipt of materials.

For the enumeration exercise, samples had to be homogenized, settled in a sedimentation chamber and cells counted in the whole chamber, the cell count should be given in cells per litre and the spiked organism identified to species level. Each analyst was sent two samples.

The taxonomic quiz (Annex II) comprised 8 questions and a total of 300 marks, participants had to answer a series of questions regarding the images and diagrams supplied in the quiz and also identify the phytoplankton to either genus or sometimes species level.

All participants were asked to keep a copy of all their results and to post the originals to the Marine Institute Phytoplankton unit senior analyst.

All required results had to be returned in the official results sheets, FORM 3_Enumeration Hardcopy results Bequalm 08 MI1.pdf (Annex IV) and Form 4_Taxonomic quiz Bequalm 08 MI1.pdf (Annex II) via post.

4.3 **Statistical analysis**

Independent statistical analysis was carried out by Dr John Newell from the Department of Mathematics at the National University of Ireland, Galway. The approach taken on this intercomparison was to compile the data from the enumeration results of the different labs and calculate Z-scores (+/- 3 sigma limits) against a reference or true value. This reference value was calculated by the Galway lab from a set of 30 samples set up and analysed in the same manner as the test samples sent to the participants. The reference data was analysed for normality and bias before it was used to compare this value with the participants results. The results were then plotted against the mean reference value +/- 3 sigma limits. Results within the 3 sigma limits would show repeatability between labs and also within labs where there was more than one analyst taking part. Also a series of typical statistical analysis was carried out to test the data levels of agreement, difference and significance of the variance. Results are found in Appendixes III and IV.

The identification exercise results were given in percentages. The pass mark was set at 70%. Results were analysed overall but also different questions were grouped and together analysed to investigate whether analysts where better at answering certain type of questions than others, in relation to identifying certain phytoplankton groups.

5. Results and Discussion

As all participants were given detailed instructions in the setting up and analysis of the samples to be test, and all the test samples were homogenized and set up in the same manner, the variance between the results should mainly be due to individual factors – such as sample set-up, homogenization and counting bias in the analysis of the samples.

5.1 **Phytoplankton cell Counts**

All enumeration results were collated and different aspects of the data were examined statistically. First, to determine the normality of the dataset generated by the Galway lab which would be used as the reference value for all the results, a number of descriptive statistical tests were used.

The Reference Galway data set (Table 1, Appendix III) was plotted using the Anderson-Darling Normality test. See appendix III, Graph 1. This test shows that the reference data suggests symmetry and normality, skewed slightly towards higher cell counts, all this means that the data although has some variance, this variance doesn't indicate a big enough difference between cell counts, so all the cell counts could belong to the same population. This mean is also very close to the gold standard that was set up to achieve the 800 cells/ml or 32000cells/L (mean=30865cells/l), which suggests that the methodology used for homogenizing and setting up samples worked well. The mean or the median in this case could have been used to become the reference value for all results but the mean in this case was used to calculate the Z-scores.

The bias of the reference data was plotted using a boxplot to study the variability, spread and symmetry of the Galway dataset. This is shown in appendix III, graph2. The data is shown as cells/litre and in percentages. The data shows symmetry of the data and spread of around 12% (stdev).

A graph of the reference Galway dataset individual values was plotted showing the mean and the confidence limits. Appendix III Graph 3. This shows the spread of the galway dataset within the 3 sigma limits. These sigma limits were used to mark the upper and lower confidence limits for the exercise.

Table2 in appendix III show the individual results of all the participants. The two charts Graph 4, Appendix III shows the 1st sample cell counts and Graph 5, Appendix III shows the 2nd sample cell counts against the Galway dataset mean and confidence intervals. Both these graphs show that only 3 results were outside the confidence limits.

Graph 6, Appendix III shows a scatter plot of the total dataset of 90 samples in sequential order of sample number to investigate the possibility of a quenching effect on cell concentration when setting up the samples. The results suggest that there is a concentration effect in the samples. Samples were purposely spiked sequentially in sample tubes numbered from 1 to 90 to be able to investigate this effect. Samples with sample numbers from 45 to 90 achieved a slightly higher cell concentration than samples 1 with sample numbers 1 to 45, this would suggest that using 1ml subsamples is too small a volume to spike samples and that a bigger volume should be used in future exercises to avoid this effect.

A test for equal variances for the Galway dataset against the participating labs was used to investigate whether there was a significant difference variance between Galway results and those from the other labs. Graph 7, Appendix III shows that there is a slight significant difference between Galway results and the other labs. There is a systematic mean bias across the participating labs. However this difference is not big enough to suggest that the participating labs were counting a different population to the Galway dataset, but it indicates that are some factors or bias which have affected the cell counts.

The final results of all the analysts have been expressed as Z-scores. See Graph 8, Appendix III. This shows that most results are within 3 sigma limits of the mean. The closest, the value is to zero the better, the agreement with the Galway dataset.

5.2 **Taxonomic quiz**

The taxonomic quiz consisted of 8 questions, each question contained several photographs and/or diagrams and participants were asked to identify to genus/species level or to answer some questions in reference to taxonomic features. Each question had different marks. The total number of marks was 300.

Incorrect answers were given a zero, but no negative marks were given to incorrect answers.

The final results have been tabulated as an overall percentage of correct answers from the total. See Appendix IV, table 1

The quiz results were analysed statistically to study first of all, how the participants did overall but also to investigate whether some participants were better at answering question on any particular phytoplankton groups.

The exercise could be divided in four areas. Question 1 is a standalone question on *Dinophysis spp.*, a very important group of toxic Dinoflagellates. Q2, 3 & 4 could be pooled together as questions on armoured Dinoflagellates. Q5, 6 & 7 were questions on diatoms and Q8 a question on naked Dinoflagellates.

Table 2, Appendix IV shows the descriptive statistics in terms of percentages on these 4 group of questions. It shows that the percentage of correct answers for all questions was high, but it shows that this percentage was lower in questions 5, 6, 7 & 8.

Graph 1, Appendix IV is the graphical representation with boxplots of table 2.

Table 3, Appendix IV shows the cumulative percentage of analysts and how they perform in the different group of questions. In question 1, only one analyst performed below 70%, in Questions 2, 3 & 4 only one analyst performed below 70%. In questions 5, 6 & 7 five analysts performed below 70% and in Q8 7 analysts were below the 70% mark. This suggests that questions 5 to 8 were either tougher questions than 1 to 4 or/and that analysts are better at identifying armoured Dinoflagellates than they are at identifying diatoms and naked Dinoflagellates.

Table 4, Appendix IV is a one way Anova test, this test assumes that the data is normally distributed, the samples are independent and the variance of the population must be equal. As the F- value is greater than 1 (5.97) that would indicate that differences between mean classes exist, that is differences between question groupings, as the P value is very small (P=0.001) it indicates that the probability of this difference is not due to chance alone. That means, that there is a difference between the mean classes.

Graph 2, Appendix IV illustrates very well this point. The fitted means of the four question groupings suggest that participants answered better the first 4 questions and less well questions 5 to 8.

Table 5, Appendix IV shows the participants marks as a percentage in graphical form, to show that most participants scored quite high. Only 6 participants scored below the 80% mark.

5.3 **Performance evaluation**

In the Enumeration part of the exercise, most of the analysts taking part performed to within 3 sigma limits of the mean as set by a reference value from the Galway Dataset.
2 analysts out of 29 were outside the 3 sigma limits of the population.

In the identification part of the exercise, most analysts exceeded the 70% pass mark. Overall, 16 analysts out of 29 scored over 90%, 7 analysts scored over 80%, 3 analysts above the pass mark of 70% and 3 analysts scored below 70%.
The score in question 1 was very good with only 1 analyst scoring below 70% and 18 analysts scoring full marks/.
In questions 2, 3 & 4 only 1 analyst scored below 70% with 27 analysts scoring above 80 and 90%.
In questions 5, 6 & 7 five analysts scoring below 70% and 16 analysts above 80 and 90% mark.
In question 8 seven analysts scored below 70% and 22 analysts above 80 and 90% mark.
Overall, the scores were quite high, but participants tended to score less well in questions 5, 6, 7 & 8.

6. Conclusions and Recommendations

In summary, the enumeration exercise shows that the Galway dataset is normally distributed, that there isn't significant mean bias of the Galway results compared to the Gold standard.
It shows that the repeatability between labs is quite good and that sample homogenization worked well, although there seem to be some kind of order effect on the Galway dataset which would suggest that some concentration quenching could have taken place when subsampling and this could be due to the small amount of spiked material pippeted out per sample. This effect must be avoided in future exercises by pippeting out larger volumes of spiked material.
There is also some evidence, but not too strong that there was a systematic mean bias across the participating labs compared to the Galway dataset.

The identification exercise shows that overall all participants did quite well, with over 50% of the participants scoring above 90%.

The group of questions 1 and questions 2, 3 & 4 were significantly different in average to the group of questions 5, 6 & 7 and question 8. The mean of correct answers for the 1 to 4 questions was in general higher than the mean of correct answers for the 5 to 8 questions.

Question 1 obtained the highest score of correct answers and question 8 the lowest.

In general, this exercise suggests that participants seem to be more knowledgeable on the identification of armoured Dinoflagellates (Q1 to 4) against the identification of diatoms (Q5 to 7) and naked Dinoflagellates (Q8).

Overall, this proficiency test has proven very successful both in terms of interest from labs involved in phytoplankton analysis and overall results.

On the 17th of April, 2008 the Marine Institute hosted the 3rd workshop of the BEQUALM Phytoplankton Intercomparison PHY-ICN-08-MI1. At this meeting, the results of the intercomparison and future directions of the exercise were discussed. See Annex I the agenda for the workshop.

Some recommendations were put forward by the participants to improve and further enhance this proficiency testing scheme. It was suggested that a statistician should be appointed at the experimental design stage of the exercise to help setting up the exercise from the statistical view point.

It was also suggested that both the enumeration and identification exercise could be done together by spiking a sample with several phytoplankton species.

It was suggested, that in order to test reproducibility of a sample between and within labs, all participants should analyse the same set of samples for the exercise. In theory, participants thought that this would be a good experiment, but in practical terms it would be difficult to work it out as all participants would have to be flown to the same lab to complete the analysis.

It was also suggested to test the cell counting method by asking analysts to use different counting strategies. Also, to use different type of organisms to spike samples with, like chain forming organisms, to use larger cell concentrations to test the robustness of the method and to study sample inversion and settling bias of the method.

The Marine Institute will be discussing these ideas in preparation for the next exercise which will take place in the 1st quarter of 2009

Appendix I: Participating laboratories

Table showing participating laboratories in the proficiency test PHY-ICN-08-MI1.

Laboratory	Country	No. Of Participants
Marine Institute, Bantry	Ireland	1
Marine Institute, Galway	Ireland	3
Environmental Protection Agency, Dublin	Ireland	2
Environment & Heritage Service, Lisburn	N. Ireland	1
AFBI, Belfast	N. Ireland	4
FRS Marine Laboratory, Aberdeen	Scotland	5
SEPA, Riccarton	Scotland	3
SAMS, Oban	Scotland	2
CEFAS Laboratory, Lowestoft	England	4
CEFAS Laboratory, Weymouth	England	1
Department of Local Government and the Environment (DLGE)	Isle of Man	1
Intecmar	Spain	1
Jacobs Aquatic Ltd	England	1
	TOTAL	**29**

Appendix II: Instructions

Marine Institute BEQUALM Phytoplankton Proficiency Test PHY-ICN-08-MI1

Instructions for Sample Preparation, Counting, Calculations and Identification

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

1. Introduction

This 4th Phytoplankton Ring Test is being conducted to determine any inter-laboratory variations in the enumeration and identification of Marine Phytoplankton species within and between labs. Please adhere to the following instructions strictly. Please note that these instructions are specific for this ring test.

2. Preliminary Check and Time Restrictions

Upon receipt of the samples please make sure that you have received everything listed in the Return Slip form (Form 1). Complete the form and send it by Fax to the Marine Institute, Galway. Fax No. 00353 91 387237. A receipt of Fax is necessary for the Marine Institute to validate the test process for your lab.

Hardcopy results (forms 3 & 4) **must be received** by the Marine Institute by **February 29th 2008**. ***Hardcopy results received after February 29th 2008 will not be included in the final report.***

3. Equipment

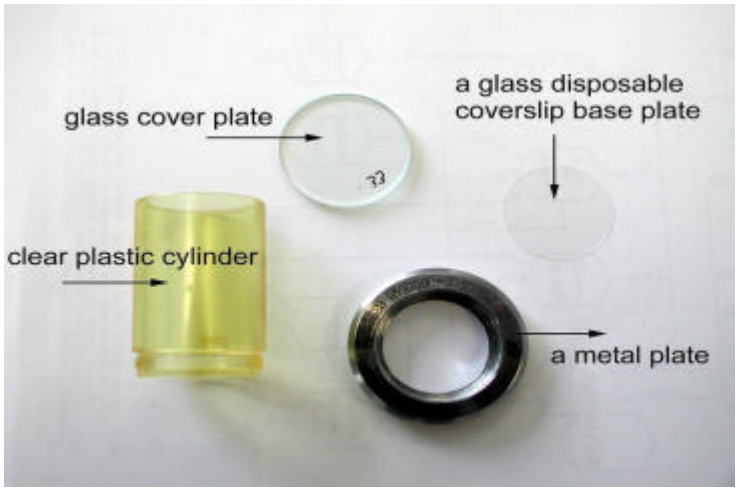
- Two Utermöhl counting chambers. **25ml sedimentation chambers should be used preferably.**
- Base plates and glass covers.

- Inverted Microscope equipped with long distance working lenses and condenser of Numerical Aperture (NA) of 0.3 or similar.

4. 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). Two sets will be required.

Fig 1: Sedimentation counting chamber



4.1 Place a disposable glass base plate on a cleaned metal plate.

4.2 Screw the plastic cylinder into the metal plate. Extra care should be taken when setting up chambers. Glass base plates are fragile and break easily causing cuts and grazes. Careless handling can easily damage metal plates, and render them unusable.

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

4.3 To set up a sample for analyses invert the sample tube gently at least five times to ensure that the contents are evenly distributed throughout the sample. Do not shake the tube to avoid air bubbles.

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

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

4.3.3 Label the sedimentation chamber which should correspond with the sample label.

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

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

4.5 Set the chamber on the inverted microscope and analyse.

4.6 Enumeration results for each sample are to be entered on **Form 3** Enumeration Hardcopy Results Sheet.

5. Counting Strategy

For this test a whole base plate count should be conducted.

5.1 The whole base plate of the chamber is counted by enumerating all cells within a continuous motion of field of view for the entire area of the base plate. This can be done by going from left to right or top to bottom, in a continuous series of sinuous movements in such a manner that the whole base plate is observed (Fig 2 and 3). Make sure the field of view does not exclude any uncounted area or overlap any area already counted.

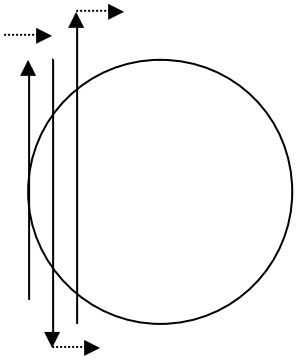


Fig 2

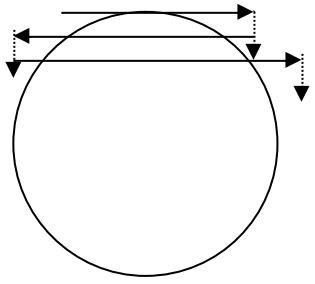


Fig 3

6. Cells

The samples for this intercomparison have been spiked with live cell culture material. This material 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.

A set of subsamples will be used to set the true value of the sample with 2 SD. As all the subsamples should be representative of the original sample, the purpose of this study would be to study reproducibility of results between and within labs.

One marine phytoplankton species have been spiked for the purpose of enumeration on this exercise. This species should be identified positively before continuing the cell counts.

It is very important to **spend some time** becoming familiar with 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 slightly different to the known standard vegetative cell type. Also note that cells will vary in size and shape. Some cells will appear smaller than others, this is normal in culture conditions, please make sure to count these.

Cells undergoing division that are not fully separated should be counted as one cell although they may appear as 2 distinctive cells jointed dorsally.

Each sample should contain approximately a volume of 26ml, this means that a very small amount of sample may be left behind in the sample tube when the sample is poured into the 25ml sedimentation chamber. This is normal and should be the same for all the samples. 25ml Sedimentation chambers have a volume uncertainty of +/- 1ml.

A 26ml sample should be sufficient to fill a 25ml sedimentation chamber to the top. Although some evaporation may occur during transport and settlement this should be minimal.

Please note: when converting cells per sample to cells per litre, use 25ml as the chamber volume rather than 26ml

As soon as samples are received participants are asked to check the samples for leaks or breakages. If a sample appears half full or completely broken, please inform Rafael.salas@marine.ie so we can send you another set of samples straightaway.

7. Conversion Calculations of Cell Counts

The number of cells found should be converted to cells per Litre.

Please show calculation step in Form 3, section A.

8. Identification

A taxonomic quiz has been designed for the identification exercise. A number of photomicrographs, diagrams and pictures will be provided for the exercise.

The purpose of this exercise is to identify the characteristics that allow you to positively identify marine phytoplankton rather than only just to identify the species from the photomicrographs.

In this exercise participants will have to identify not only the species name of marine phytoplankton species but also to identify correctly morphological and taxonomic characteristics unique to these marine phytoplankton species.

Please identify and include your results on the Identification Sheet (**Form 4**).

The identification exercise carries a total of 300 marks.

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.

Examples of this are: *Prorocentrum cordatum* better known as *P.minimum* or *Akashiwo sanguinea* also known as *Gymnodinium splendens*

9. Points to Remember:

1. **All results must be the analysts own work. Conferring with other analysts is not allowed.**
2. **Before sending the original results in the post, make a copy of your own results just in case they get lost in the post.**
3. Form 3: Enumeration Hardcopy Results Sheet, and Form 4: taxonomic quiz must be received by the Marine Institute, Phytoplankton unit by **Friday Feb 29th 2008.**

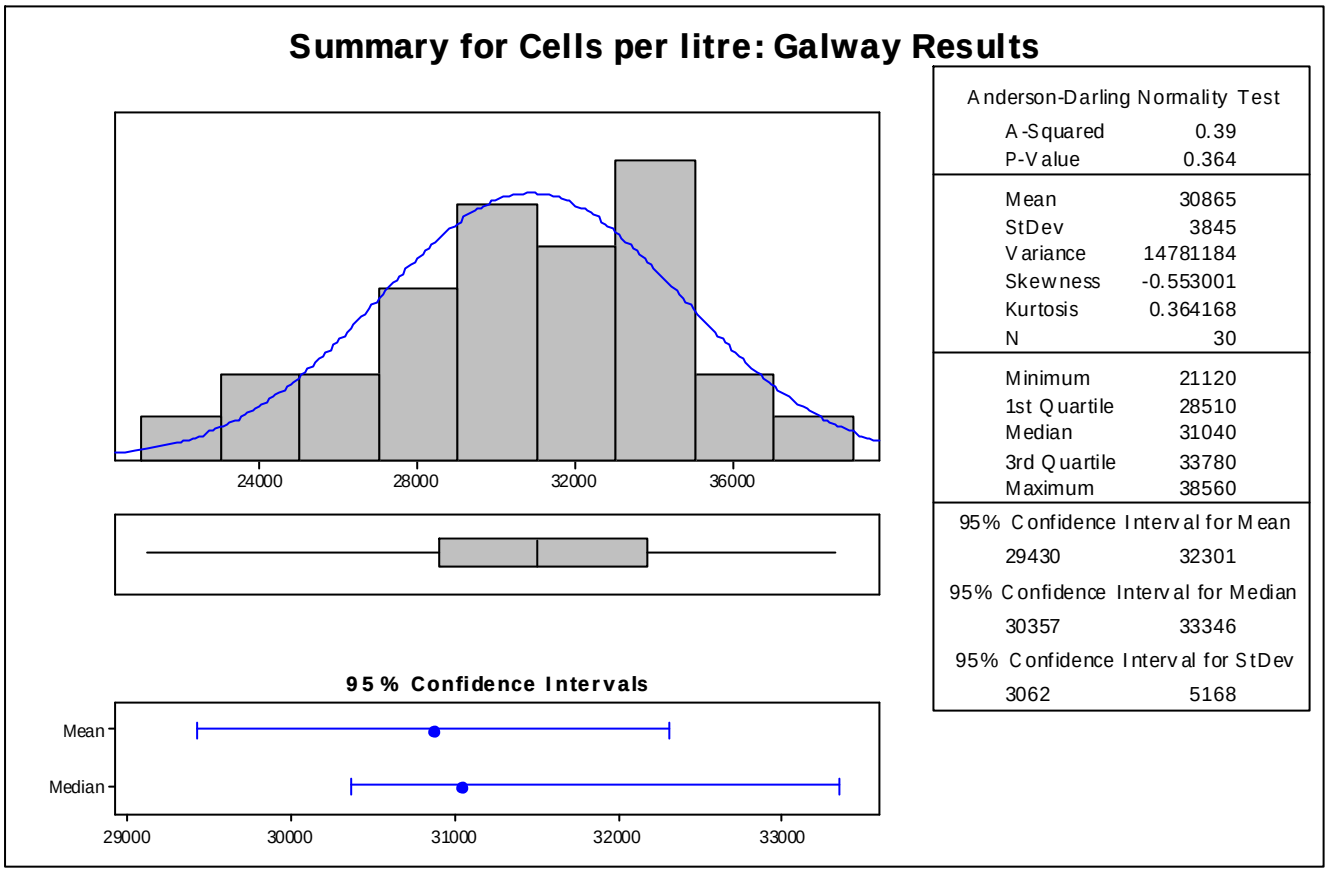
Appendix III: Detailed results of the enumeration test

Table 1: Galway Lab Sample dataset count used as reference value

Inter-comparison Samples 2008 **Analyst: Rafael Salas**

	Sample No	Date Settled	Date Analysed	Count	Comments
1	3	29/01/2008	30/01/2008	766	30640
2	64	29/01/2008	30/01/2008	890	35600
3	38	29/01/2008	30/01/2008	855	34200
4	40	29/01/2008	30/01/2008	623	24920
5	44	29/01/2008	30/01/2008	592	23680
6	26	29/01/2008	30/01/2008	528	21120
7	13	05/01/2008	07/02/2008	663	26520
8	25	05/01/2008	07/02/2008	774	30960
9	63	05/01/2008	07/02/2008	762	30480
10	57	05/01/2008	07/02/2008	683	27320
11	89	05/01/2008	07/02/2008	844	33760
12	43	05/01/2008	08/02/2008	779	31160
13	19	05/01/2008	08/02/2008	782	31280
14	6	05/01/2008	08/02/2008	694	27760
15	28	05/01/2008	08/02/2008	670	26800
16	47	11/02/2008	12/01/2008	758	30320
17	82	11/02/2008	12/01/2008	819	32760
18	35	11/02/2008	12/01/2008	778	31120
19	59	11/02/2008	12/01/2008	768	30720
20	67	11/02/2008	14/02/2008	846	33840
21	60	11/02/2008	14/02/2008	719	28760
22	84	11/02/2008	14/02/2008	838	33520
23	87	11/02/2008	14/02/2008	964	38560
24	55	11/02/2008	14/02/2008	870	34800
25	78	13/02/2008	15/02/2008	882	35280
26	2	13/02/2008	15/02/2008	721	28840
27	69	13/02/2008	15/02/2008	763	30520
28	30	13/02/2008	15/02/2008	814	32560
29	45	15/02/2008	18/02/2008	864	34560
30	71	15/02/2008	18/02/2008	840	33600
				Mean	30865

Graph 1: Anderson-Darling Normality Test of Galway Sample set count



Anderson-Darling Normality Test	
A-Squared	0.39
P-Value	0.364
Mean	30865
StDev	3845
Variance	14781184
Skewness	-0.553001
Kurtosis	0.364168
N	30
Minimum	21120
1st Quartile	28510
Median	31040
3rd Quartile	33780
Maximum	38560
95% Confidence Interval for Mean	
29430	32301
95% Confidence Interval for Median	
30357	33346
95% Confidence Interval for StDev	
3062	5168

Graph 2: Bias of Galway results

Descriptive Statistics: Bias, Bias(%)

Variable	N	Mean	StDev	Median
Bias	30	-1135	3845	-960
Bias(%)	30	-3.55	12.01	-3.00

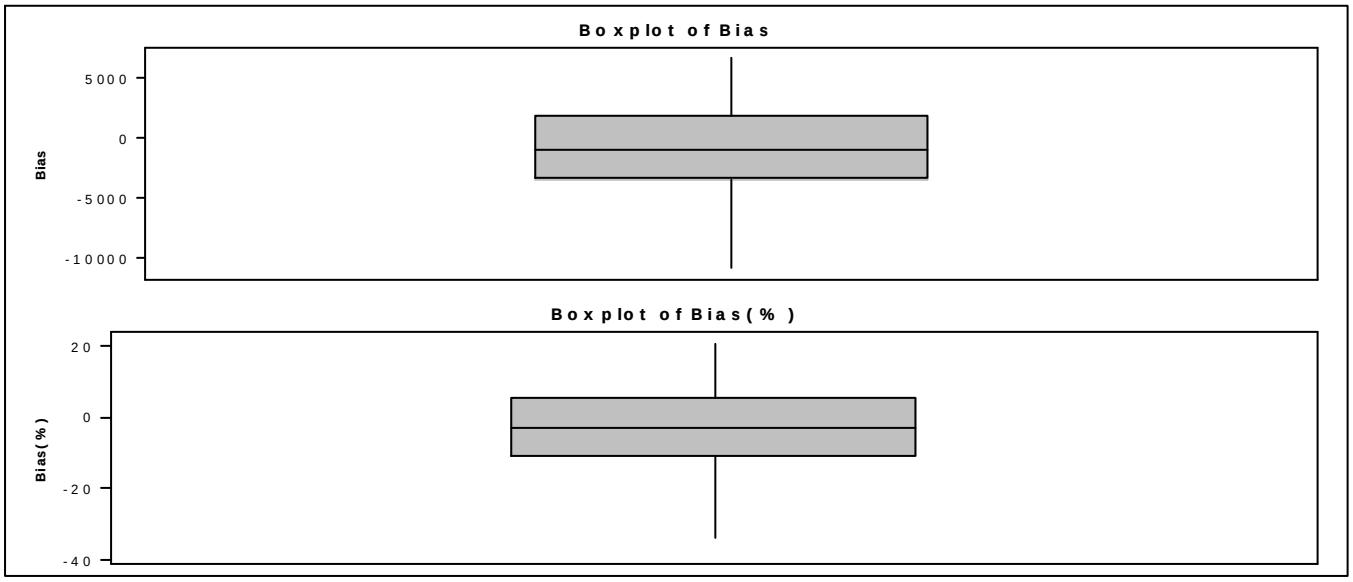
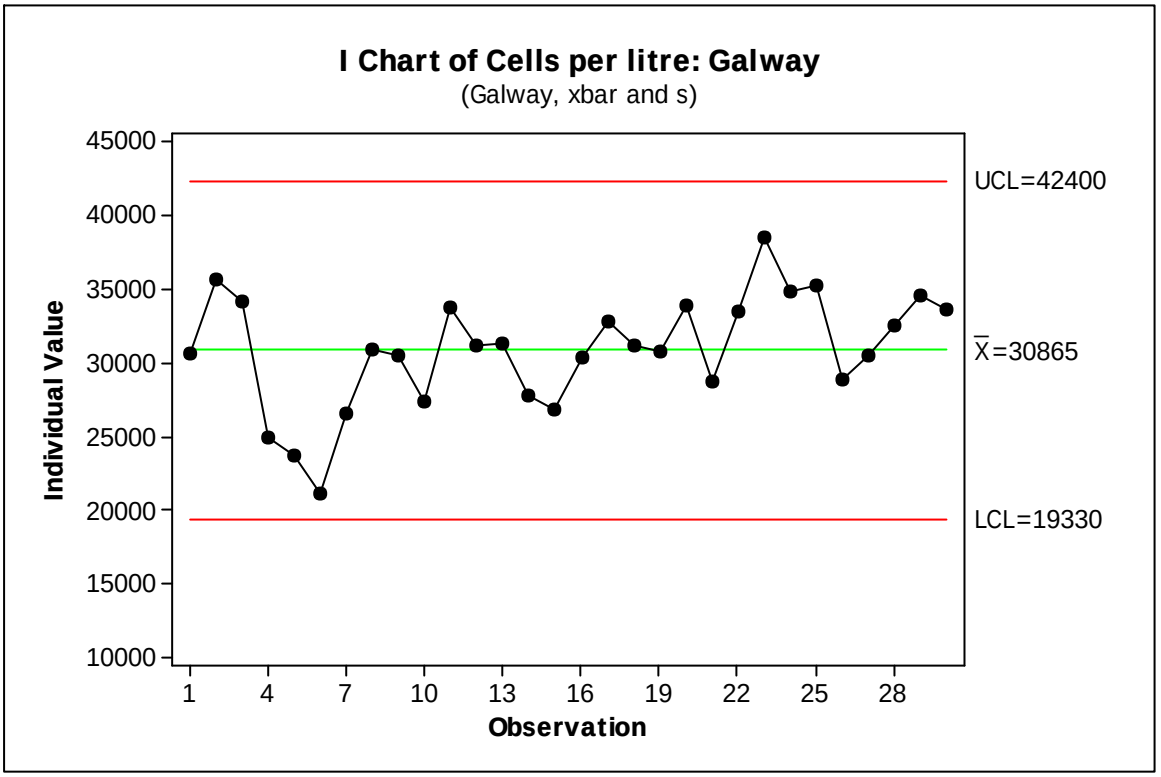


Table 2: Participants sample cell counts and concentration in cells per litre.

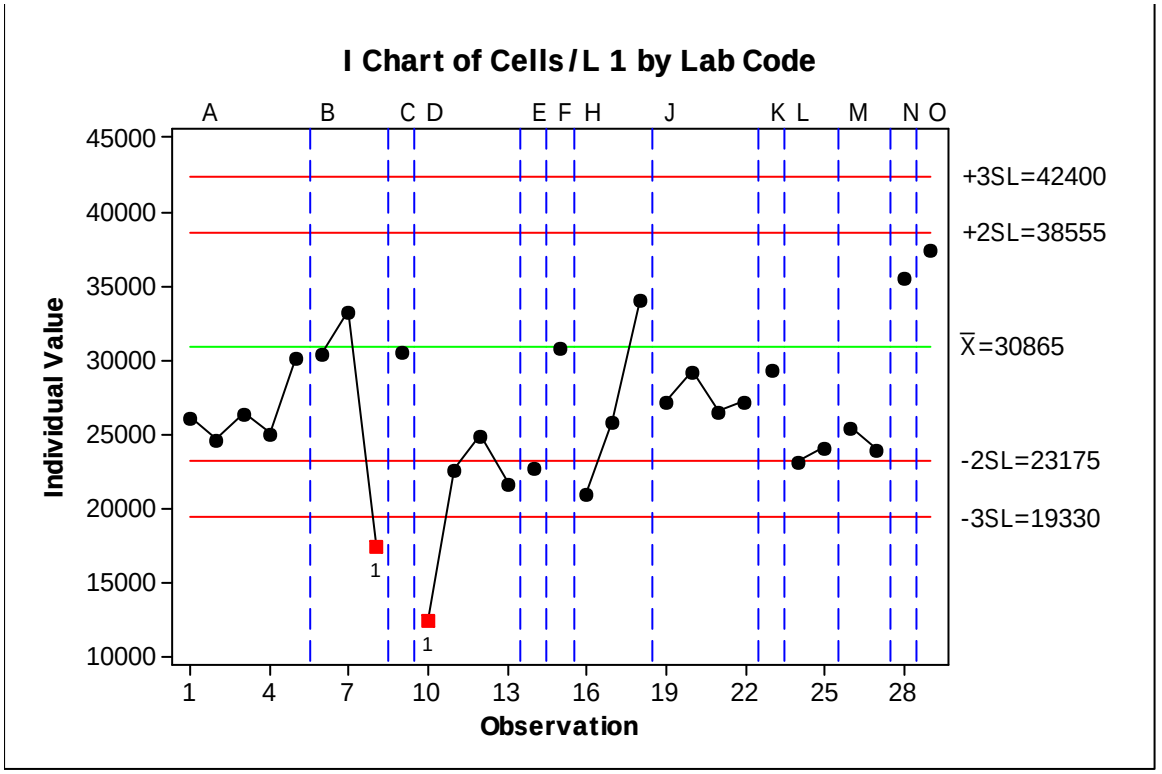
ANALYST CODE	SAMPLE CODE		Number of cells		Cells/L		ANALYST CODE	SAMPLE CODE		Number of cells		Cells/L	
			Cell count	Cell count	Cell count	Cell count				Cell count	Cell count	Cell count	Cell count
c	1	34	260	220	26000	22000	r	41	74	522	917	20880	36680
i	7	65	614	669	24560	26760	a	36	62	644	498	25760	19920
p	24	86	263	393	26300	39300	o	49	52	849	753	33960	30120
v	23	79	250	376	25000	37600	e	16	26	678	655	27120	26200
q	5	48	301	322	30100	32200	m	29	53	727	692	29080	27680
b	9	51	760	905	30400	36200	s	14	54	660	762	26400	30480
j	4	70	829	884	33160	35360	x	31	76	676	741	27040	29640
y	10	42	433	no count	17320	no count	f	17	80	731	865	29240	34600
k	33	85	763	715	30520	28600	h	20	77	577	690	23080	27600
d	12	46	308	444	12320	17760	t	21	90	240	369	24000	36900
l	58	59	564	762	22560	30480	g	37	61	633	663	25320	26520
w	27	88	621	813	24840	32520	n	22	75	597	809	23880	32360
ch	25	89	539	798	21560	31920	u	39	72	886	873	35440	34920
ñ	32	66	567	616	22680	24640	ß	3	38	373	982	37300	39280
z	11	56	767	772	30680	30880							

*Please note that most analysts used 25ml sedimentation chambers but some analysts used 10ml sedimentation chambers to carry out the cell counts. Analysts c, p, v, q, and t used 10ml sedimentation chambers (Multiplication factor(MF)=100 to achieve cells/litre). Analyst i used a reduction step and analysed 25ml in a 10ml sedimentation chamber (MF=40). Analyst ß analysed one sample in a 25ml sedimentation Chamber and one sample in a 10ml sedimentation Chamber.

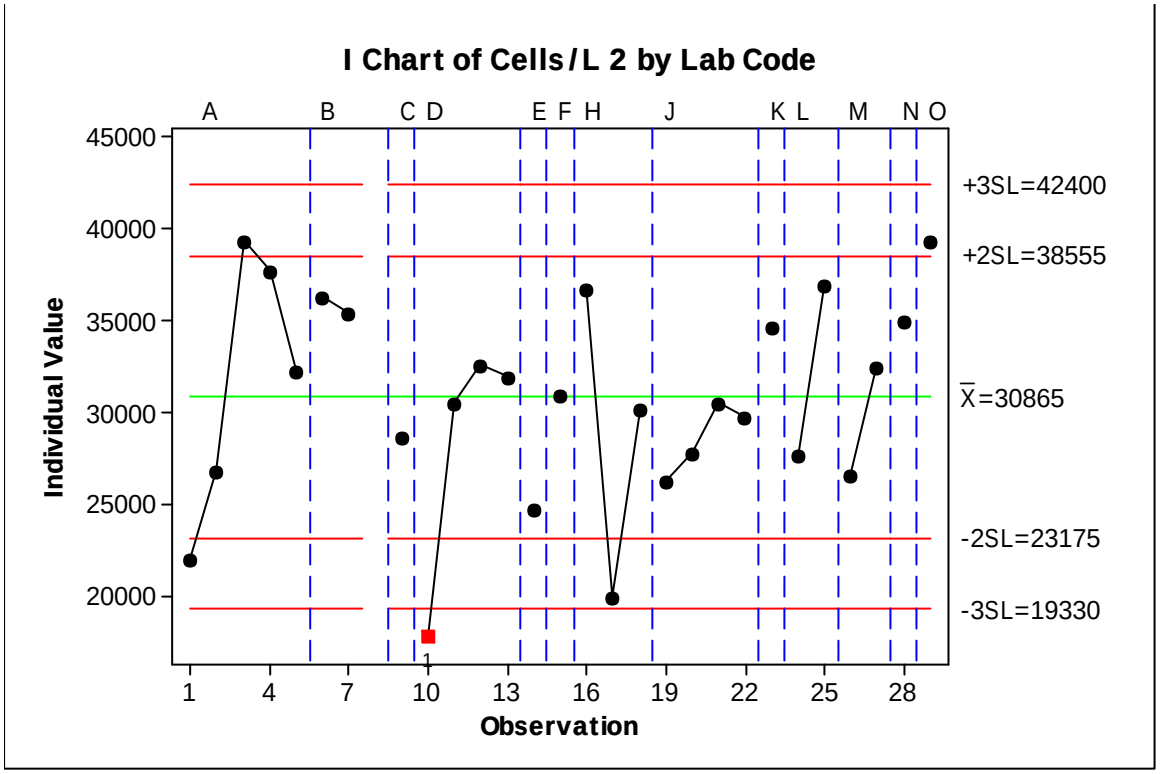
Graph 3 : Plot of the Galway counts based on Mean +/- 3 sigma limits



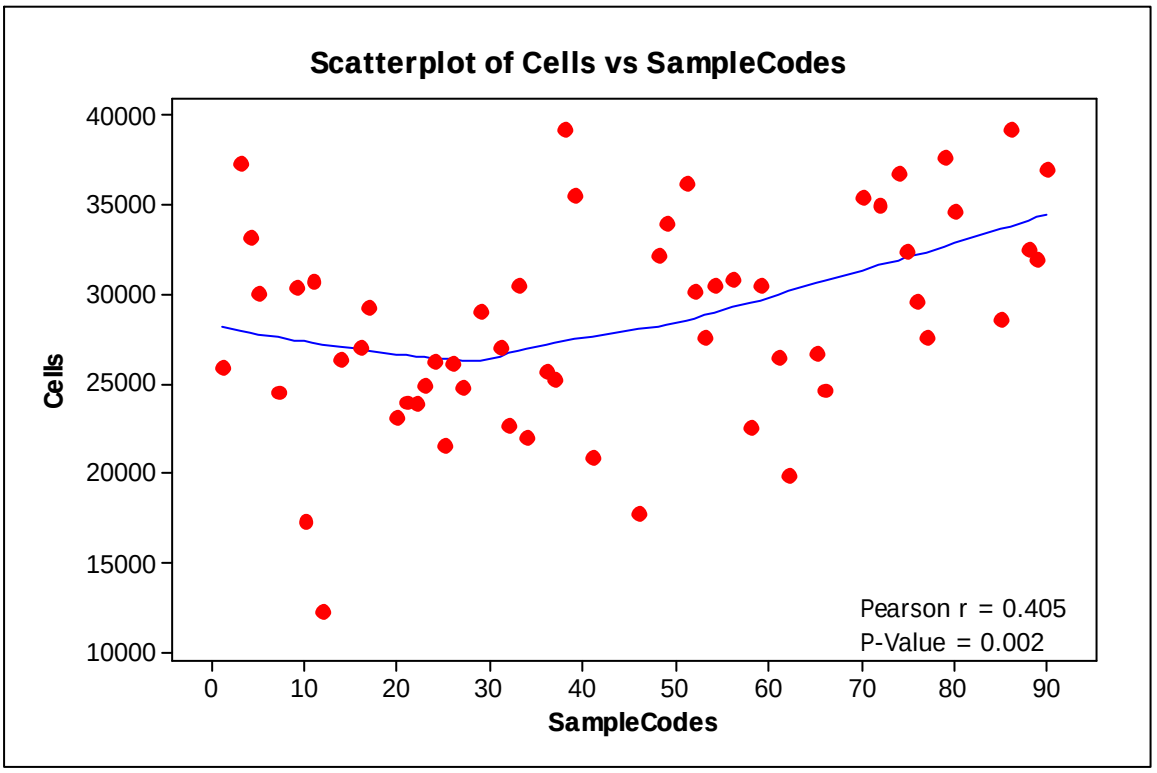
Graph 4: Plot of Participant labs 1st sample results against Galway Mean +/- 2SL and +/- 3 Sigma limits



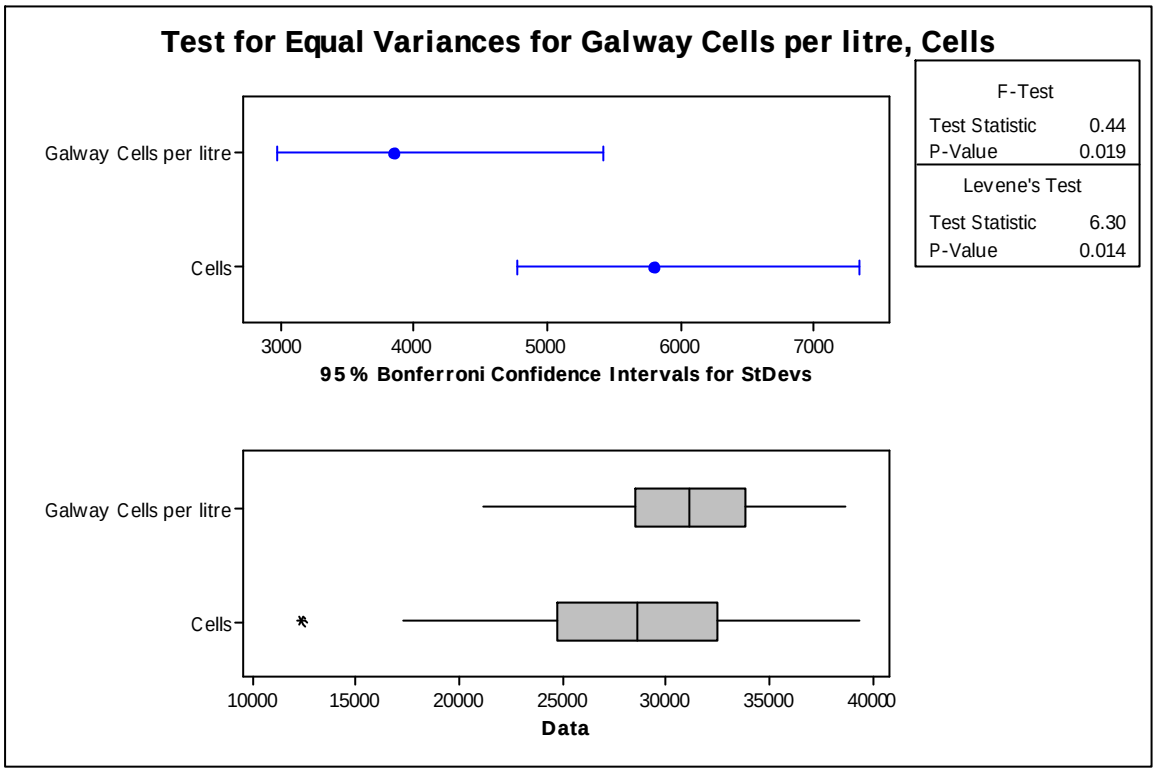
Graph 5: Plot of Participant labs 2nd sample results against Galway Mean +/- 2SD and +/- 3 Sigma limits



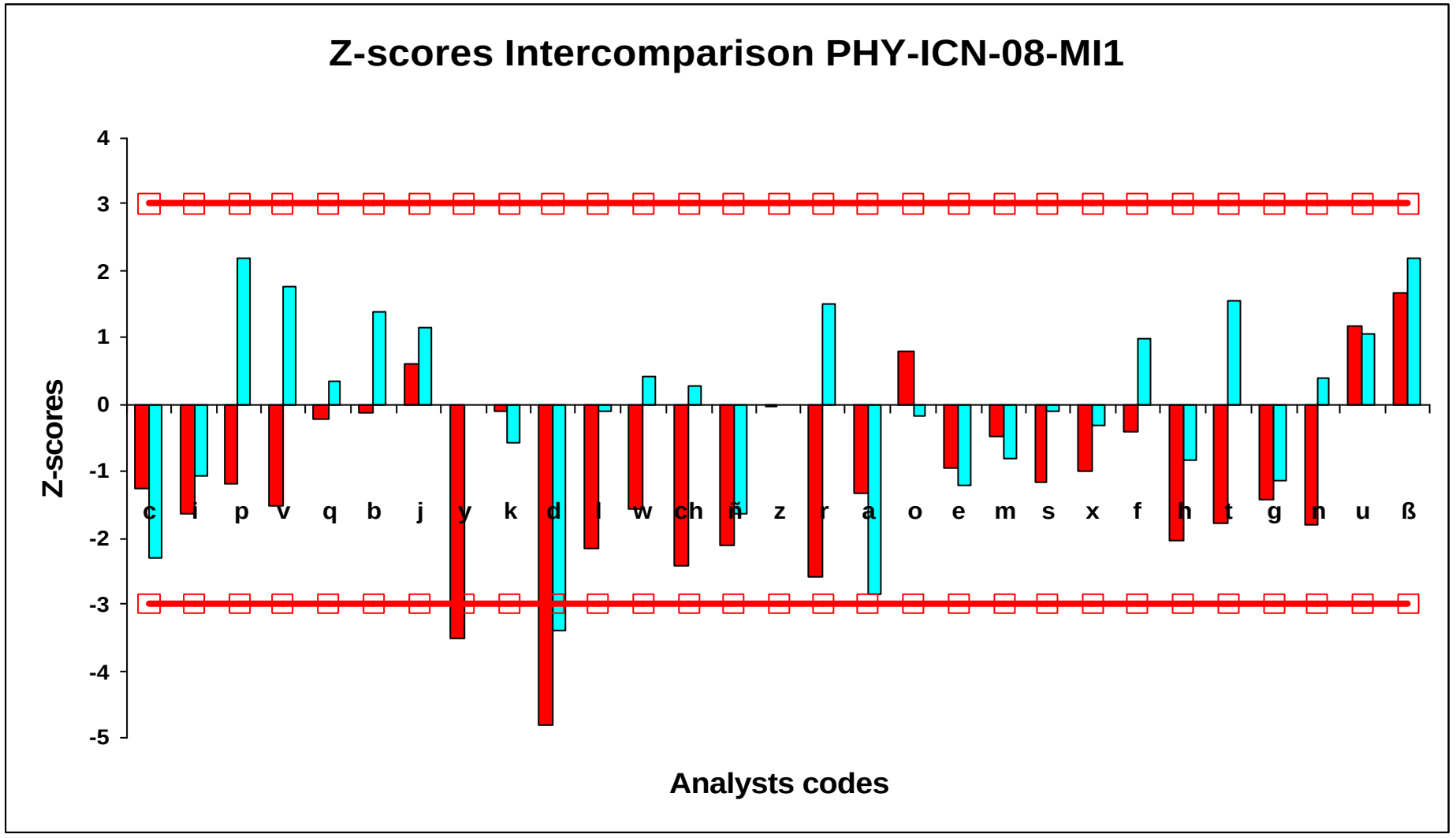
Graph 6: Scatter plot of total data set of 90 samples in sequential order



Graph 7: Test for equal variances between Galway dataset versus participating labs



Graph 8: Z-scores of participants by analyst code



Appendix IV: Detailed results of the identification test

Table 1: Identification results by analyst code

ANALYST CODE	Question 1 (60 marks)												Question 2 (20 marks)			Question 3 (20 marks)				Question 4 (50 marks)									
	A		B		C		D		E		F									A		B		C		D		E	
	sp.	ft	sp.	ft	sp.	ft	sp.	ft	sp.	ft	sp.	ft	1a	1b	2a	a	b	c	d	gen	sp.	gen	sp.	gen	sp.	gen	sp.	gen	sp.
c	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
i	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	5	5	5	5	0	5	5	5	5	5
p	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	0	5	5	5	0	5	5	5	5	5	5	5	5	5
v	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	0	5	5	5	5	5	5	5	5	5
q	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	0	5	5	5	5	5	5	5	5	5
b	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	5	5	5	5	0	5	5	5	5	5
j	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	5	5	5	5	0	5	5	5	5	5
y	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	0	0	5	5	5	5	5	5	5	5	5	5	5	5
k	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	5	5	5	5	0	5	5	5	5	5
d	5	5	5	0	5	5	5	5	5	5	5	5	5	5	10	5	0	0	5	5	5	5	5	5	0	5	5	5	5	5
l	5	5	5	5	5	5	5	0	0	5	5	5	5	5	0	5	5	5	5	5	5	5	5	5	0	5	5	5	5	5
w	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	5	5	5	5	5	5	5	5	5	0	5	5	5	5	5
ch	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	5	0	5	5	5	5	5	0	5	5	5	5	5
ñ	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	0	0	5	5	5	0	5	5	5	5	5
z	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	0	5	5	5	5	5	5	5	5	5
r	5	5	5	5	5	5	5	5	5	5	5	0	5	5	10	5	5	0	0	5	5	5	5	5	5	5	5	5	5	5
a	5	5	5	0	5	5	5	0	5	5	5	5	5	5	10	5	5	0	0	5	5	5	5	5	5	5	5	5	5	5
o	0	5	0	5	0	5	5	5	5	5	5	5	5	5	10	5	5	0	5	5	5	5	5	5	0	5	5	5	5	5
e	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
m	5	5	5	0	5	5	5	5	5	5	5	5	5	5	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
s	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	5	5	5	5	0	5	5	5	5	5
x	5	5	0	0	5	5	5	0	5	0	5	5	0	0	0	5	0	0	5	5	5	5	5	5	0	5	5	5	5	0
f	5	5	5	0	5	5	5	5	0	5	5	5	5	5	10	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
h	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	0	5	5	5	5	5	5	5	5	5	5	5	5
t	5	5	5	5	0	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
g	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
n	5	5	5	5	5	5	5	0	5	5	5	5	5	5	0	5	5	5	5	5	0	5	5	5	5	5	5	5	5	5
u	5	5	5	5	5	5	5	5	5	5	5	5	5	5	10	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
ß	5	5	5	0	5	0	5	5	5	5	5	5	5	5	5	5	5	5	5	0	0	5	5	5	5	5	5	5	5	5

Table 1: Identification results by analyst code (Continue from previous page)

Question 5 (70 marks)														Question 6 (20 marks)	Question 7 (30 marks)			Question 8 (30 marks)						Total out of 300marks	%	ANALYST CODE
A		B		C		D		E		F		G														
gen	sp.	gen	sp.	gen	sp.	gen	sp.	gen	sp.	gen	sp.	gen	sp.	Circle answer	A	B	C	A	B	C	D	E	F			
5	0	5	0	5	5	5	5	5	5	5	0	5	5	20	10	10	10	5	5	5	5	5	5	285	95	c
5	5	5	5	5	5	5	5	5	5	5	0	5	5	20	10	10	10	5	0	5	5	5	5	285	95	i
5	0	5	5	5	5	5	5	5	5	5	5	5	5	20	10	10	0	5	5	5	5	5	5	275	92	p
5	5	5	5	5	5	5	5	5	5	0	0	5	5	20	10	10	10	5	0	5	5	5	5	280	93	v
5	0	5	5	5	5	5	5	5	5	0	0	5	5	20	10	10	0	5	5	5	5	5	5	270	90	q
5	0	5	5	5	5	5	5	5	5	5	5	5	5	20	10	10	10	5	5	5	5	5	5	290	97	b
5	0	5	5	5	5	5	5	5	5	5	5	5	5	20	10	10	10	5	5	5	5	5	5	290	97	j
5	0	5	0	5	5	5	5	5	5	5	0	5	5	20	10	10	0	5	0	0	5	5	5	255	85	y
5	0	5	5	5	5	5	5	5	5	5	5	5	5	20	10	10	10	5	0	0	5	5	5	280	93	k
5	0	5	0	5	5	5	5	5	5	5	5	5	5	20	10	10	0	5	0	5	5	5	5	255	85	d
5	0	5	0	5	5	0	0	5	5	5	0	5	5	20	10	10	10	0	0	0	5	5	5	235	78	l
5	5	5	0	5	0	5	5	5	5	5	5	5	5	20	10	10	10	5	5	5	5	5	5	275	92	w
5	5	5	0	5	0	5	5	5	5	5	0	5	0	20	10	10	10	5	0	0	0	5	5	245	82	ch
5	0	5	5	5	5	5	5	5	5	5	0	5	5	20	10	10	10	5	5	5	5	5	5	275	92	ñ
5	0	5	0	5	5	5	5	5	5	5	0	5	5	20	10	10	0	5	5	5	5	5	5	270	90	z
5	0	5	5	5	5	0	0	5	5	5	5	5	5	20	10	10	0	5	0	5	5	5	5	255	85	r
5	0	5	0	5	5	5	5	5	5	5	5	5	5	0	10	10	0	5	0	5	5	5	5	235	78	a
5	0	5	5	5	5	5	5	5	5	5	0	5	0	0	0	0	0	5	0	5	5	5	5	205	68	o
5	5	5	5	5	5	5	5	5	5	5	5	5	5	20	10	10	0	5	0	5	5	5	5	285	95	e
5	0	5	0	5	5	0	0	5	5	5	0	5	5	20	0	0	0	5	0	5	5	5	0	220	73	m
5	5	5	5	5	5	5	5	5	5	5	5	5	5	20	10	10	10	5	0	5	5	5	5	290	97	s
0	0	5	0	5	5	5	5	5	5	5	0	5	0	20	0	0	0	5	5	5	5	5	5	185	62	x
5	0	5	0	5	5	0	0	5	5	5	0	5	5	20	10	10	10	5	5	5	5	5	5	265	88	f
5	0	5	5	5	5	5	5	5	5	0	0	5	5	20	10	10	0	5	0	5	5	5	5	265	88	h
5	5	5	5	5	5	5	5	5	5	5	0	5	5	20	10	10	0	5	5	5	5	5	5	280	93	t
5	0	5	0	5	5	0	0	5	5	5	5	5	5	20	10	10	10	5	5	5	5	5	5	280	93	g
5	0	5	0	5	5	0	0	5	5	0	0	5	5	20	10	10	10	5	0	0	5	5	5	240	80	n
5	5	5	0	5	0	5	5	5	5	5	5	5	5	20	10	10	10	5	5	5	5	5	5	290	97	u
5	0	5	0	5	0	0	0	5	5	5	0	5	5	0	0	0	0	5	0	5	5	0	5	185	62	ß

Table 2: Descriptive statistics for each question grouping

Descriptive Statistics: Q1(%), Q2,3,4(%), Q5,6,7 (%), Q8(%)

Variable	N	Mean	StDev	Q1	Median	Q3
Q1(%)	29	94.25	8.94	91.67	100.00	100.00
Q2,3,4(%)	29	90.23	9.23	83.33	94.44	94.44
Q5,6,7 (%)	29	80.60	15.99	79.17	83.33	91.67
Q8(%)	29	85.63	15.89	75.00	83.33	100.00

Graph 1: Boxplot of Identification score (%) for each question grouping

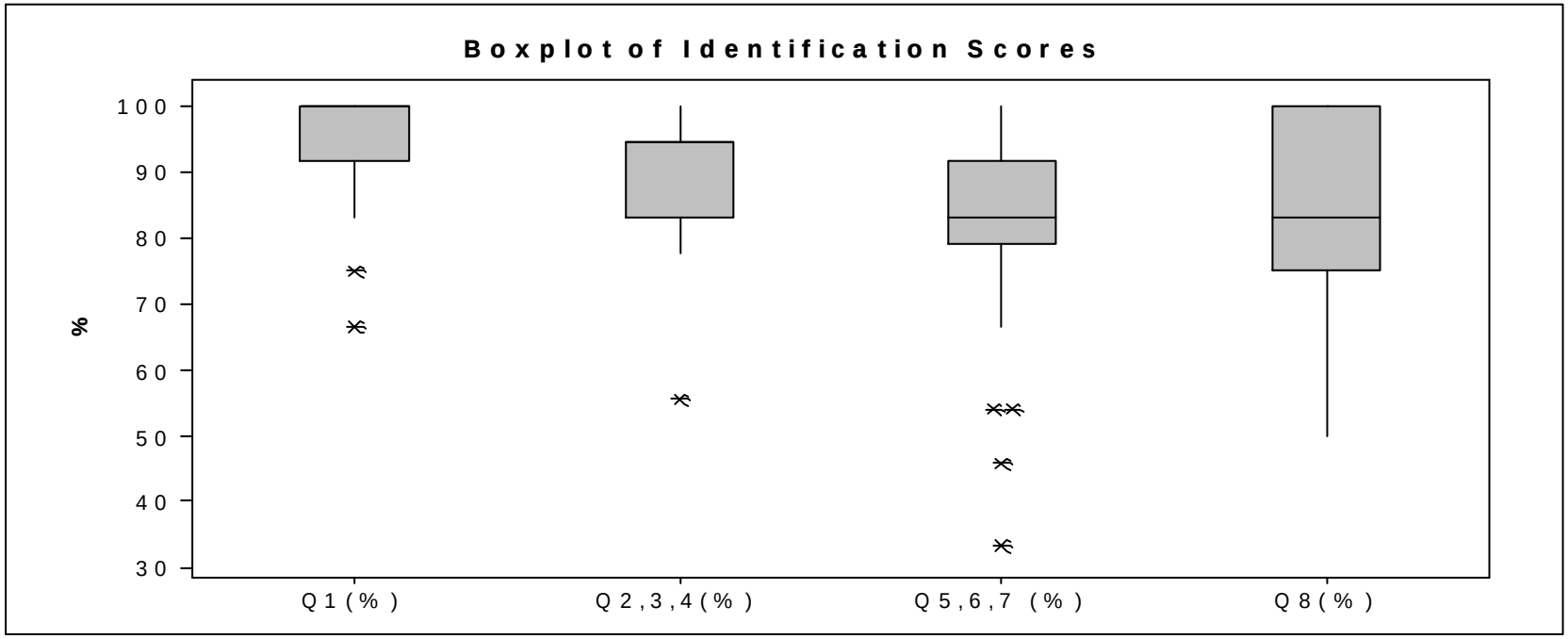


Table 3: Analysts Cumulative percentage of correct answers per group of questions

Tally for Discrete Variables: Q1(%), Q2,3,4(%), Q5,6,7 (%), Q8(%)

Q1(%)	Count	CumPct
66.667	1	3.45
75.000	1	6.90
83.333	4	20.69
91.667	5	37.93
100.000	18	100.00
N=	29	

Q2,3,4(%)	Count	CumPct
55.556	1	3.45
77.778	1	6.90
83.333	6	27.59
88.889	6	48.28
94.444	9	79.31
100.000	6	100.00
N=	29	

Q5,6,7 (%)	Count	CumPct
33.333	1	3.45
45.833	1	6.90
54.167	2	13.79
66.667	1	17.24
75.000	1	20.69
79.167	7	44.83
83.333	3	55.17
87.500	3	65.52
91.667	5	82.76
95.833	4	96.55
100.000	1	100.00
N=	29	

Q8(%)	Count	CumPct
50.000	2	6.90
66.667	5	24.14
83.333	9	55.17
100.000	13	100.00
N=	29	

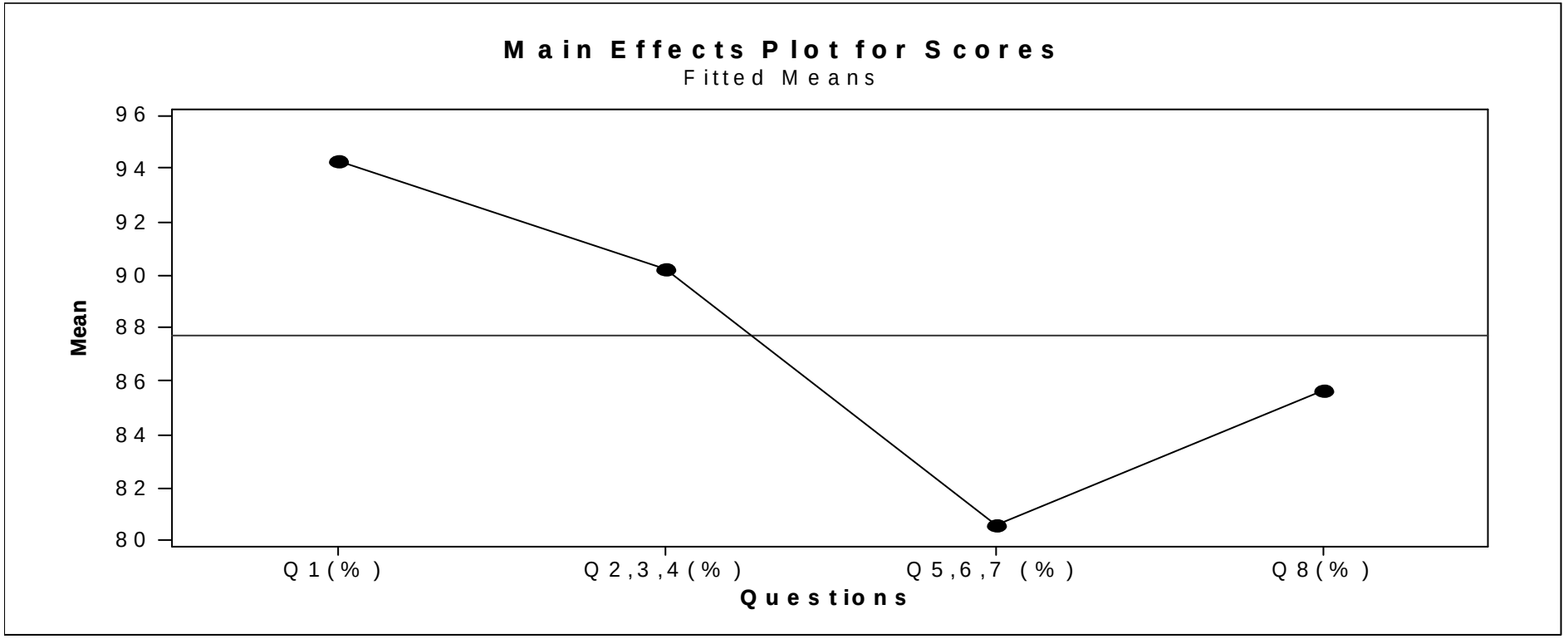
Table 4: One Way Anova test

One-way ANOVA: Q1(%), Q2,3,4(%), Q5,6,7 (%), Q8(%)

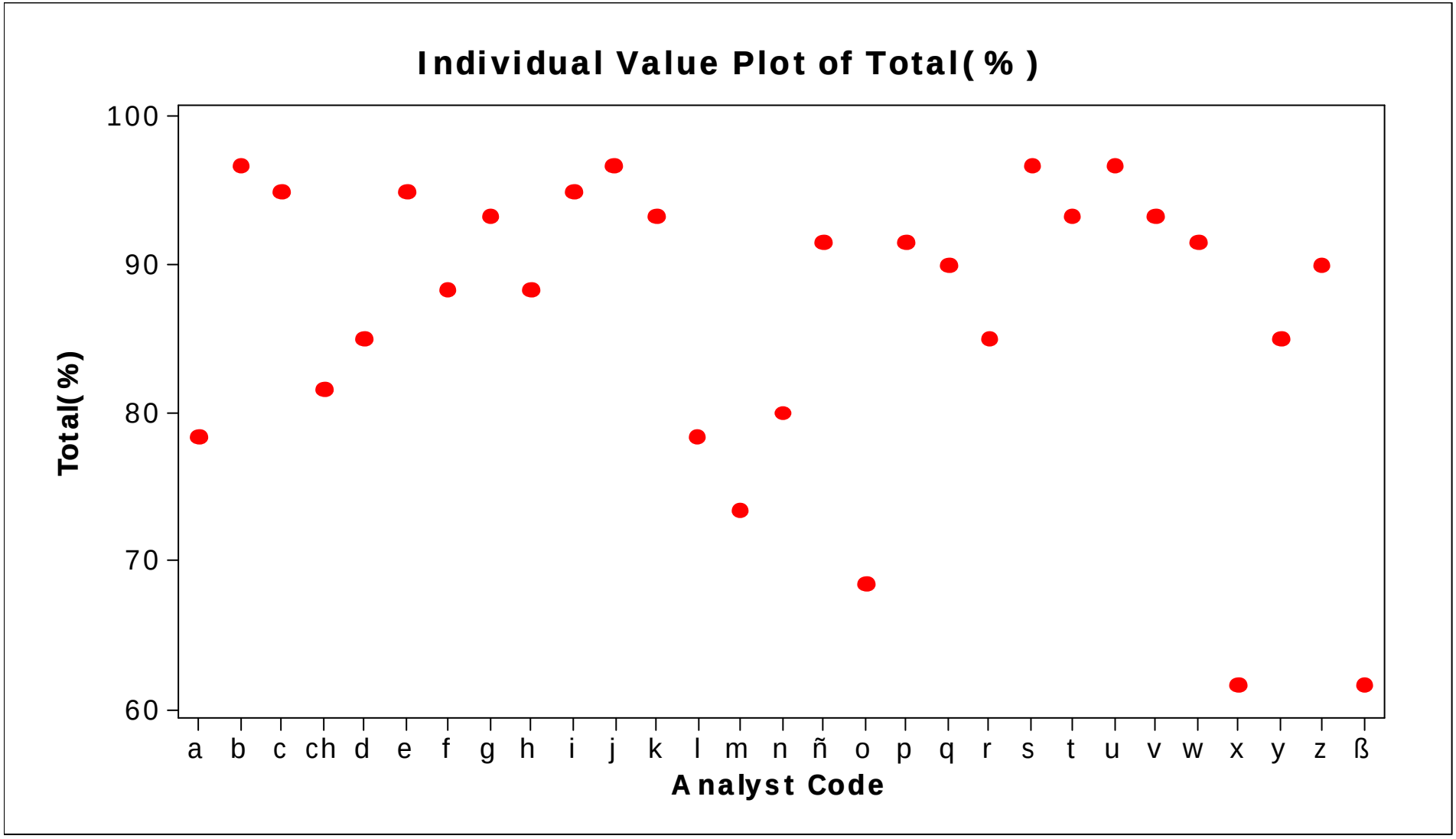
Source	DF	SS	MS	F	P
Factor	3	3015	1005	5.97	0.001
Error	112	18854	168		
Total	115	21869			

S = 12.97 R-Sq = 13.79% R-Sq(adj) = 11.48%

Graph 2: Fitted means of the 4 question groupings



Graph 3: Participants percentage total score



ANNEX I: Workshop Agenda

BEQUALM / National Marine Biological Analytical Quality Control Scheme
Phytoplankton ring test PHY-ICN-08-MI1 2008

Workshop

Thursday, 17th April 2008,
Marine Institute Brendan the Navigator Auditorium

Agenda

09:45 **Introductions / Welcome**

10:00 **Intercomparison exercise PHY-ICN-08-MI1**

Materials and Methodology

A: Enumeration exercise
B: Identification exercise
Questions and answers session

11:30 Coffee Break

12:00 **Statistical analysis of ICN exercise: results of enumeration and identification exercise**
John Newell NUIG Mathematics department

13:00 Lunch in Marine Institute Restaurant

14:00 **'Living dinoflagellates: from theca to cyst' Part 1**
Professor Jane Lewis,
Dean, School of Biosciences
School of Biosciences
University of Westminster

14:45 **Diatoms: Pseudonitzschia spp. The Basics**
Dr. Caroline Cusack
Research scientist

Climate Change Phytoplankton Team

15:30 Coffee Break

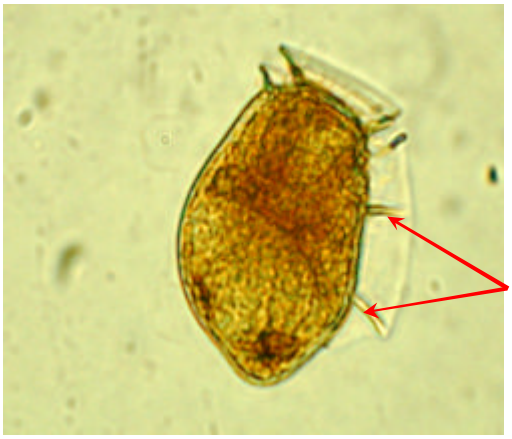
16:00 **'Living dinoflagellates: from Cyst to theca ' Part 2**
Professor Jane Lewis,
Dean, School of Biosciences
School of Biosciences
University of Westminster

16:30 **Results Discussion: Future developments of ICN 2009**

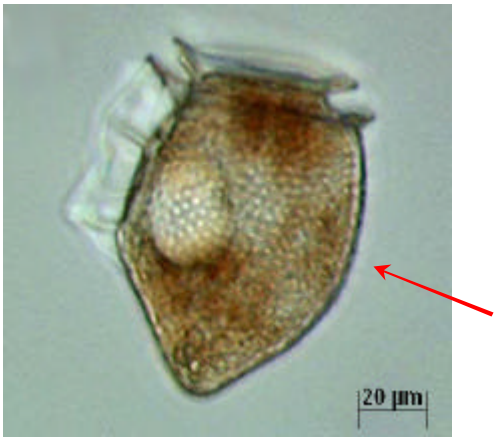
ANNEX II: Taxonomic Quiz

FORM 4: TAXONOMIC QUIZ BEQUALM PHY-ICN-08-MI1

QUESTION 1: The following photomicrographs belong to the genus *Dinophysis*. Participants are asked to name the species and the morphological features that the arrows are pointing at. This question is worth 60 marks. 5 marks/ species named correctly and 5 marks/ features named properly.

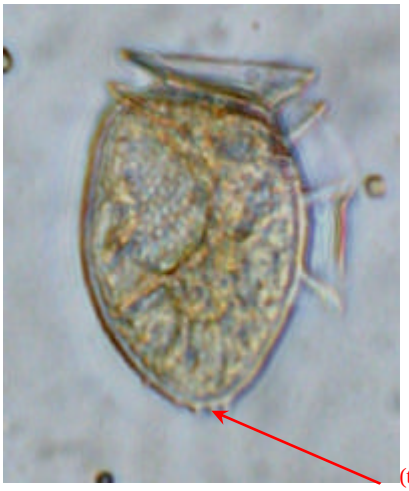


A. ***Dinophysis*_____**
Size: L: 85.0, W: 55.0 µm



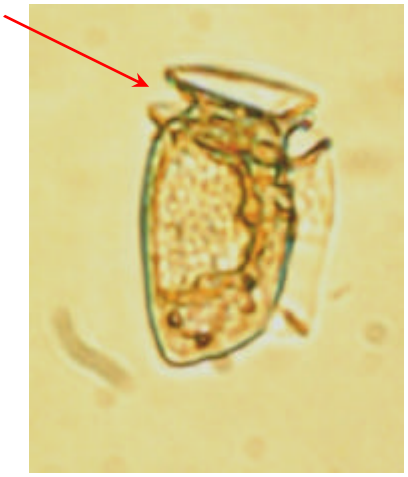
B. ***Dinophysis*_____**

C. ***Dinophysis*_____**
Size: L: 74, W: 58 µm

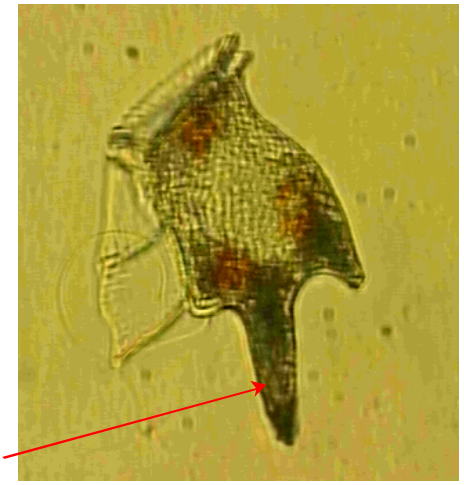


(the small bulgy things)

D. ***Dinophysis*_____**
Size: L: 44.8, W: 31.2 µm



E. ***Dinophysis*_____**
Size: L: 52.5, W: 32.5µm



F. ***Dinophysis*_____**
Size: L: 95.0, W: 55.0 µm

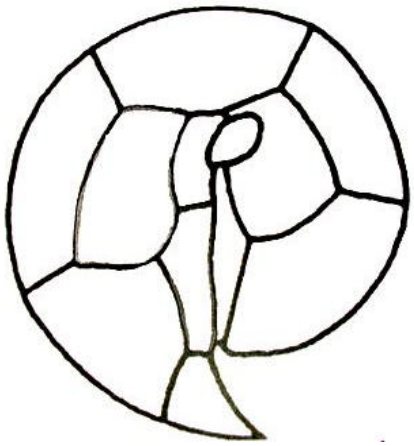
QUESTION 2: The following diagrams show the **kofoidean tabulation** of two different armoured dinoflagellates in apical view.

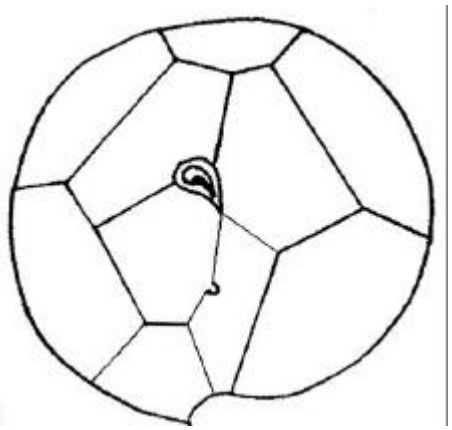
This question is worth 20 marks. 10 marks/question

You are asked:

1) Which armoured dinoflagellates genera do these diagrams represent? ***Write answer under each diagram***

2) Which are the main plate differences between these two genera? ***Name the plates that are different and point at them with arrows***



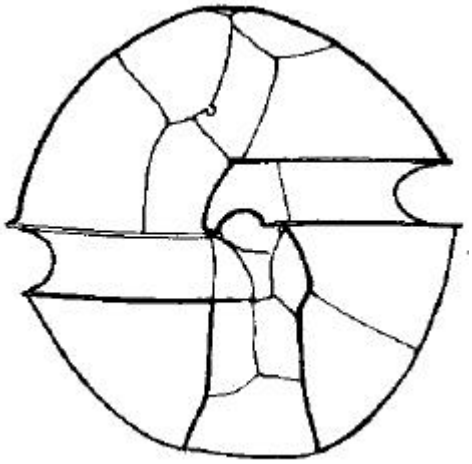


QUESTION 3: The following diagrams represent an armoured dinoflagellate plate structure in ventral and apical view. Could you with the help of arrows point to the following features:

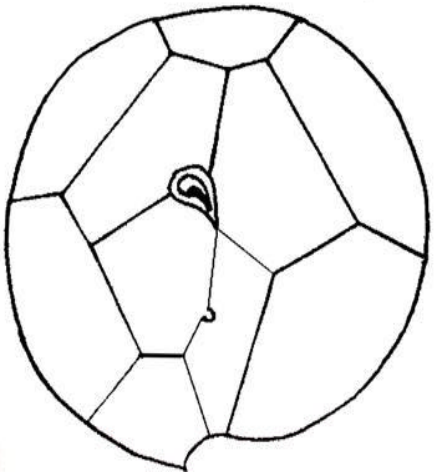
a) the 1' (apical) plate
b) the 6'' (cingular) plate
c) the ventral pore
d) the sulcal plate

Use either diagram to point to the features

(**This question is worth 20 marks, 5 marks/correct feature**)

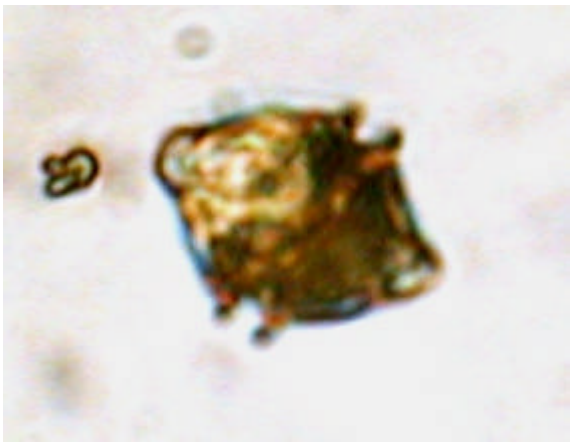


Ventral view

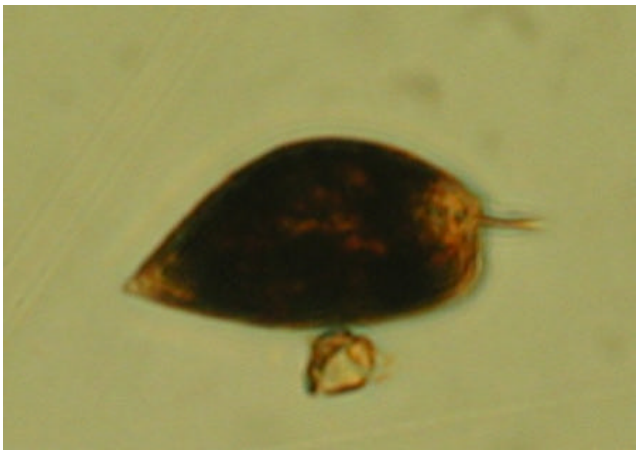


Apical view

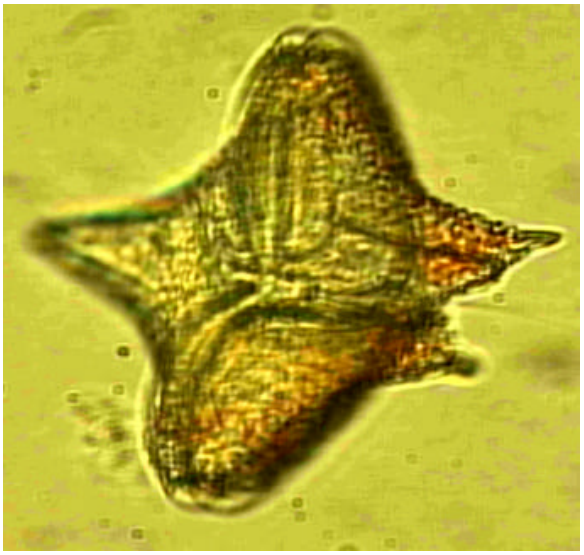
QUESTION 4: **Identify to species level** the following pictures of armoured dinoflagellates.
Cell size is given in microns, first number indicates length and second number is width of the cell.
Each correct genus answer carries 5 marks. Each correct species answer carries 5 marks. If the genus is named incorrectly, no marks will be awarded for the species name. This question is worth 50 marks.



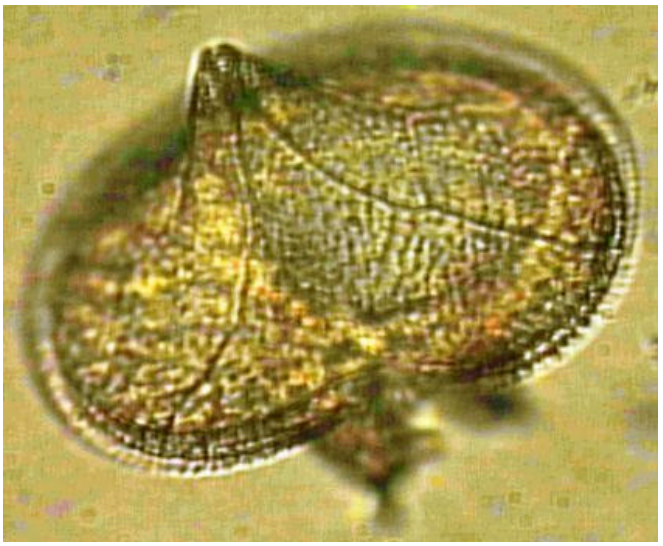
A. Size: L:25, W:20 µm (cell rounded)
Name:



B. Size: L: 65, W: 30 µm
Name:



C. Size: L: 100, W: 105 µm
Name:

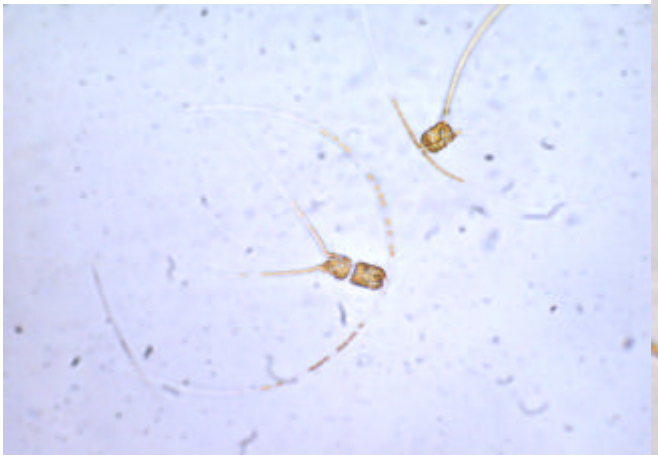


second image showing plate structure

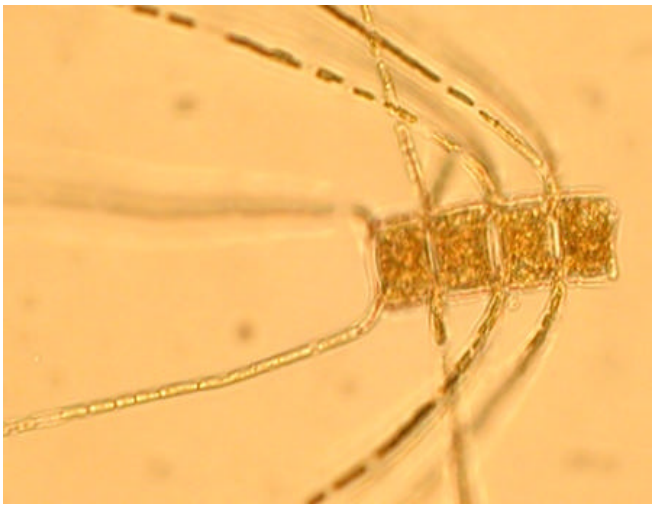
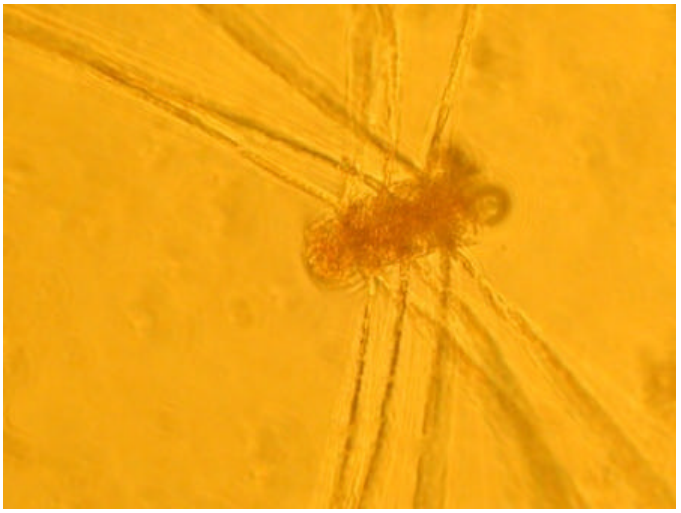
D. Size: L: 47.5, W: 32.5 µm
Name:

E. Size: L: 64, W: 38 µm
Name:

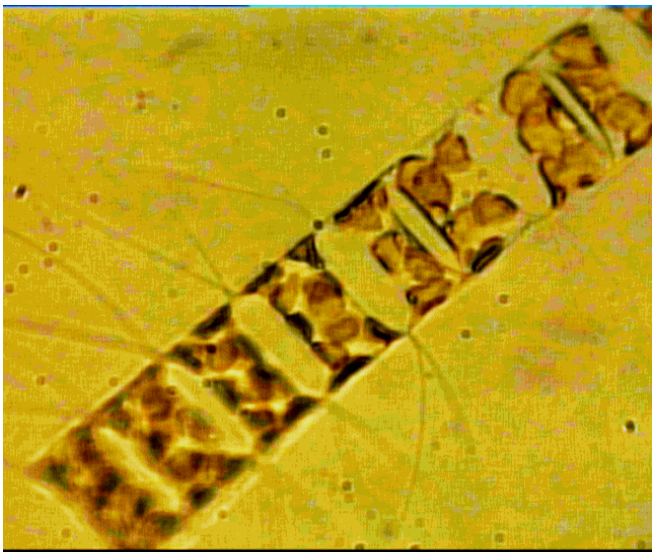
QUESTION 5: Name the following diatoms to **species level**
Each correct genus answer carries 5 marks. Each correct species answer carries 5 marks. If the genus is named incorrectly, no marks will be given for the species name. This question is worth 70 marks.




2 images of the same organism (not chain forming):
A. Name:

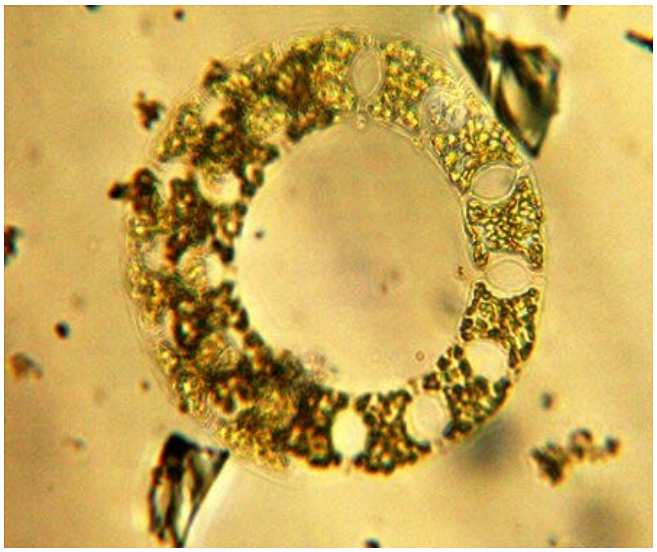



2 images of the same organism
B. Name:

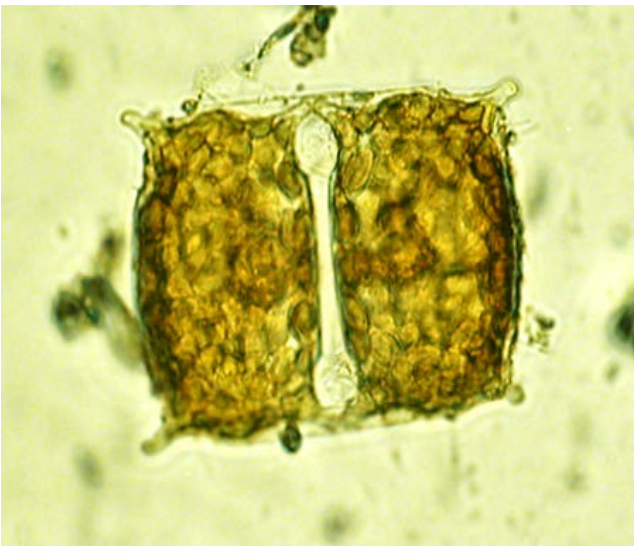



C. Name:

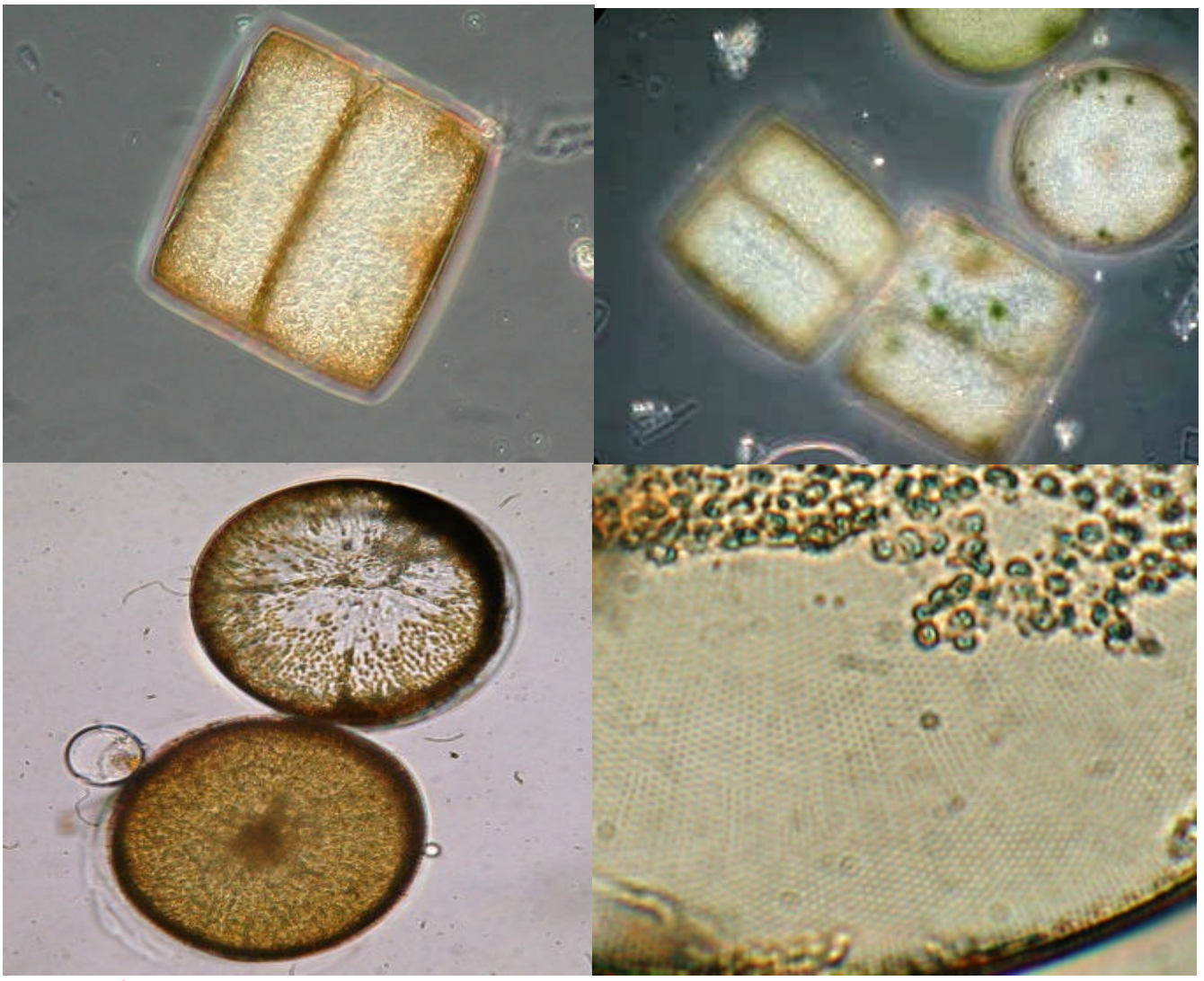
D. Name:
Size: L: 650, W: 100 µm



E. Name:



F. Name:

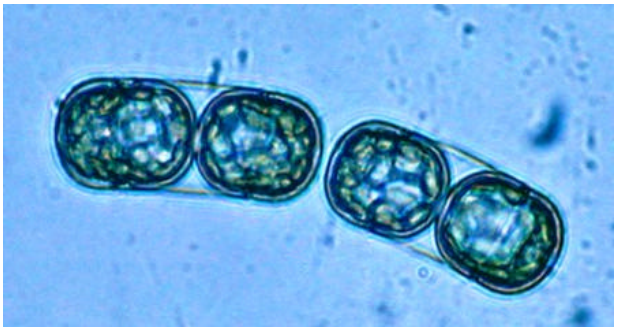


4 images of the same organism. (300 µm diameter)

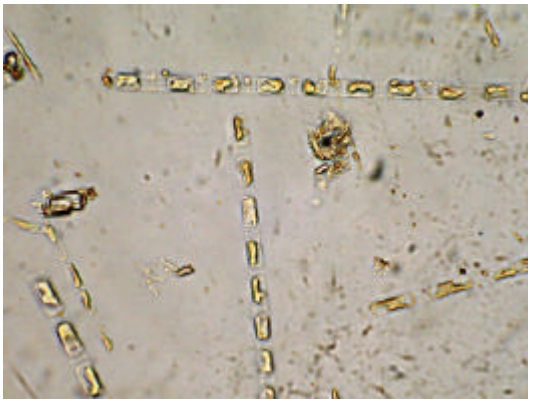
Areolae details

G. Name :

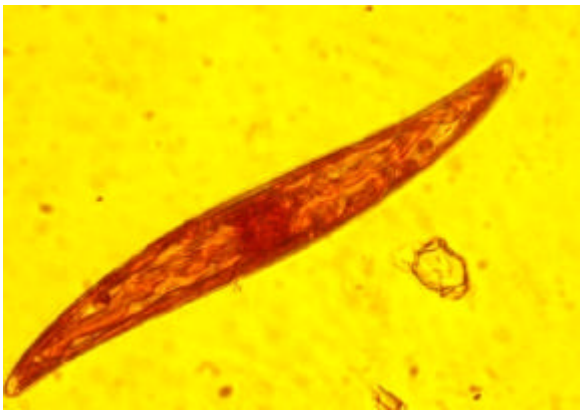
QUESTION 6: Could you circle the odd one out?
This question is worth 20 marks



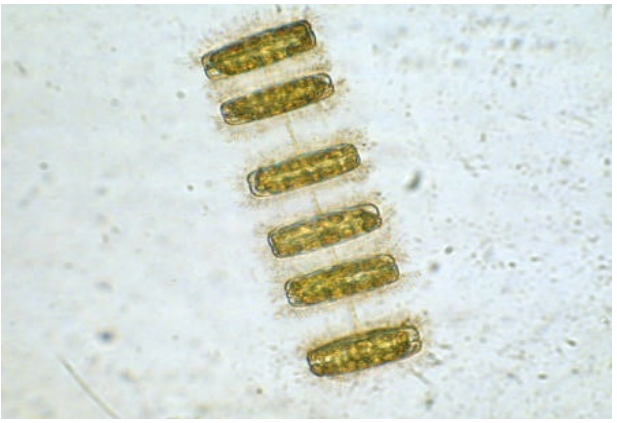
A



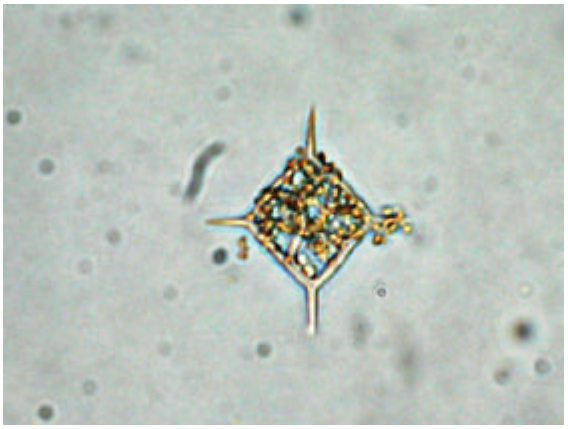
B



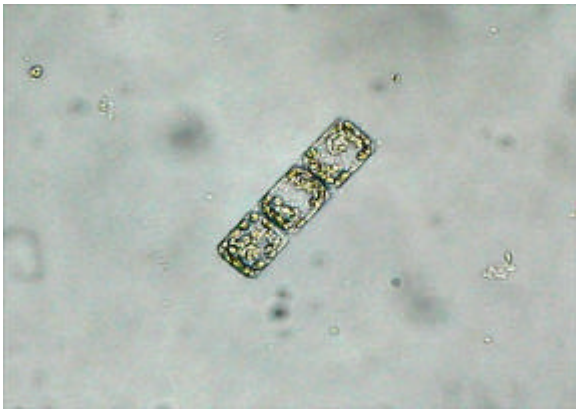
C



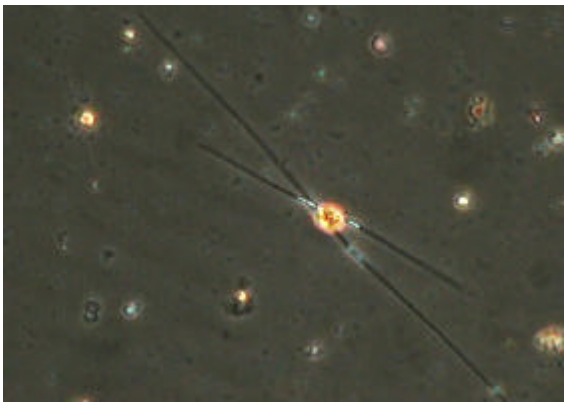
D



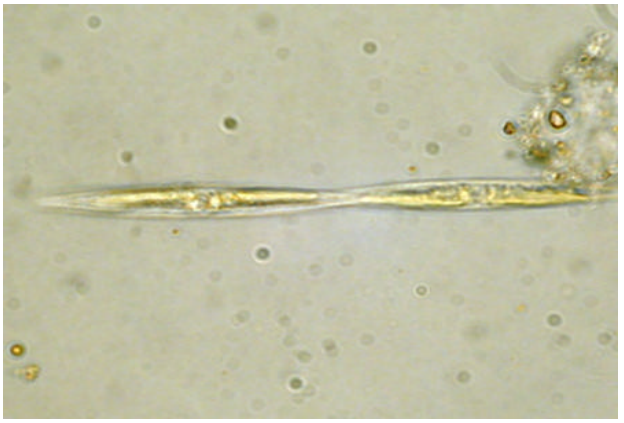
E



F



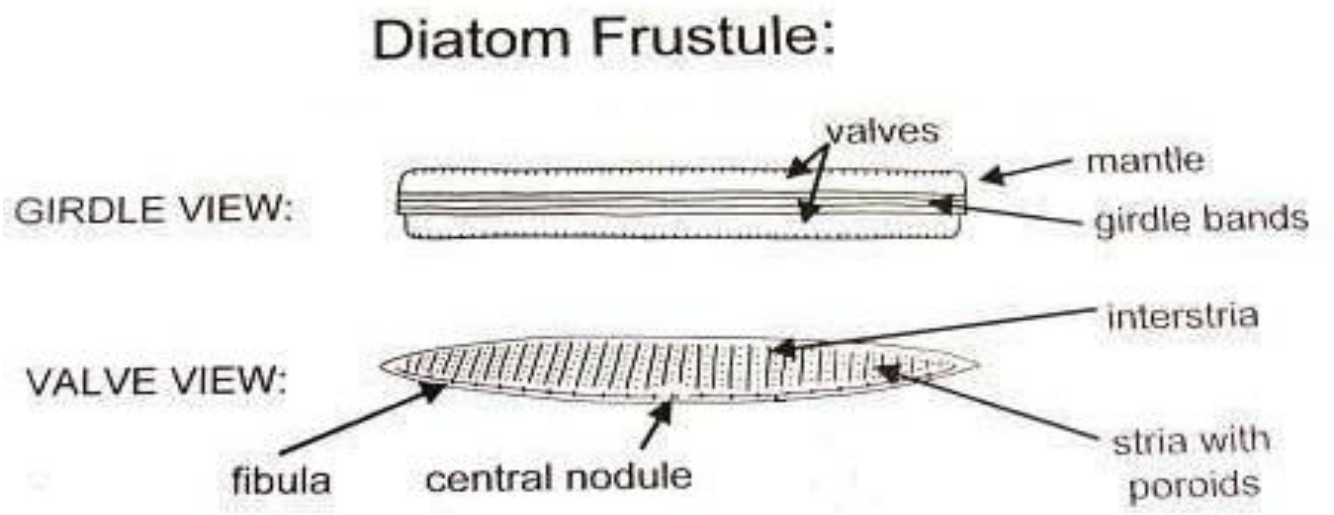
G



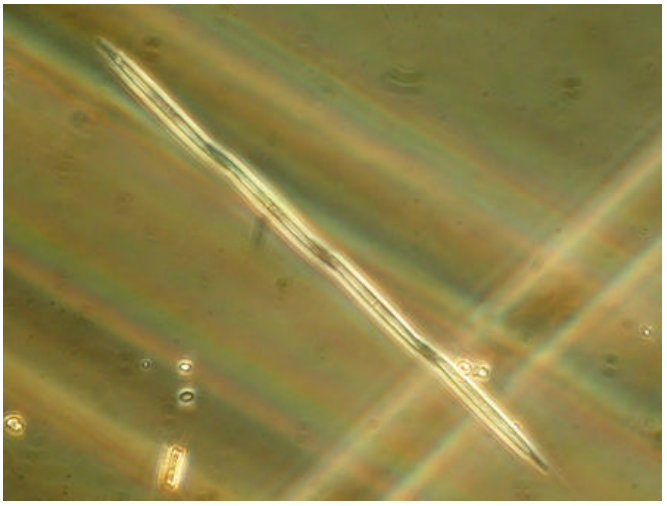
H

QUESTION 7: The following diagrams show a schematic picture of a *Pseudonitzschia* cell in valve and girdle view. **A)** If you were to measure the 'width' of a *pseudonitzschia* cell, which **view** would you choose to do this? (Draw a line showing where you would measure the cell's 'width') **B)** And give a reason why you would choose that particular view to measure the width of the cell?

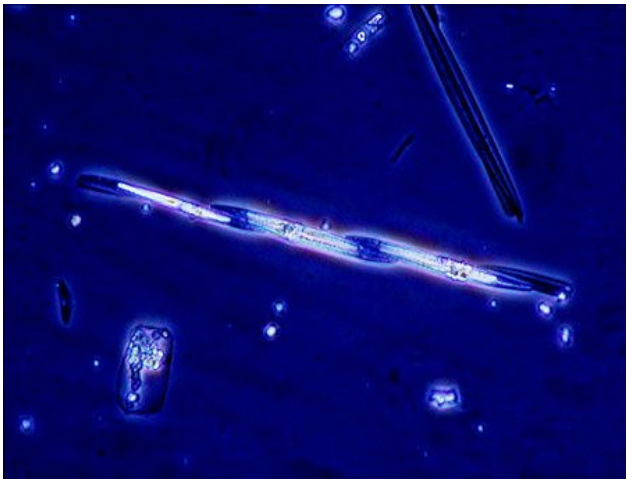
This question is worth 30 marks. 10 marks/correct answer.



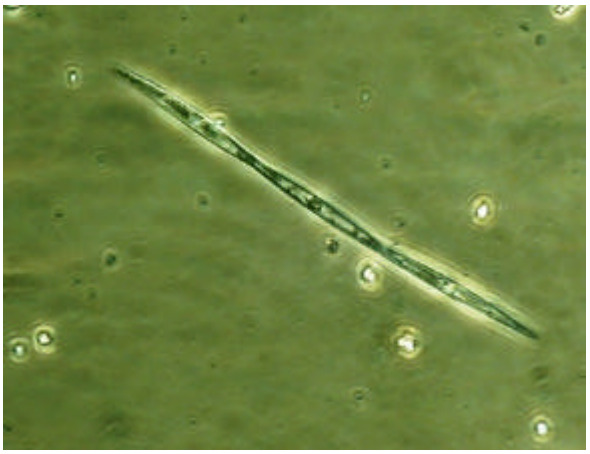
C) Taking into account the answers to **A** and **B**. which of the following photographs of *pseudonitzschia* cells would you choose to carry out a width measurement?



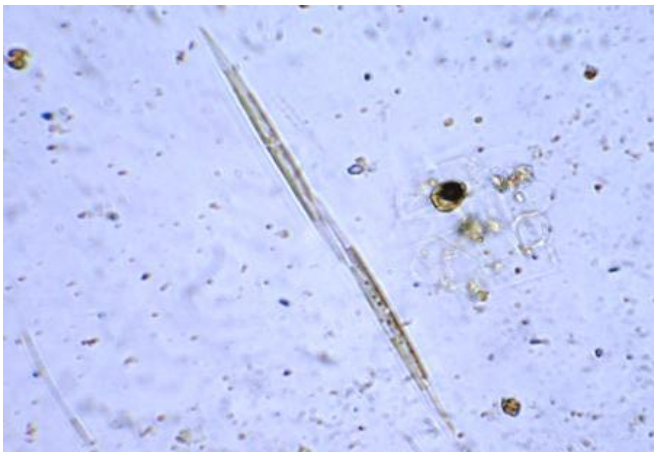
A



B

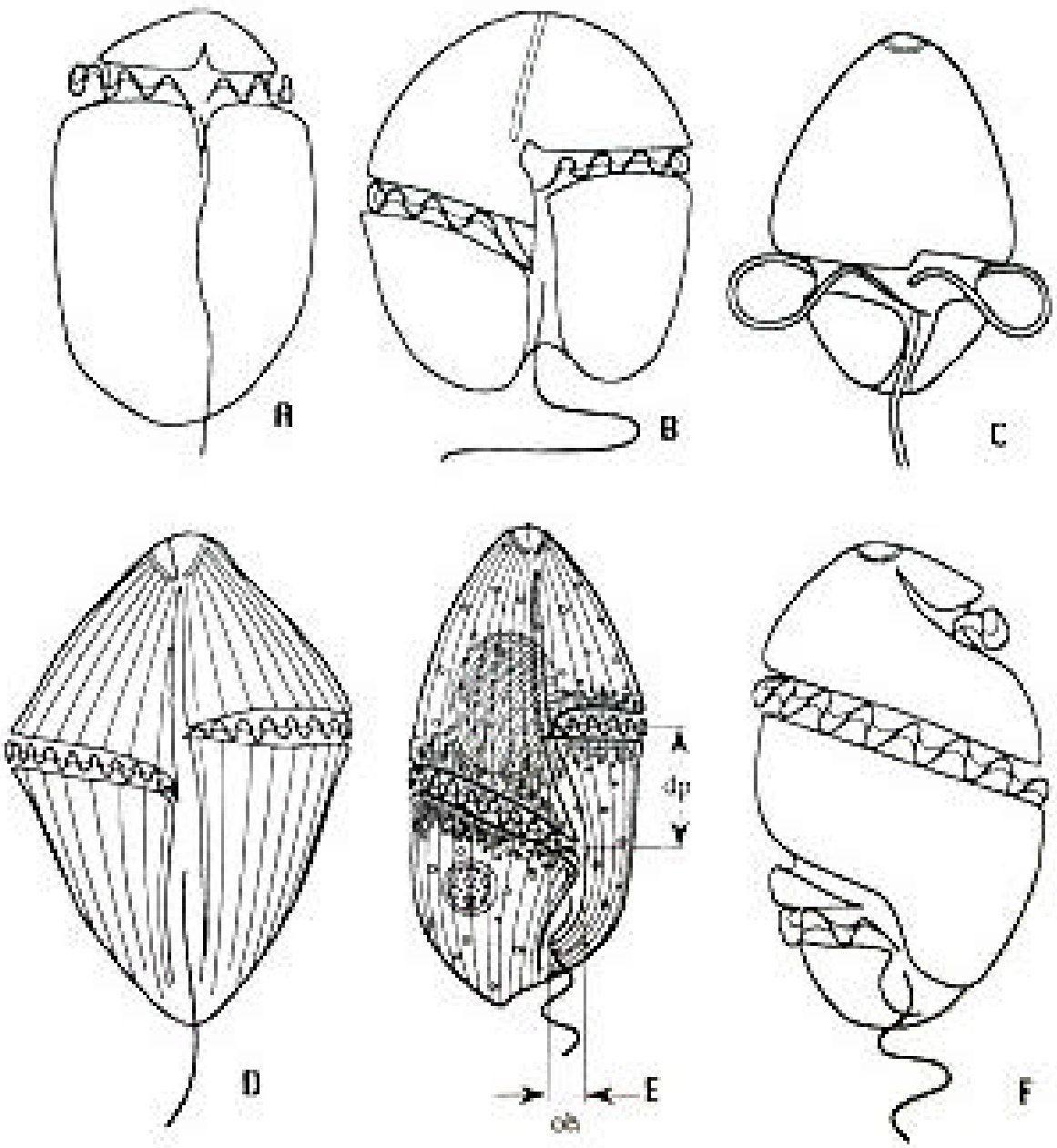


C



D

QUESTION 8: Which **Genera** do these organisms belong to?
This question is worth 30 marks. 5 marks/correct answer
A:
B:
C:
D:
E:
F:



Signed:_____

Date:_____

Annex III: FORM 1_Checklist to Fax bequalm 08 MI1.pdf

Bequalm Intercomparison PHYICN-08-MI1
FORM 1: RETURN SLIP AND CHECKLIST

ATTENTION: Rafael Salas

Please ensure to complete the table below upon receipt of samples, and fax immediately to the Marine Institute. 00353 91 387237		
Name of Analyst:		
Laboratory Name:		
Analyst Code Assigned :		
Contact Tel. No. / e-mail		
CHECKLIST OF ITEMS RECEIVED	**(Please circle the relevant answer)**	
Sample _____	YES	NO
Sample _____	YES	NO
Set of Instructions	YES	NO
Enumeration Result Sheet **(Form 3)**	YES	NO
Identification Sheet **(Form 4)**	YES	NO
One MI Addressed Envelope	YES	NO

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

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

SIGNED: _____

DATE: _____

Annex IV: , FORM 3_Enumeration Hardcopy results Bequalm 08 MI1.pdf

Bequalm Intercomparison PHYICN-08-MI1

FORM 3: ENUMERATION HARD COPY RESULTS SHEET

Name of Analyst:	
Laboratory Name:	
Analyst Code :	

Section A: Enumeration exercise

Sample No	Date of Settlement	Date of Analysis	No. of cells	Volume Chamber (ml)	Calculations	Number cells/L

Signed: _____

Date: _____

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 2008

Participant details:

Name of organisation: «OrgName»
Participant: «NMP_Participant»
Year of joining: «YearJoined»
Years of participation: «YearsInScheme»

Statement Issued: «Issued»
Statement Number: «CertificateID»

Summary of results:

Component Name	Exercise	Subcontracted	Results Z-score (+/- 3 Sigma limits)	
			Sample No:	Sample No:
Phytoplankton Enumeration	PHY-ICN-08-MI1	Marine Institute		
			Results Pass Mark 70% (over 90% proficient)	
Phytoplankton Identification	PHY-ICN-08-MI1	Marine Institute		

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: «Note»

Details certified by:

Section Manager
Joe Silke (MI)

Senior Lab Analyst
Rafael Salas (MI)

scription of Scheme components and associated performance standards

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 qualm 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
hytoplankton Enumeration Exercise	1	To assess the performance of participants when undertaking analysis of a prepared sample/s of Seawater preserved in Lugol's iodine and spiked using biological or synthetic subjects using the Utermohl cell counting method.	Prepared marine water sample/s distributed to participants for Phytoplankton enumeration analysis and calculation of counts in cells per litre	Participants are required to enumerate the spiked material and give a result to within ±3SD or sigma limits of the true value. The true value is the mean calculated from a sample population of the total as calculated by the organising laboratory. This data has to demonstrate normality to become the reference data for the exercise.
hytoplankton dentification 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 diagrams supplied. In addition, certain taxonomic details need to be identified as well as in some cases genus and species name of the organism. This exercise may also include a combined identification plus enumeration exercise.	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.