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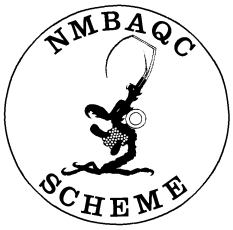

PHYTOPLANKTON ENUMERATION AND IDENTIFICATION ANALYSIS

Ring Test PHY-ICN-10-MI1 Exercise Report, June 2010

Rafael Gallardo Salas

Marine Institute
Rinville
Oranmore
Co. Galway
Ireland

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Phytoplankton Enumeration and Identification Proficiency Test

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

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1. Summary of results

Identification exercise

- All analysts performed extremely well in this exercise
- All analysts achieved over 90% overall score
- No evidence of significant differences between the means of correct answers of Question 1, Questions 2-3-4, Questions 5-6-7 and Question 8
- Q8 was the top scored question.
- The mean of correct answers of the questions on diatoms (5, 6 & 7) was slightly better than the questions on armoured dinoflagellates (2, 3 & 4)
- Scores on Questions (1) and (2,3,4) on dinoflagellates are comparable with questions (5,6,7) on diatoms

Enumeration exercise

Summary of low cell density samples:

- Low cell concentrations: Analysts used mainly 2 sub-sample volumes 10ml and 25ml.
- Most analysts used the Whole Chamber strategy for their cell counts.
- There are differences in the mean concentration between 10 and 25ml methods but the difference is not significant.
- The Standard deviation for those using the 25ml method however is smaller, which suggests there is less variation in their cell counts.

Summary of High cell density samples:

- High cell concentrations: Analysts used mainly 10 or 25ml sub-samples
- Analysts used mainly either Transect (TR) or Whole Chamber (WC) counts for their cell counting strategies
- There are significant differences in the mean concentration between 10 and 25ml methods and between TR and WC counting strategies
- This difference is significantly larger in 10ml than in 25ml and larger in TR than in WC

Summary Learning effects:

- There is no evidence of learning effects between sample replicates at low or high cell concentrations by analysts.
- Could have this Learning effect been confounded by cell concentrations?

Summary Z-scores:

- All analysts performed within the mean +/- 3 SD of all the results, both for the low counts and for the high counts.
- The mean and 3SD is calculated from all the results sent by the analysts in this Intercomparison.
- The variability is larger at the higher cell concentrations. Is this variability acceptable?

Summary Hypothesised means:

- Hypothesized means are the hypothesized true densities for the low and high cell concentrations set by a number of 10 replicate analysis of 1ml aliquots in Sedgewick-Rafter counting chambers.
- Hypothesized means indicate method effects tend to underestimate final concentration for both the low and high cell concentration samples.
- The method effects seem to be related to the sub-sample volume and the counting strategy chosen.
- The Bias of the hypothesised values for Low and High cell densities is negative. This indicates that the method effects tend to underestimate cell counts. Should correction factors be introduced?
- Low cell count bias and High cell count bias are comparable as a Z-score. The Z-score bias tends to be similar at both concentrations.

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 exercises are to compare the performance of laboratories engaged in national official/non-official phytoplankton monitoring programmes and other labs working in the area of phytoplankton (see bequalm website www.bequalm.org).

The objectives of the NMBAQC Bequalm Intercomparison for phytoplankton are to mirror what we do in the lab and that is to analyse marine water samples for phytoplankton enumeration and identification to the highest taxonomic level possible and to test our routine monitoring test method.

Most labs in Europe use the Utermöhl cell counting test method with small variations; these are usually related to the volume of the sub-sample used and cell counting strategies. We are looking to test the method and its limitations in terms of their limit of detection, quantification, bias, robustness, accuracy, precision, specificity, reproducibility, repeatability, stability, etc. Each Intercomparison exercise in a given year is designed to test one or various aspects of the test method.

Bequalm is a proficiency testing scheme, which is perceived by labs engaged in Phytoplankton monitoring as of being of good quality and where participating labs

have an active input on the scheme through workshops and direct communications with the Marine Institute phytoplankton unit.

The participation in this type of schemes is becoming an essential requirement for National phytoplankton monitoring labs in order to achieve accreditation for their methods.

Since 2008, we also certify the participation of individual analysts on the scheme by issuing statement performance certificates.

This year is the 5th Phytoplankton Bequalm intercalibration exercise and for the first time we have participating labs from outside of Europe. Two labs from South America have taken part in the exercise, one from Peru and one from Argentina. Also, we have had an increase number of enquiries from new labs around Europe. In the Mediterranean area, we had two labs from Croatia taking part and a lab from Sweden. There have also been enquiries from further afield from Asian and African countries.

The Marine Institute Phytoplankton lab is accredited to ISO 17025 for Toxic Marine phytoplankton identification and enumeration since 2004 and it is audited annually on the continuation of the award by the Irish National Accreditation Board (INAB). This recognizes that regular Quality Control assessments are crucial to ensure a high quality output of Phytoplankton data.

In 2009, INAB auditors were very complementary on the Phytoplankton Proficiency testing scheme Bequalm and asked us if we were considering applying for accreditation of the scheme under ISO/IEC 17043 which is an International Standard for the requirements of Proficiency testing schemes.

At present, we are looking into the requirements of this standard and we are hoping to be in a position to apply for accreditation in 2011. This is in my opinion a necessary step forward towards the recognition of the scheme as a quality assured proficiency testing scheme by international standards, which I think should add value to the exercise and to the certification of performance for the analysts.

3. Participants

In 2010, we had 39 analysts from 21 laboratories mostly from Europe but we had 2 labs from South America participating in the exercise PHY-ICN-10-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, UK, Spain, Croatia, Holland, Sweden and Germany. Also 2 labs from the South American region took part; Argentina and Peru.

A complete list of the participating laboratories is given in Annex I.

This is, again an increase in the number of labs and analysts taking part in this exercise from previous years.

4. Materials and Methodology

4.1 Study design

The 2010 Bequalm exercise was divided in two sections as in previous years: an enumeration exercise comprising 6 samples spiked with cultured material at two cell concentrations and an identification exercise comprising a taxonomic quiz.

4.2 Taxonomic Quiz

The identification exercise or taxonomic quiz was a repeat of the exercise from 2008 and the reason behind using this exercise again was first of all because participants felt that the full exercise in 2009 was too cumbersome and it needed to be trimmed down, but also I was felt that given the number of new entrants for 2010 (19 analysts), this exercise was a good basic taxonomic exercise for new entrants.

This meant that analysts that participated in 2008 were exempted from completing this part of the exercise.

The identification exercise is custom made from 'scratch' and comprises 8 questions and 300 marks. The pass mark for the exercise was set at 70%. It uses photographs and line drawings of marine phytoplankton species. The photographs are our own to avoid copyright issues and the line drawings are used to be able to view taxonomic features that otherwise would not be apparent in a photograph (e.g. Dinoflagellates plate structure).

This exercise is a basic identification exercise which is purposely biased towards Diatoms and Dinoflagellates marine phytoplankton species, biased towards toxic/harmful species and designed to test participants' basic Phytoplankton taxonomy knowledge.

4.3 Phytoplankton samples: Enumeration exercise

The enumeration exercise Beq 2010 has been designed to be strictly a counting exercise only. No identification of the spiked cell culture material was needed.
Two different cell concentrations were used in triplicates to obtain balanced and robust data for statistical analysis.

No gold standard or reference values have been set for this exercise as we have already proven from previous exercises that there are no significant differences between the reference data analysed by the organising lab and the data from the participating labs. This means that the data generated by the participants will be used to set the mean and the Sigma limits for the sample population.

In previous years, a particular counting strategy, volume and methodology was prescribed to be used to analyse the samples but this year in order to avoid the proficiency test becoming a way of validating a particular methodology labs and analysts were asked to carry out the analysis according to their in-house methodologies which they use in the routine monitoring of samples and would be a truer reflection of their sample analysis.

Participants were asked to read carefully the instructions and were also asked on receipt of the samples to send back the return slip and checklist form (see Annex V:

Form1: Checklist to Fax) to the organising laboratory that the samples have been received in good condition.

Analysts were asked to carry out cell counts on 6 spiked samples with cultured material, using their in-house methodologies and techniques. Analysts have to return results within a 4 weeks deadline.

They were also given the option to analyse a field sample for a full Phytoplankton community analysis and to send images of the identifications. The results of this sample won't be published in this report because it was the first time that a sample of this kind was sent to the participants and the sample was not validated and there weren't enough replicates so no statistical inferences could be made of the results. This is clear from the instructions sent to the participants of the compulsory work of the exercise and the optional work.

However it was agreed that another report on the results of this sample will follow in order to discuss in particular the issues relating to this type of samples, what rules should apply regarding the naming and identifying of species, which species list should be used, how the results should be processed and how these samples could be validated among other considerations.

The 6 spiked samples consisted of a *Scrippsiella sp.* culture kept in the Marine Institute culture collection. The samples were preserved using Lugol's iodine.
The 6 samples were triplicates of low cell density aliquots and high cell density aliquots.

A master mix was made for each density using a 500ml borosilicate glass screw top bottle and the aliquots were taken with a 1ml Gilson Pipette after homogenising the sample at least 100 times for each aliquot.

Preliminary cell counts for each cell density were carried out using a Sedgewick-Rafter cell counting chamber to ascertain the approximate densities in the samples. (See Table 18).

The 1ml aliquots at low cell concentrations contained 200 cells approximately and 10000 cells in the high cell concentration (hypothesised values based on 10 replicate counts).

The aliquots were dispensed into sterilin tubes containing a volume of 29ml sterile filtered seawater. The final concentration was hypothesised to be approximately 6 cells/ml and 333 cells/ml for each sample type.

The hypothesized values are based on 10 replicate counts of 1ml aliquots using a Sedgewick-Rafter cell counting chamber.

Overall, 300 samples were aliquot for each cell density, that is 300ml were aliquot from a Master mix of 500ml in total.

All samples used in this exercise were chosen randomly using Minitab software Vr15.0. Each sample was given a number and randomly assigned to a lab by the programme by using the randomization tool. What is important is that any sample from the sample population had the same chance to end up in any of the participating labs.

4.4 Forms and Instructions

4.4.1 Couriers and materials

All the necessary forms and instructions to complete the exercise were sent to all the participating analysts.

Each lab received apart from a set of 6 x samples, 1 x natural sample and a taxonomic quiz (Form 3, Annex III) per analyst, a set of instructions (Annex II) and several forms, a form for writing the enumeration results in (Form 2 : Enumeration hardcopy results. Annex VI) and a form to confirm receipt of materials (Form 1: Fax checklist. Annex V).

The samples were sent via courier to all the labs on the same day and the forms were sent to the participants via e-mail. Upon receipt of these materials all participants were asked to check the samples and the documentation for missing forms or leaked samples. Usually, a small number of samples have to be replaced each year. This is done on an individual basis with the labs.

4.4.2 Instructions

A set of concise instructions was sent with the rest of the materials, labs and analysts were asked to read and follow the instructions before commencing the test and to give themselves plenty of time to limit the number of errors due to tiredness and stress. See instructions in annex II.

4.5 Utermöhl cell counting method

The Utermöhl cell counting method is the standard methodology used by participants in this Intercomparison exercise. This methodology is based on preserved water samples that are homogenised by agitation and poured into a sedimentation chamber or Utermöhl chamber where the sample is let to settle overtime to allow phytoplankton species to drop to the bottom of the chamber before identification and cell counting can take place. Once the organisms are settled at the bottom of the chamber, these can be viewed using inverted light microscopy.

There are different type of sedimentation chambers and chamber volumes. We are not prescribing the use of one over the other at this stage. The most usual chamber volumes used are 10ml, 25ml and 50ml and within these you have sliding chambers and fixed chambers.

In the Marine Institute phytoplankton lab we are accredited to use 25ml fixed sedimentation chambers.

A variety of different methods have been developed to enumerate phytoplankton over the years. Descriptions of these can be found in two UNESCO-produced volumes: The *Phytoplankton manual*, edited by Sournia in 1978 and The *Manual on Harmful*

Marine Microalgae edited by Hallegraeff *et al.* was first published in 1995, with a revised second edition published in 2003.

An intercalibration workshop comparing a variety of different methods for the identification and enumeration of the dinoflagellate *Alexandrium fundyense* was held at Kristineberg Marine Research Station, Fiskebäckskil, Sweden in 2005. The results of this workshop are presented in Godhe *et al.* (2007). This concludes that the most reliable method for cell counting is the traditional count by the Utermöhl method.

Also this year, the IOC-UNESCO has published a Manual of Microscopic and molecular methods for quantitative phytoplankton analysis. This manual can be found in the IOC website at the following address: http://ioc-unesco.org/hab/index.php?option=com_oe&task=viewDocumentRecord&docID=5440

4.6 Statistical analysis

The objective of the statistical analysis of this intercalibration exercise Bequalm 2010 was to obtain Phytoplankton quantitative and qualitative data of the participating labs and analysts to compare results.

The qualitative exercise was in the form of a taxonomic quiz where participants have to answer questions on marine phytoplankton species based on photographs of species and also by using line drawings and diagrams of species to show particular taxonomic characteristics.

The participants' final results are given as a percentage of correct answers from the total. Some set of questions in the exercise were compared against other sets, for example questions 2,3 and 4 on Dinoflagellates were compared against questions 5,6 and 7 on Diatoms. The main statistics used for this exercise were descriptive statistics of the main question groupings to ascertain whether analysts answer particular questions better than others. Box plots to show graphically whether there were any differences between answers to certain questions, the cumulative percentage of correct answers to provide a yardstick for the exercise on where the pass mark for the

exercise should be set, the individual values of each analysts to compare how well participants did and finally the ranking of analysts in the exercise.

The quantitative exercise was designed to compare cell concentrations on samples spiked with cultured material. There were triplicate samples of low and high cell concentration samples; these were sent on triplicates to obtain robust statistical data of the measurand and enable us to carry out ANOVA statistics.

Learning effects between replicates were looked at through box plots, individual value plots and descriptive statistics, also significant differences in cell concentrations depending on the volume sub-sampled and the cell counting strategy used were studied through the use of two sample T-tests, paired T-tests, ANOVA, interaction plots for factors and General Linear Models.

Hypothesised values were also used to make assumptions about the samples true value and how these values compared with the analysts. This allowed us to discuss method effects.

Finally, mean values for each concentration were plotted and results were compared between analysts and labs. The final score was given as a Z-score using the mean of all the results and 3 sigma limits as a measure of dispersion.

4.7 Bequalm Workshop 2010

The workshop Bequalm 2010 was held this year in Vilaxoan, Pontevedra, Galicia Spain and it was held at INTECMAR (Instituto Tecnologico para el Control del Medio marino).

This workshop was held on the 27th of May 2010 (see Annex V: Workshop Agenda). The workshop was opened by the Director in INTECMAR, Dr Covadonga Salgado where she welcomed the participants to the workshop and gave a presentation about the work that is routinely carried out in INTECMAR. A tour of the lab facilities took place afterwards where participants had the chance to talk to the technical staff in the labs and had a chance to ask questions about phytoplankton monitoring in Galicia.

After the lab visit, the participants settled inside the auditorium where Mr Rafael Salas presented the results of the Intercomparison. These were divided in three different presentations, the first one on the taxonomy quiz results, the second one on the enumeration results and the third one on some aspects of the Natural sample results for future Intercomparison exercises.

After lunch, our taxonomy expert and guest speaker Dr Santiago Fraga from the IEO (Instituto Español de Oceanografia) in Vigo gave a presentation on 'Species Concept on HABs monitoring'.

The lecture touched in various aspects of the biological species concept from a historical perspective and compared these ideas with the most modern use of the ecological and phylogenetic concepts. How the phylogenetic concept sometimes clashes with the most classical ecological concept on what defines a species and it gives the example of the problems encountered to define the genus *Alexandrium spp.*, how morphological features used to identify *Alexandrium spp.* to species level like the presence /absence of ventral pore, the formation of chains and the anterior/posterior compression of cells might not be enough to define species apart, specially those toxic ones from the non-toxic ones.

Then, went onto discuss various important toxic phytoplankton genera. He explained briefly the problems facing the *Dinophysis spp.* concept (Edvarsen et al. 2003), *Coolia and Ostreopsis* (Penna et al. 2010, Fraga et al. 2008) and *Gambierdiscus* (Richlen et al. 2008, Litaker et al. 2009).

Finally, he finished by saying that the use of molecular tools is strongly recommended in monitoring of harmful algae.

Dr. Yolanda Pazos from INTECMAR showed a poster by MIDTAL (Microarrays for the detection of Toxic Algae). The objectives of this project are to test and optimise existing rRNA probes for toxic species and antibodies for toxins for their application to a microarray, to design and test the specificity of any new probe needed, to construct a universal microarray from the probes tested and optimized by all of the

partners for the detection of harmful algae and their toxins, to provide national monitoring agencies with a rapid molecular tool to monitor toxic algae, to validate or replace traditional methods for monitoring for toxic algae and to integrate European efforts to monitor coastal waters for toxic algal species.

After, an open questions and answers session on the future development of the Bequalm scheme and any future recommendations took place (See conclusion and recommendations chapter)

The Bequalm workshop was concluded after thanking our hosts INTECMAR, our guest speaker Dr Santiago Fraga and the participants for making the effort to travel to the workshop in Galicia.

5. Results and discussion

5.1 Phytoplankton identification results

The Phytoplankton identification exercise Bequalm 2010 was a repeat of the Taxonomic quiz exercise from 2008 (see Annex VII: Taxonomic quiz Beq 2010). There were 19 new entrants for this exercise in 2010. The other 20 analysts were exempted from doing this part of the exercise as they have already completed it in 2008.

The taxonomic quiz consisted of 8 questions, each question contained several photographs and/or illustrations and participants were asked to identify to genus/species level or to answer some questions in reference to taxonomic features of the species. Each question had different marks. The total number of marks was 300. Incorrect answers were given a zero, but no negative marks were given.

The quiz results were analysed to study how the participants did overall and also to investigate whether some participants performed better at identifying particular phytoplankton species over others. See Annex III for the correct answers to the taxonomic exercise.

Q1 was a question particularly on *Dinophysis spp.,* a very important group of toxic Dinoflagellates. Q2, 3 & 4 were questions on armoured Dinoflagellates. Q5, 6 & 7 were questions on diatoms and Q8 a question on naked Dinoflagellates.

Q1 was a general question on the toxic armoured Dinoflagellates of the genus *Dinophysis*. The analysts had to identify the images provided to species level and name a structural feature of the species marked with an arrow. All analysts performed very well in this question with percentages above 90% of correct answers. Only 6 analysts answered incorrectly one of the sub-sections (See Table 1).

Table 1: Participants results Beq2010 Q1

ANALYST CODE	Question 1 (60 marks)											
	A		B		C		D		E		F	
	sp.	ft	sp.	ft	sp.	ft	sp.	ft	sp.	ft	sp.	ft
33	5	5	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5	5	5
38	5	5	5	5	5	0	5	5	5	5	5	5
27	5	5	5	5	5	5	5	5	5	5	5	5
16	5	5	5	5	5	5	5	5	5	5	5	5
8	5	5	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	0	5	5
35	5	5	5	5	5	5	5	5	5	5	5	5
9	5	5	5	5	0	5	5	5	5	5	5	5
11	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
19	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	0	5	5	5	5	5	5	5	5	5
28	5	5	5	5	5	5	5	5	5	0	5	5
15	5	5	5	5	5	5	0	5	5	5	5	5
12	5	5	5	5	5	5	5	5	5	5	5	5
22	5	5	5	5	5	5	5	5	5	5	5	5
29	5	5	5	5	5	5	5	5	5	5	5	5
40	5	5	5	5	5	5	5	5	5	5	5	5

Q2, 3 and 4 dealt with armoured dinoflagellates, in Q2 analysts were asked using illustrations to differentiate between the Kofoidean tabulation of 2 armoured dinoflagellates and to name the genus they represented.

Q3 showed an illustration of the thecal structure of *Alexandrium spp.* in ventral and apical view. Analysts were asked to name certain plates and structures typical of this genus. Most analysts performed very well in these 2 questions and only 3 analysts made a small number of errors (See table 2).

Table 2: Participants results Beq2010 Q2 & 3

ANALYST CODE	Question 2 (20 marks)			Question 3 (20 marks)			
	1a	1b	2a	a	b	c	d
33	5	5	5	5	5	5	5
4	5	5	10	5	5	5	5
38	5	5	10	5	5	5	5
27	5	5	10	5	5	5	5
16	5	5	10	5	5	5	5
8	5	5	10	5	5	5	5
2	5	5	10	5	5	5	5
35	5	5	10	5	5	5	5
9	5	5	0	5	5	5	0
11	5	5	10	5	5	5	5
10	5	5	10	5	5	5	5
19	5	5	10	5	5	5	5
3	5	5	0	5	5	5	5
28	5	5	0	5	5	5	0
15	5	5	10	5	5	5	5
12	5	5	10	5	5	5	5
22	5	5	10	5	5	5	5
29	5	5	10	5	5	5	5
40	5	5	10	5	5	5	5

Q4 asked analysts to identify to species level a number of images representing armoured Dinoflagellates, again all analysts performed very well. Only 5 analysts made errors in Q4 but 4 out of the 5 analysts answered incorrectly image A. See table 3.

Table 3: Participants results Beq2010 Q4

ANALYST CODE	Question 4 (50 marks)									
	A		B		C		D		E	
	gen	sp.	gen	sp.	gen	sp.	gen	sp.	gen	sp.
33	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5
38	5	5	5	5	5	5	5	5	5	5
27	5	0	5	5	5	5	5	5	5	5
16	5	5	5	5	5	5	5	5	5	5
8	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	0	5	5	5	5
35	5	5	5	5	5	5	5	5	5	5
9	5	5	5	5	5	5	5	5	5	5
11	5	5	5	5	5	5	5	5	5	5
10	0	0	5	5	5	5	5	5	5	5
19	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5
28	5	5	5	5	5	5	5	5	5	5
15	5	5	5	5	5	5	5	5	5	5
12	5	5	5	5	5	5	5	5	5	5
22	5	5	5	5	5	5	5	5	5	5
29	0	0	5	5	5	5	5	5	5	5
40	0	0	5	5	5	5	5	5	5	5

Q5, 6 and 7 were questions on Diatoms. Q5 were diatom images and participants were asked to identify these to species level. Most analysts returned near perfect results.
8 analysts had difficulty going to species level with image B and 4 analysts had problems identifying to species level image A. 3 analysts answered incorrectly both images A and B. See table 4

Table 4: Participants results Beq2010 Q5

ANALYST CODE	Question 5 (70 marks)													
	A		B		C		D		E		F		G	
	gen	sp.	gen	sp.	gen	sp.	gen	sp.	gen	sp.	gen	sp.	gen	sp.
33	5	5	5	5	5	5	0	0	5	5	5	5	5	5
4	5	0	5	0	5	5	5	5	5	5	5	5	5	5
38	5	5	5	5	5	5	5	5	5	5	5	5	5	5
27	5	0	5	0	5	5	5	5	5	5	5	5	5	5
16	5	5	5	0	5	5	5	5	5	5	5	5	5	5
8	5	5	5	0	5	5	5	5	5	5	5	5	5	5
2	5	5	5	0	5	5	5	5	5	5	5	5	5	5
35	5	5	5	5	5	5	5	5	5	5	5	5	5	5
9	5	5	5	5	5	5	5	5	5	5	5	5	5	5
11	5	0	5	5	5	5	5	5	5	5	5	5	5	5
10	5	5	5	0	5	5	5	5	5	5	5	5	5	5
19	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5
28	5	0	5	5	5	5	5	5	5	5	5	5	5	5
15	5	5	5	5	5	5	5	5	5	5	5	5	5	5
12	5	5	5	0	5	5	5	5	5	5	5	5	5	5
22	5	5	5	0	5	5	5	5	5	5	5	5	5	5
29	5	5	5	5	5	5	5	5	5	5	5	5	5	5
40	5	5	5	5	5	5	5	5	5	5	5	5	5	5

In Q6 participants were asked to name the image that was the odd one out. The Question contained 8 images one of which did not belong to a Diatom. The correct answer was image E depicting a silicoflagellate. All analysts answered correctly. See table 5.

Q7 was a specific question on *Pseudo-nitzschia spp.* First, participants were asked using the illustration depicting a silica frustule of this diatom in valve and girdle view to draw where a width measurement should be taken and in which view. The answer was in valve view.

The follow up question asks participants given that the valve view is used to measure the width in these species, which of the images shown depicts this view and the answer is image C. There were only 2 incorrect answers to this question.

Q8 was a question about naked dinoflagellates, the illustration depicts 7 different genera and participants are asked to give the name of the genus that each illustration represented based on morphological features like the cingulum displacement, the presence/absence of ventral pores and their typical apical groove. All analysts received full marks on this question. See table 5.

Table 5: Participants results Beq2010 Q6, 7 & 8

ANALYST CODE	Question 6 (20 marks)	Question 7 (30 marks)			Question 8 (30 marks)						
	Circle answer	A	B	C	A	B	C	D	E	F	G
33	15	10	10	10	5	5	5	5	5	5	5
4	15	10	10	10	5	5	5	5	5	5	5
38	15	10	10	10	5	5	5	5	5	5	5
27	15	10	10	10	5	5	5	5	5	5	5
16	15	10	10	10	5	5	5	5	5	5	5
8	15	10	10	10	5	5	5	5	5	5	5
2	15	10	10	0	5	5	5	5	5	5	5
35	15	10	10	10	5	5	5	5	5	5	5
9	15	10	10	10	5	5	5	5	5	5	5
11	15	10	10	10	5	5	5	5	5	5	5
10	15	10	10	10	5	5	5	5	5	5	5
19	15	10	10	10	5	5	5	5	5	5	5
3	15	10	10	10	5	5	5	5	5	5	5
28	15	10	10	0	5	5	5	5	5	5	5
15	15	10	10	10	5	5	5	5	5	5	5
12	15	10	10	10	5	5	5	5	5	5	5
22	15	10	10	10	5	5	5	5	5	5	5
29	15	10	10	10	5	5	5	5	5	5	5
40	15	10	10	10	5	5	5	5	5	5	5

The overall results have been ranked per analyst and tabulated as a percentage of correct answers from the total. See tables 6 and 7.

Table 8 shows the descriptive statistics of all the participants' results in the Intercomparison. The overall mean of correct answers for all the questions is very high indicating nearly perfect results. The highest result is for Q8 followed by Q1, the groupings Q2, 3, 4 (armoured dinoflagellates) and Q5, 6, 7 (diatoms) are slightly lower than the other 2 with a higher percentage of correct answers on diatoms but not significantly higher. This is shown graphically with box plots in Graph 1: Box plot of identification scores.

Table 6: Overall score

Analyst Code	Q1(%)	Q2,3,4(%)	Q5,6,7(%)	Q8(%)	Total(%)
33	100	94	93	100	97
4	100	100	93	100	98
38	92	100	100	100	98
27	100	94	93	100	97
16	100	100	96	100	99
8	100	100	96	100	99
2	92	94	85	100	93
35	100	100	100	100	100
9	92	83	100	100	94
11	100	100	96	100	99
10	100	89	96	100	96
19	100	100	100	100	100
3	92	89	100	100	95
28	92	83	85	100	90
15	92	100	100	100	98
12	100	100	96	100	99
22	100	100	96	100	99
29	100	89	100	100	97
40	100	89	100	100	97

Table 7: Analysts' rank

%	ANALYST CODE	Position
100	35	1
100	19	1
99	16	2
99	8	2
99	11	2
99	12	2
99	22	2
98	4	3
98	38	3
98	15	3
97	29	4
97	40	4
97	33	4
97	27	4
96	10	5
95	3	6
94	9	7
93	2	8
90	28	9

Table 8: Descriptive statistics of quiz results

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

Variable	N	Mean	SE Mean	StDev	Q1	Median	Q3
Q1 (%)	19	97.368	0.913	3.980	91.667	100.00	100.00
Q2,3,4 (%)	19	95.03	1.40	6.11	88.89	100.00	100.00
Q5,6,7 (%)	19	96.20	1.06	4.63	92.86	96.43	100.00
Q8 (%)	19	100.00	0.00	0.00	100.00	100.00	100.00

Graph 1: Box plot of identification scores

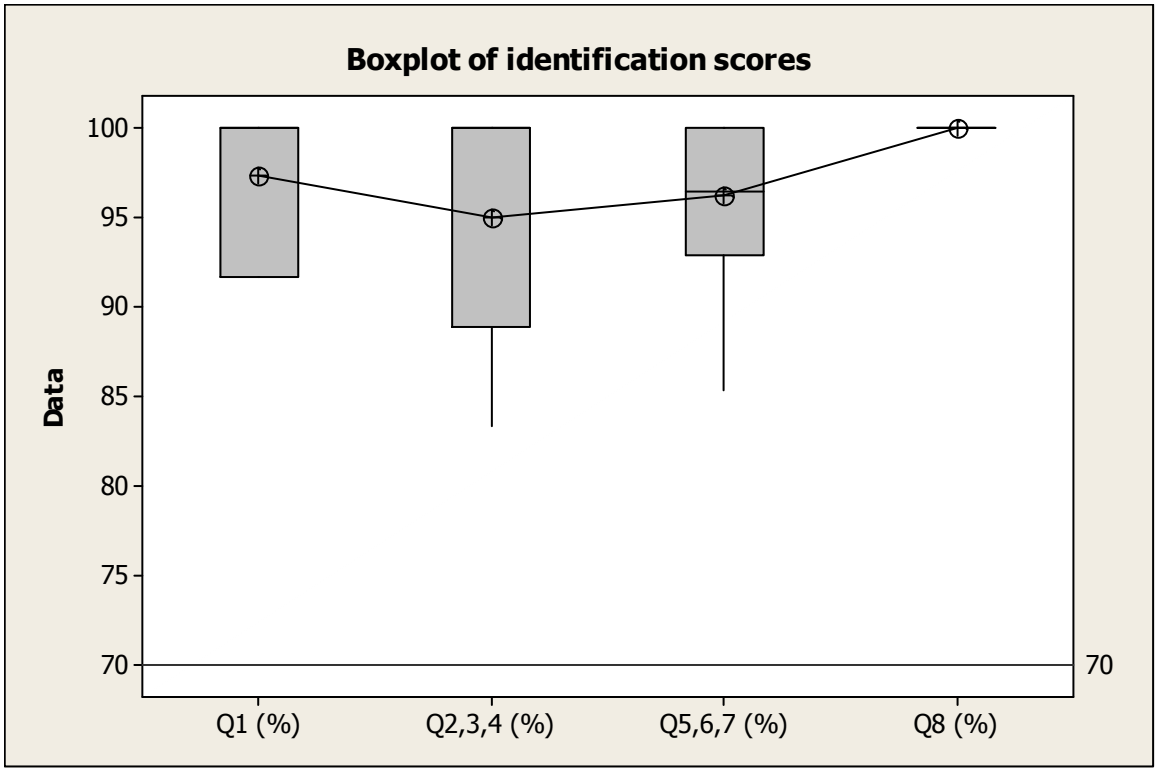


Table 9 shows the cumulative percentage of correct answers. The tally for discrete variables shows that most analysts would be above the 90% mark in most questions with a small number of analysts just below the 90% mark in Q2, 3, 4 and Q5, 6, 7.

Table 9: Cumulative percentage of correct answers

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

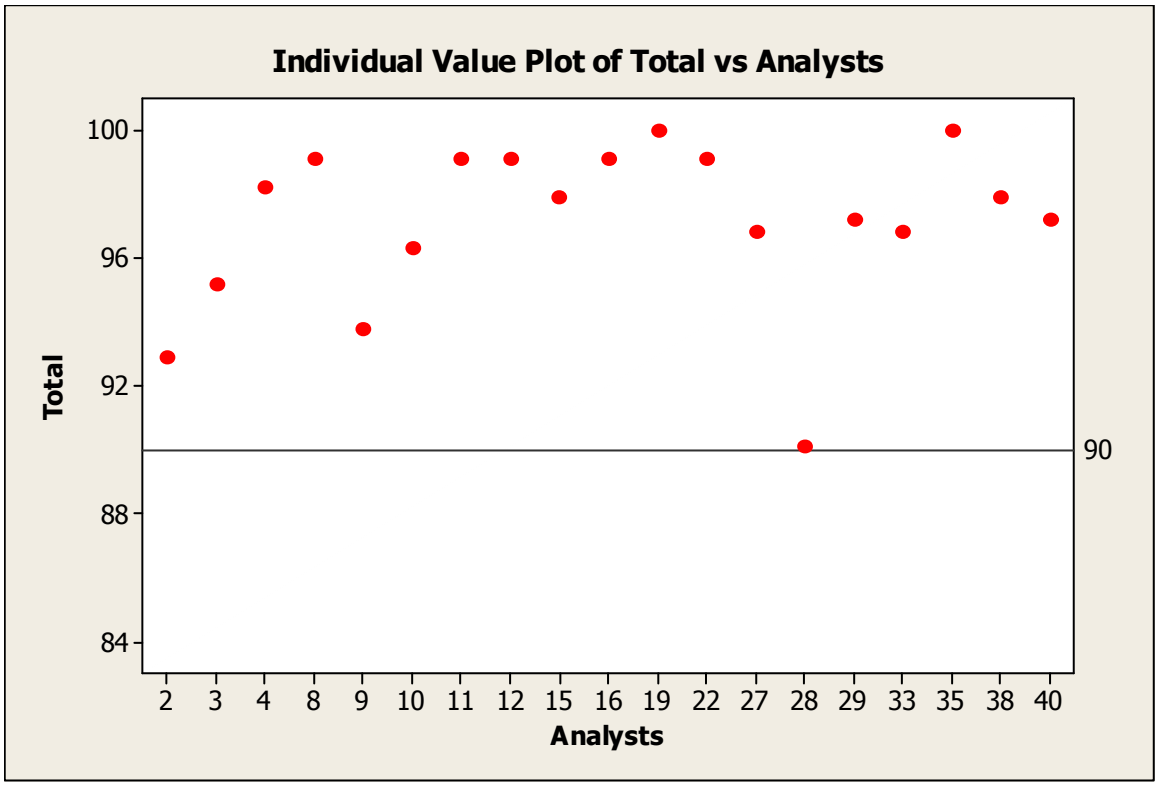
Q1 (%)	Count	CumPct
92	6	31.58
100	13	100.00
N=	19	

Q2,3,4 (%)	Count	CumPct
83	2	10.53
89	4	31.58
94	3	47.37
100	10	100.00
N=	19	

Q5,6,7 (%)	Count	CumPct
85	2	10.53
93	3	26.32
96	6	57.89
100	8	100.00
N=	19	

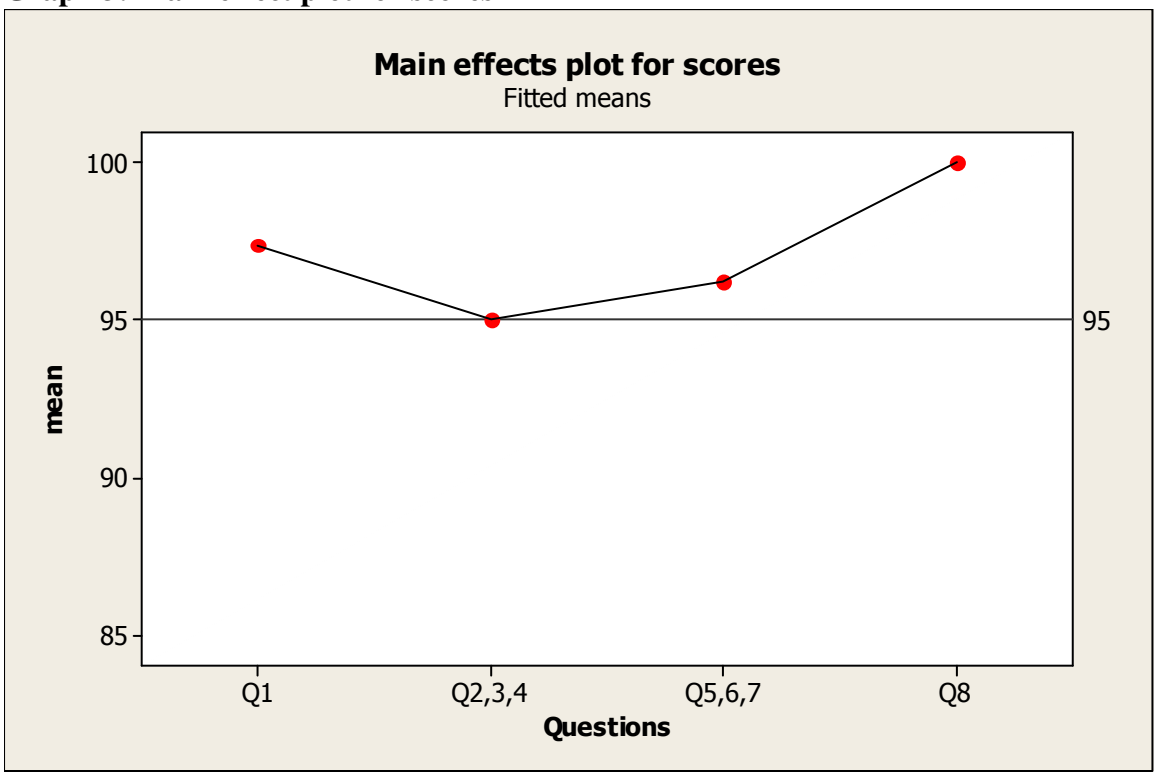
Q8 (%)	Count	CumPct
100	19	100.00
N=	19	

Graph 2: Overall % correct answers by individual analysts



Graph 2 shows that if the pass mark was 90% all analysts will still pass the test with most analysts in the high 95-96% mark and one analyst around the 90% mark. Graph 3: Main effect plot for scores shows that the mean of correct answers per question is above 95%

Graph 3: Main effect plot for scores



Overall, the analysts that completed the taxonomic quiz performed to a very high standard and the overall results with 2 analysts on perfect scores (100%) and another 8 analysts in 98% or above with the other 9 analysts above 90% suggests that all analysts showed proficiency in this exercise.

5.2 Phytoplankton enumeration results

The Phytoplankton enumeration exercise Bequalm 2010 was designed to be solely a counting exercise, so no identification of the spiked culture material was needed as in the previous exercise in 2009. In this exercise one species *Scrippsiella spp.* an armoured Dinoflagellate was spiked in the samples at two different cell concentrations and in triplicates. All analysts received 6 samples for this part of the exercise and the results obtained are tabulated in Table 10. All analysts used the Utermöhl test method for this exercise but different sedimentation chamber volumes and counting strategies were used.

Table 10: Analysts cell concentration counts Bequalm 2010

ANALYST CODE	SAMPLE CODES						Cells/L					
							Cell count (Low			Cell count (High density)		
10	35	39	151	82	90	112	0	5000	2000	101000	205000	168000
37	88	132	133	15	17	25		1600	3300	158000	125200	153000
13	86	201	293	190	255	276	6400	4900	4500	186100	326000	277000
17	55	161	265	11	99	245	6000	4500	4400	155200	167000	402486
30	13	249	275	43	80	93	4000	4400	5000	149300	148000	169500
21	21	77	124	69	147	289	3800	3100	3800	160000	139700	244700
9	129	250	287	254	257	297	5915	6097	5733	387512	374376	443340
11	3	18	66	44	120	185	5500	5800	4067	219567	209833	436333
15	2	54	159	283	16	67	5800	5400	6700	723900	315400	266000
33	64	179	246	97	177	272	4160	3360	4480	157480	108600	227400
4	72	87	209	14	169	238	3160	3360	2225	161480	163480	211920
38	176	230	291	103	237	292	3960	4280	3880	175880	166000	151920
24	49	101	294	126	212	296	1840		2080	173160	142880	207500
26	119	140	223	52	23	228	4080	2920	3200	166120	139640	102240
39	102	150	225	183	187	229	4010	3952	4192	105752	124930	141915
34	38	236	279	5	123	298	3640	3920	2960	157560	90280	181320
5	24	211	273	28	85	197	4240	4240	4320	184560	184400	197520
25	47	36	263	40	51	58	3640	3680	4760	153520	169320	170400
35	232	60	12	207	252	194	5000	5280	4000	136200	158200	151440
28	29	94	121	4	10	239	4038	3482	3667	114334	164815	21260
27	41	37	171	248	160	186	3360	2760	2880	55720	120080	126240
18	95	226	166	56	19	135	4400	5280	5680	132183	190931	171606
7	193	235	26	1	266	227	3400	2960	4160	142975	284760	187093
20	100	231	281	62	167	240	2360	1440	4494	90629	138565	229447
36	68	259	284	163	195	234	3240	2824	3960	86216	107448	101795
16	111	46	61	261	27	92	4840	5520	5440	296678	239659	294413
8	115	162	241	7	84	131	5667	4173	5280	243041	238374	256734
2	152	157	203	148	224	274	5040	4320	4920	317130	284456	219108
19	110	130	286	114	153	256	5160	3840	3400	180112	224726	190026
3	8	210	191	105	50	178	4600	4360		197000	258280	284760
29	75	196	214	6	53	220	5280	4440	5080	231679	175473	291997
40	280	113	138	206	70	117	6080	4120	5720	204330	211870	288776
12	34	149	180	59	202	300	5280	4560	4400	158064	221432	278392
14	108	144	264	122	158	174	5445	4455	5940	305415	390555	242055
23	104	258	290	73	127	295	4120	5360	5320	329618	266875	242149
32	143	156	285	165	189	253	3480	2880	4280	191212	122922	245844

Table 10 shows the analysts codes, the sample codes and the cell concentrations in the low and high density samples. There are few gaps in the data and this indicates that analysts did not return all the cell counts, in the case of analyst 37 and 24 their samples leaked from the sedimentation chamber and in the case of analyst 3 this analyst received 4 samples at high concentration and only 2 at low concentration which was an error on our part. In all cases it was too late to send a replacement sample before submission of results.

Table 11 shows the sub-sampled volume used by the different analysts and the cell counting strategies used for each cell concentration. Most analysts used either 10ml or 25ml aliquots to analyse bar one analyst that used 2ml aliquots. One analyst used a 27ml aliquot instead of 25ml but for statistical purposes the results have been pooled together with the 25ml analysts.

Table 11: Analysts methodologies Bequalm 2010

ANALYST CODE	Methodology	Counting strategy	
		Low	High
10	2ml sub-sample in Utermohl chamber	WC	HC
37	10 ml sub-sample in utermohl chamber	WC	WC
13	10 ml sub-sample in utermohl chamber	WC	WC
17	10 ml sub-sample in utermohl chamber	WC	WC
30	10 ml sub-sample in utermohl chamber	WC	WC
21	10 ml sub-sample in utermohl chamber	WC	WC
9	10 ml sub-sample in utermohl chamber	WC	TR
11	10 ml sub-sample in utermohl chamber	WC	TR
15	10 ml sub-sample in utermohl chamber	WC	TR
33	25ml sub-sample in Utermohl chamber	WC	WC
4	25ml sub-sample in Utermohl chamber	WC	WC
38	25ml sub-sample in Utermohl chamber	WC	WC
24	25ml sub-sample in Utermohl chamber	WC	WC
26	25ml sub-sample in Utermohl chamber	WC	WC
39	25ml sub-sample in Utermohl chamber	WC	WC
34	25ml sub-sample in Utermohl chamber	WC	WC
5	25ml sub-sample in Utermohl chamber	WC	WC
25	25ml sub-sample in Utermohl chamber	WC	WC
35	25ml sub-sample in Utermohl chamber	WC	WC
28	25ml sub-sample in Utermohl chamber	WC	WC
27	25ml sub-sample in Utermohl chamber	WC	HC
18	25ml sub-sample in Utermohl chamber	WC	TR
7	25ml sub-sample in Utermohl chamber	WC	TR
20	25ml sub-sample in Utermohl chamber	WC	TR
36	25ml sub-sample in Utermohl chamber	WC	TR
16	25ml sub-sample in Utermohl chamber	WC	TR
8	25ml sub-sample in Utermohl chamber	WC	TR
2	25ml sub-sample in Utermohl chamber	WC	TR
19	25ml sub-sample in Utermohl chamber	WC	TR
3	25ml sub-sample in Utermohl chamber	WC	TR
29	25ml sub-sample in Utermohl chamber	WC	TR
40	25ml sub-sample in Utermohl chamber	WC	TR
12	25ml sub-sample in Utermohl chamber	HC	TR
14	25ml sub-sample in Utermohl chamber	TR	TR
23	25ml sub-sample in Utermohl chamber	WC	FoV
32	25ml sub-sample in Utermohl chamber	WC	FoV

The cell counting strategy mainly used for the low cell concentration samples was a Whole chamber (WC) count by the majority of the analysts, although one analyst used a half chamber (HC) and another a Transect (TR) count.

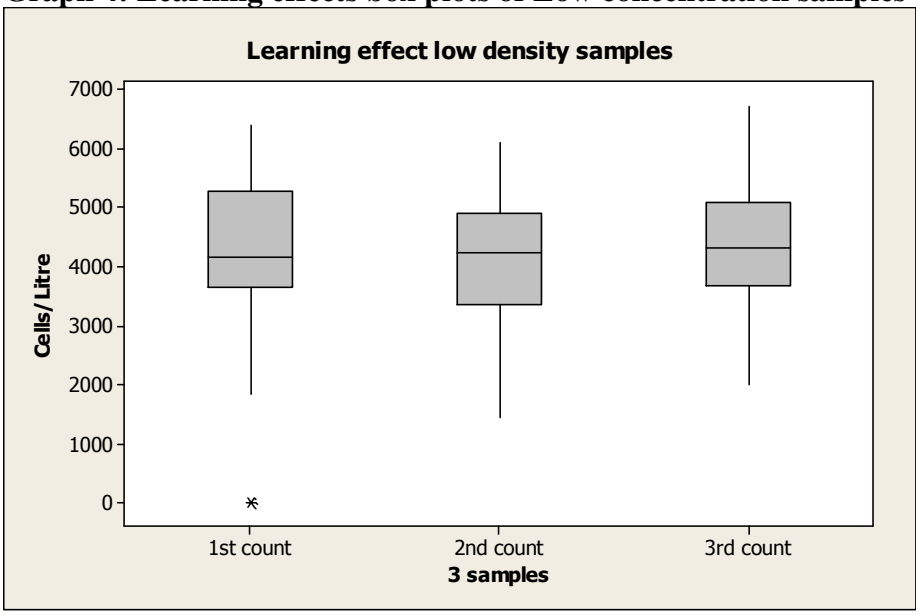
For the high concentration samples analysts were divided mainly between using WC counts or TR counts, while one analyst used a HC count and 2 analysts used a Field of View (FoV) count.

5.2.1 Learning effects

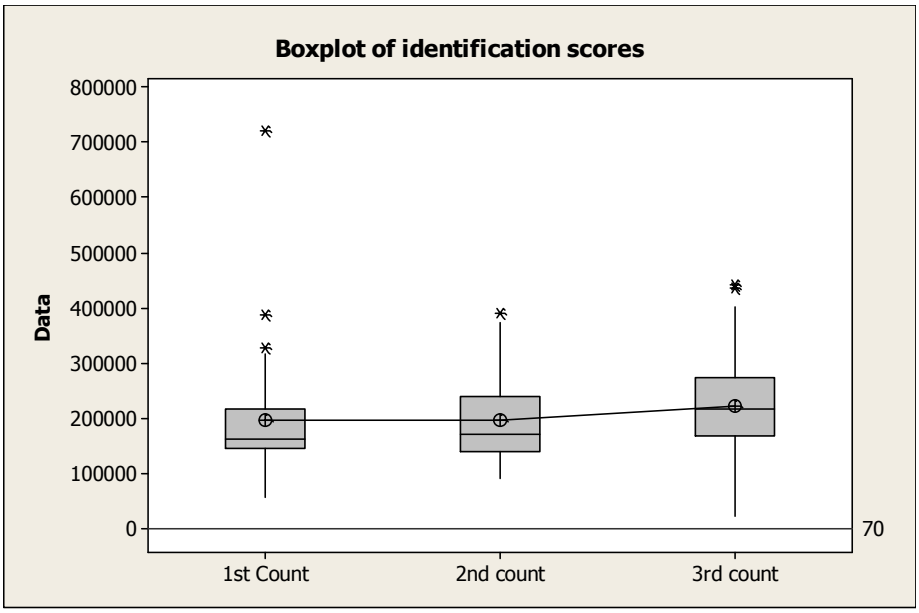
Learning effects can be caused by the ability of analysts to improve their performance when analysing successive replicate samples. For this reason, analysts were asked to number sequentially their samples as these were analysed.

Graph 4 shows the analysts mean per replicate on low cell concentration samples. This graph indicates that there are no significant learning effects between replicates, that there is no improvement in the performance either towards higher or lower cell numbers. This was done for the high cell concentration samples as well with similar results (See Graph 5). However, there were more outliers in the high cell counts.

Graph 4: Learning effects box plots of Low concentration samples



Graph 5: Learning effects box plots of High concentration samples



A paired T-test was performed to compare the analysts replicate counts. Table 12 shows the Paired T-test between the 1st and 2nd count at low concentration. The 95% Confidence Interval for the mean difference of these 2 counts is (-175,653) and a P-value of 0.248 which demonstrates that the null hypothesis is true and that there is no significant differences between replicate counts.

Table 12: Paired T-test of 1st and 2nd count at low concentration

Paired T for 1st count - 2nd count

	N	Mean	StDev	SE Mean
1st count	34	4385	1260	216
2nd count	34	4146	1025	176
Difference	34	239	1186	203

95% CI for mean difference: (-175, 653)
T-Test of mean difference = 0 (vs not = 0): T-Value = 1.18 P-Value = 0.248

This Paired T-test was performed for all cell concentrations Low and High (see table 13) and all possible permutations of sample replicates (1st Vs 2nd, 1st vs 3rd, 2nd vs 3rd) and the results indicated that there are not learning effects between replicate counts overall.

Table 13: Paired T-test of 1st and 2nd count at high concentration

Paired T-Test and CI: 1st Count, 2nd count

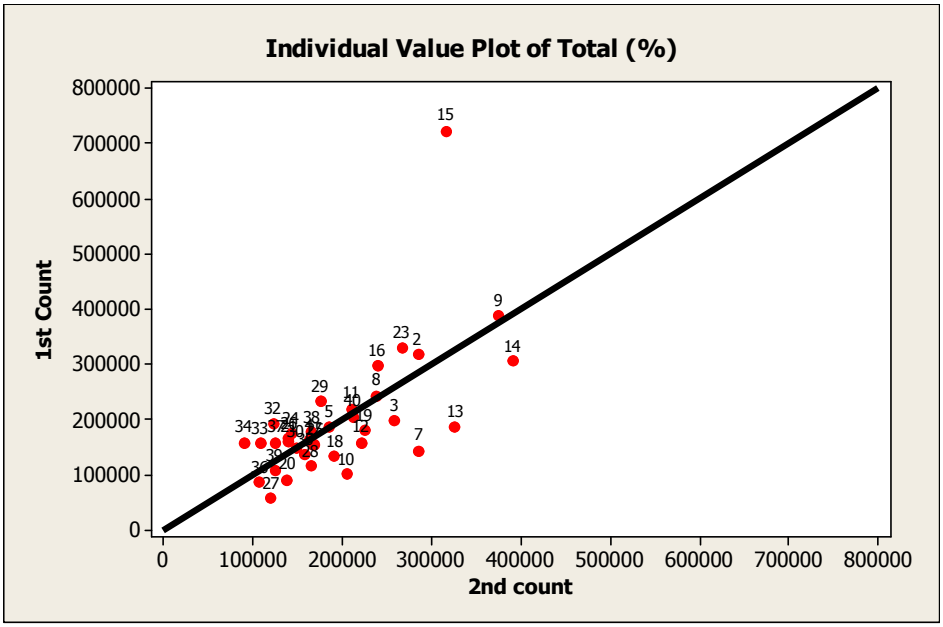
Paired T for 1st Count - 2nd count

	N	Mean	StDev	SE Mean
1st Count	36	196906	115574	19262
2nd count	36	197207	75624	12604
Difference	36	-301	88649	14775

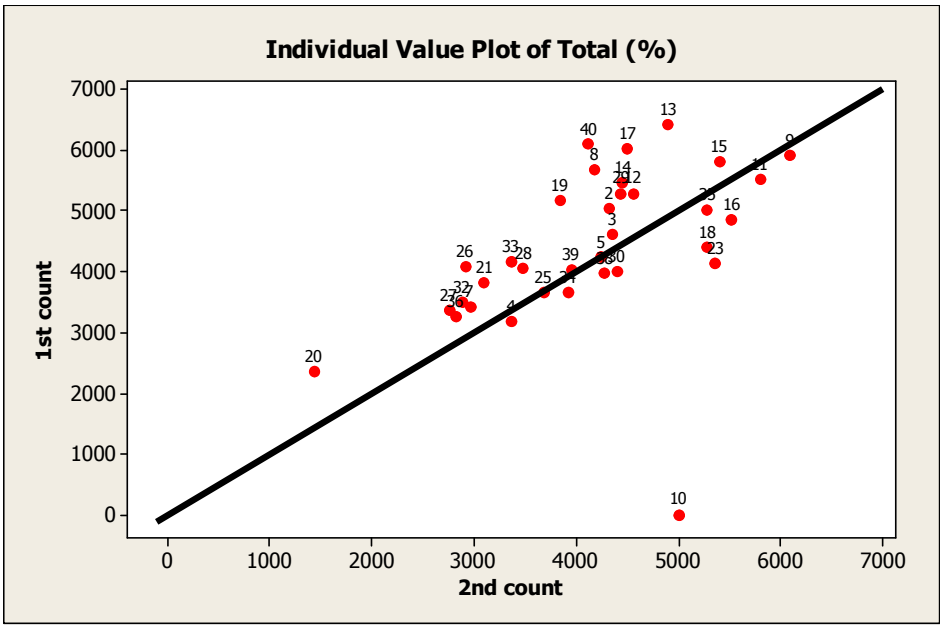
95% CI for mean difference: (-30295, 29693)
T-Test of mean difference = 0 (vs not = 0): T-Value = -0.02 P-Value = 0.984

Graph 6 & 7 shows the high concentration of the first minus the second count to compare individual analysts' performance between replicates. If both the first and second cell count were close to each other then the red dot should be on the reference line (Black line) and if all the analysts were equally precise then all the dots should appear very close to the reference line and bunched together, this would suggest good agreement between replicates and between analysts. Analyst 15 is an outlier where the 1st count is close to 700000 C/L and the second is down to just over 300000 C/L.

Graph 6: individual values by analysts 1st count-2nd count at high concentration



Graph 7: individual values by analysts 1st count-2nd count at Low concentration



Graph 7 shows the cell count differences between analysts' replicates at low concentration. In this case there is one outlier (analyst 10).

Overall, it can be summarised that learning effects have not been observed in the analysis of low or high cell concentration replicates and that there is variability between replicates within and between analysts but the paired T-test indicates that this difference is not significant.

5.2.2 Method effects

The main objective of interest in this study was to observe method effects in the analysis of the samples and compare these results. In order to do this, we needed a good design and that's why we choose 3 sample replicates per analyst as the minimum required to obtain statistically robust data.

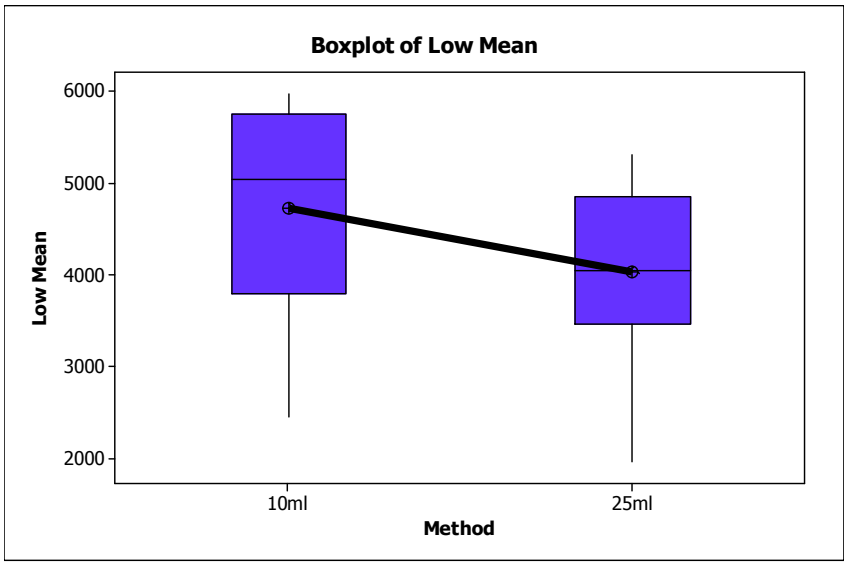
The reason for using two concentrations was to test the behaviour of the test method at particular concentration ranges. We were interested about the method choices that participants would make in the analysis depending on the cell concentration found in the samples.

Analysts were asked to follow their own protocols as this would be essentially what they would be doing routinely in their respective monitoring programmes. All analysts decided to use the Utermöhl test method.

75% of the analysts chose the 25ml sub-sample volume and the rest bar one chose the 10ml sub-sample volume. It is possible that if a bigger volume had been given instead of 30ml that some labs would have chosen to use 50ml sub-samples too.

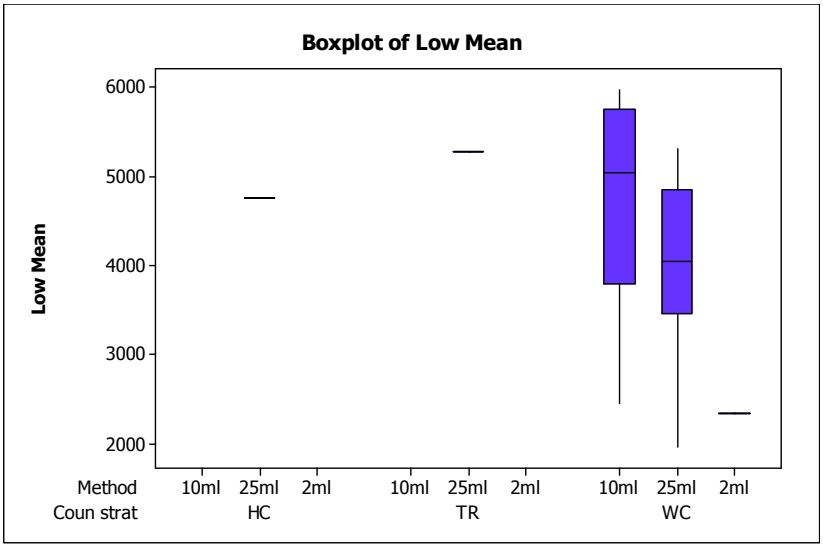
The Whole Chamber (WC) cell counting strategy was used by most analysts on the samples containing low cell concentrations (See table 11). This was most likely because based on experience the cell concentration in the sample was low enough so that it would be typical to count the total amount in the whole area of the sedimentation chamber.

Graph 8: Box plot of 10ml versus 25ml sub-samples at low concentrations



Graph 8 compared the analysts' mean concentration of low density samples using the 10ml volumes versus these using 25ml volumes.

Graph 9: Box plot of 10ml, 25ml, 2ml sub-samples at low concentrations against counting strategies



Graph 9 shows that there is insufficient data to compare the analysts using 2ml volume and the analysts using the 25ml volume but different counting strategies. There is only one data point for 2ml (WC), one for 25ml (HC) and one for 25ml (TR).

Table 14 indicates that 8 and 25 analysts performed respectively a 10ml and 25ml sample analysis on the low concentration samples and that all of them used a WC counting strategy.

Table 14: Tabulated statistics by method (volume) and counting strategy

Tabulated statistics: Coun strat, Method

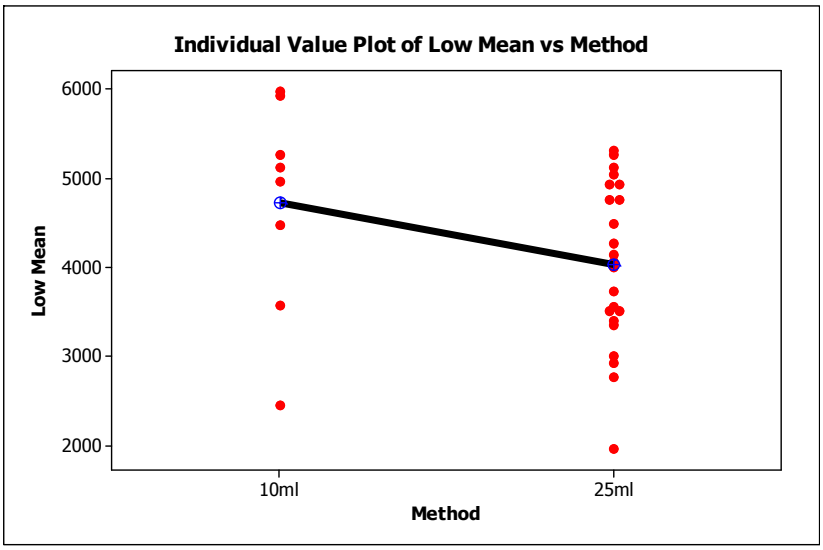
Rows: Coun strat Columns: Method

	10ml	25ml	2ml	All
HC	0	1	0	1
• TR	0	1	0	1
• WC	8	25	1	34
• All	8	27	1	36

- Cell Contents: Count

Graph 10 shows the mean values of all analysts using 10ml or 25ml volumes. The mean value is compared through a reference line in black, there seem to be differences between the mean of analysts using 10ml or 25ml sample volumes.

Graph 10: Individual value plot of low concentration samples



To test whether the differences in the mean values of 10ml versus 25ml are significant a two sample T-test is carried out (See Table 15). The result (P-value=0.170) and confidence interval (-353, 1720) suggests that the null hypothesis is true; there is no significant differences between the 10 and 25ml volume methods at low cell concentrations. The standard deviation is however smaller in the 25ml results (872) compared to the 10ml results (1198) suggesting that the variation is smaller in bigger sample volumes at this concentration.

Table 15: Two sample T 10 versus 25ml volume

Two-Sample T-Test and CI: Low Mean, Method

Two-sample T for Low Mean

Method	N	Mean	StDev	SE Mean
10ml	8	4715	1198	424
25ml	25	4032	872	174

Difference = mu (10ml) - mu (25ml)
Estimate for difference: 684
95% CI for difference: (-353, 1720)
T-Test of difference = 0 (vs not =): T-Value = 1.49 P-Value = 0.170 DF = 9

The high cell concentration samples were a bit more complex to analyse than their counterparts at low concentrations. At the high cell concentration apart from the 10ml against 25ml volumes used, there were other factors to take into account and this was also the cell counting strategy used. Table 16 are the ANOVA results for the high cell concentration samples depending on the volume and the counting strategy used. It is evident from this table that the analysts using 10ml and 25ml were divided as to what counting strategy should be used.

Table 16: ANOVA statistics for high cell concentration samples

Results for: ANOVA High counts

Tabulated statistics: count strat, Method

Rows: count strat Columns: Method

	10ml	25ml	2ml	All
FV	0	2	0	2
HC	0	1	1	2
TR	3	13	0	16
WC	5	11	0	16
All	8	27	1	36

Cell Contents: Count

Out of the 8 analysts using 10ml volumes 3 used a Transect (TR) and 5 used a Whole chamber (WC) counting strategy. Equally, those using the 25ml volumes, 13 used TR and 11 used WC counting strategies. 2 analysts used Field of View (FoV) counting strategies and one other Half Chamber (HC), but there are only a couple of data points for FoV and HC so we can not compare statistically with the others.

Therefore, the 25ml and 10ml volumes and the Transect and Whole chamber counting strategies will be compared. Graph 11 shows the Box plot of the mean results of all analysts and it shows that there are differences between methods and also between counting strategies. Is this mean difference significant?

In order to compare both the methodology used and the counting strategies we fitted a General Linear Model, Table 17 shows an ANOVA of the results where these are compared. It compares methodologies, counting strategies and both together, in all cases the P-value is below 0.05 which is significant and the alternative hypothesis is

true that is there are significant differences between methods, between counting strategies and between both taken together.

Graph 11: Box plot of Methods and counting strategies

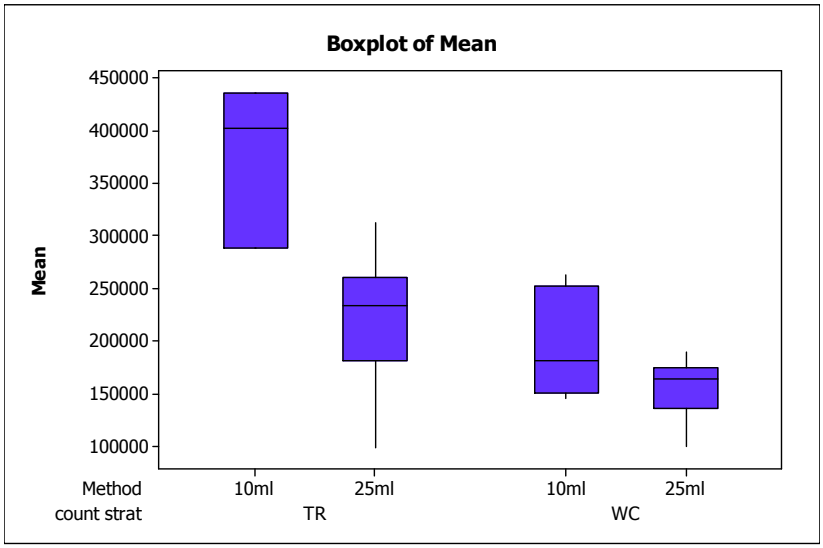


Table 17: General Linear Model of method and counting strategy

General Linear Model: Mean versus Method, count strat

Factor	Type	Levels	Values
Method	fixed	2	10ml, 25ml
count strat	fixed	2	TR, WC

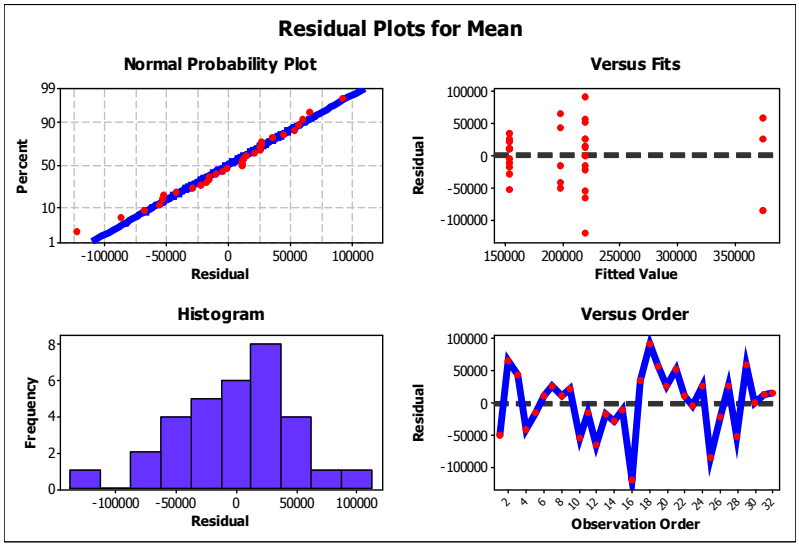
Analysis of Variance for Mean, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Method	1	33260934694	56425214036	56425214036	22.87	0.000
count strat	1	68233708189	85258666854	85258666854	34.55	0.000
Meth*c strat	1	17557939388	17557939388	17557939388	7.12	0.013
Error	28	69092177089	69092177089	2467577753		
Total	31	1.88145E+11				

- S = 49674.7 R-Sq = 63.28% R-Sq(adj) = 59.34%

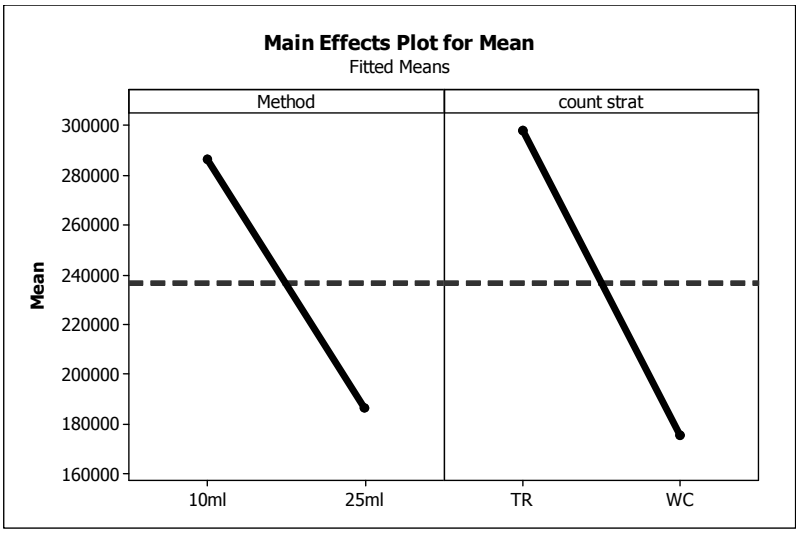
In graph 12, the residual plot of all the results show four graphs, The Normality and histogram plots show how the variation of results are distributed to either side of Zero and give us an idea of the variation on results, the Versus order plot show a random observation order and the versus fit show the results fitted according to the concentration found. This plot shows that there are four different concentration types and they correspond to the method used and the counting strategy used.

Graph 12: Residual plots of results



Graph 13 demonstrates the main effect of both factors (Method and Counting strategy) and levels (10 or 25; TR or WC) independently first. It is obvious from the graph that there are significant differences between methods and between counting strategies. The differences are larger due to the counting strategy than due to the volumes used at high concentrations. The line and slope is stepper and longer on the counting strategy panel.

Graph 13: Main effects by method and by counting strategy

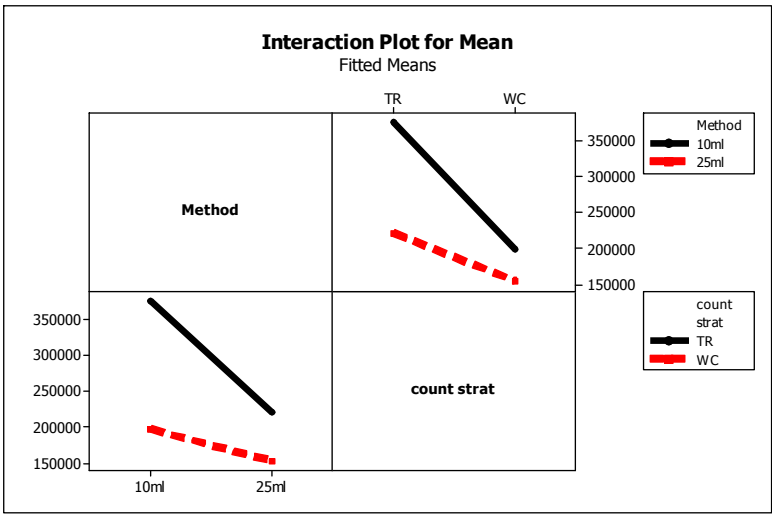


In graph 14, this interaction plot compares the differences between counting strategy and method at the same time. In the panel below left it compares the volume used with regards to the counting strategy, the panel clearly indicates that the differences

between cell counts at high cell concentrations tends to be larger if a transect count rather than a whole chamber cell count across methods. The black line is steeper.

Equally it can be summarised from the panel above right that if you use different counting strategies TR or WC the differences will be larger if you use 10ml rather than 25ml volumes.

Graph 14: Interaction plot of counting strategy, Method



5.2.3 Hypothesised means

The hypothesised means study is based on raw cell counts of 1ml aliquots dispensed from the 2 concentrations Master mix into Sedgewick-Rafter counting chambers to calculate values that hypothetically speaking could be the true values of the cell concentration on our samples.

Table 18 shows the approximate values that the organising lab produced for the low and high density samples. The Hypothetical values for the low density samples were based on 10 replicate cell counts of the total area of the chamber. The estimate for the high density samples was based again on 10 replicates but only 1/10 of the area of the chamber was counted.

It is important at this point to clarify that these hypothetical values are not to be used as reference or true value for the purpose of this Intercomparison but rather as a way

of highlighting potential method effects that may occur once aliquots are dispensed into sterilin tubes.

Table 18: Hypothesised values based on Sedgewick-Rafter cell counts

Sedgewick-Rafter cell counts		
Sample number	Cell number in 1ml	Final Cell conc. (Cells/L)
1	210	7000
2	195	6500
3	198	6600
4	189	6300
5	213	7100
6	188	6267
7	205	6833
8	202	6733
9	206	6867
10	208	6933
Mean	201	6713

Hypothesised mean= 6000
200 cells in 30ml

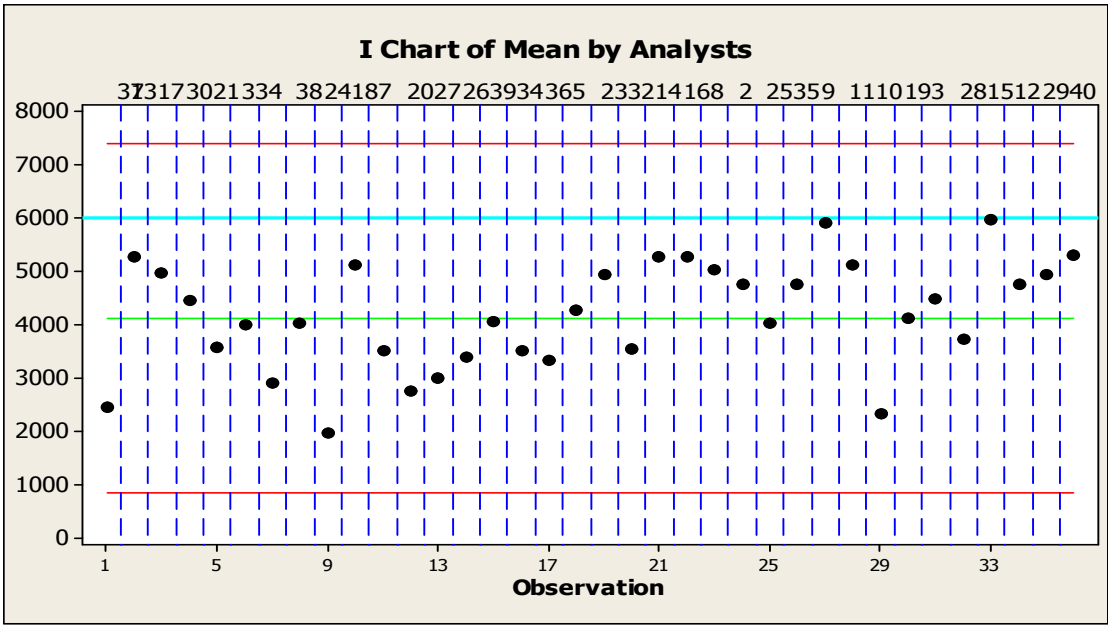
Sedgewick-Rafter cell counts			
Sample number	Cell number	Cell number* 10	Final Cell conc. (Cells/L)
1	982	9820	327333
2	1035	10350	345000
3	957	9570	319000
4	982	9820	327333
5	1001	10010	333667
6	1025	10250	341667
7	995	9950	331667
8	1015	10150	338333
9	975	9750	325000
10	1036	10360	345333
Mean	1000	10003	333433

Hypothesised mean= 333333
10000 cells approx. in 30ml

The hypothetical values arrived at for the low density samples was rounded to 6000 cells/Litre final concentration and for the high density samples was 333333 cells/Litre.

This is theoretically the samples real values before they are dispensed into sterilin tubes.

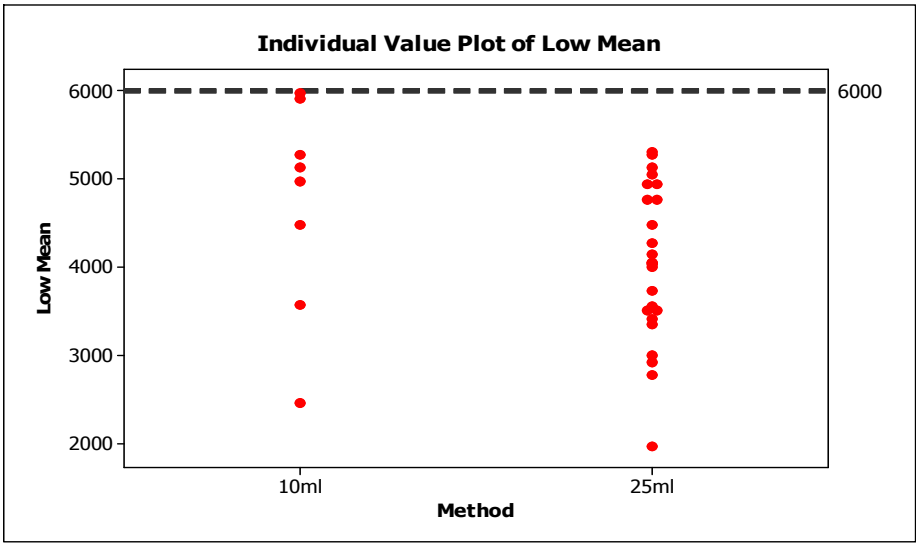
Graph 15: I chart of mean results (Low) by analysts and hypothesised mean



Graph 15 demonstrates that the values individual analysts produced do not compare very well with the hypothetical values (Blue line). Only two analysts, 9 and 15 gets close to the potential real value of the sample. The mean of all results is around the 4000 cells/L mark that is a 2000 cell underestimate of the hypothetical value.

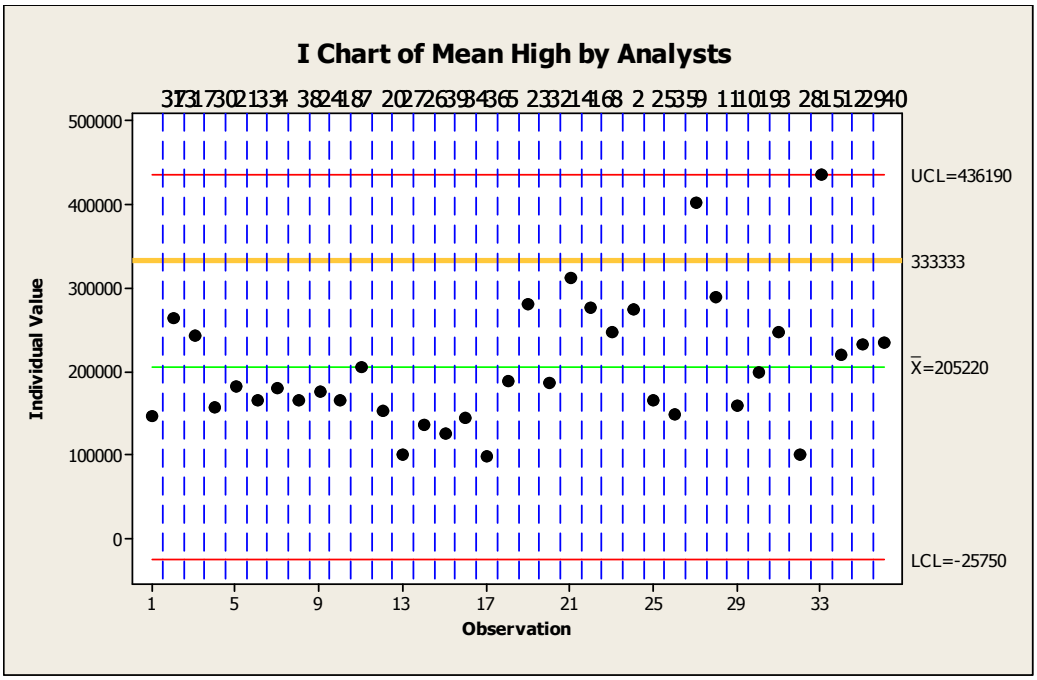
This potentially means that cells are being lost due to method effects of the sample after these have been prepared. Graph 16 demonstrates that the underestimation is independent of the method used. Analysts tend to underestimate using either 10ml or 25ml samples.

Graph 16: Individual value plot of 10 and 25ml

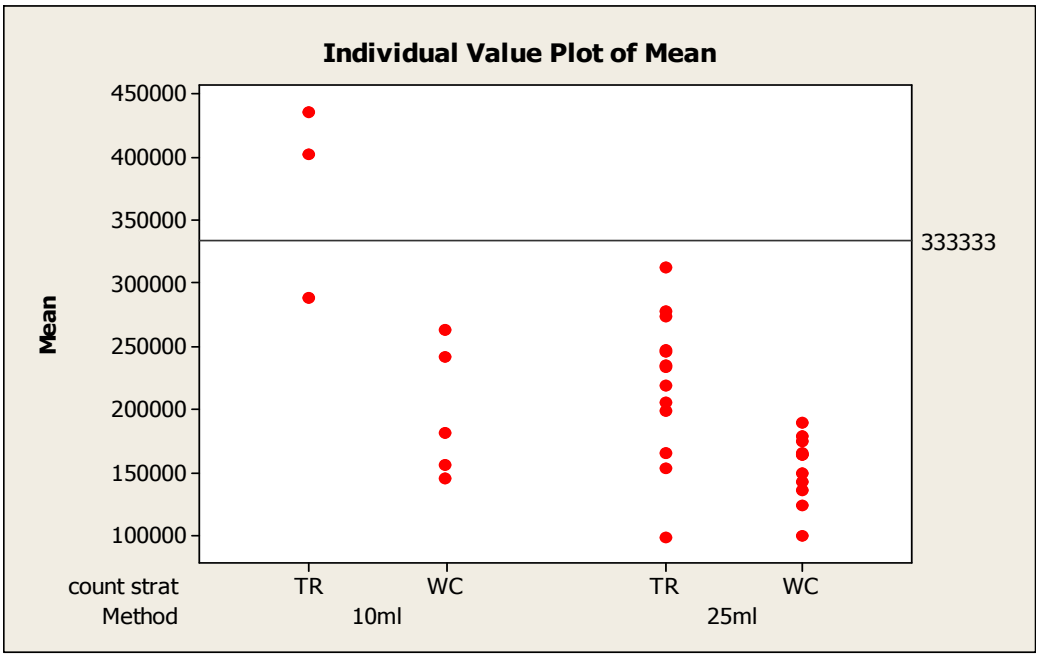


Graph 17 and 18 illustrates that the underestimation also occurs with the high density samples using either 10ml or 25ml volumes and independently of the counting strategy, be a whole chamber or a transect count. Although the results are closer to the hypothetical value for those using a 10ml aliquot and performing a transect count.

Graph 17: I chart of mean results (High) by analysts and hypothesised mean

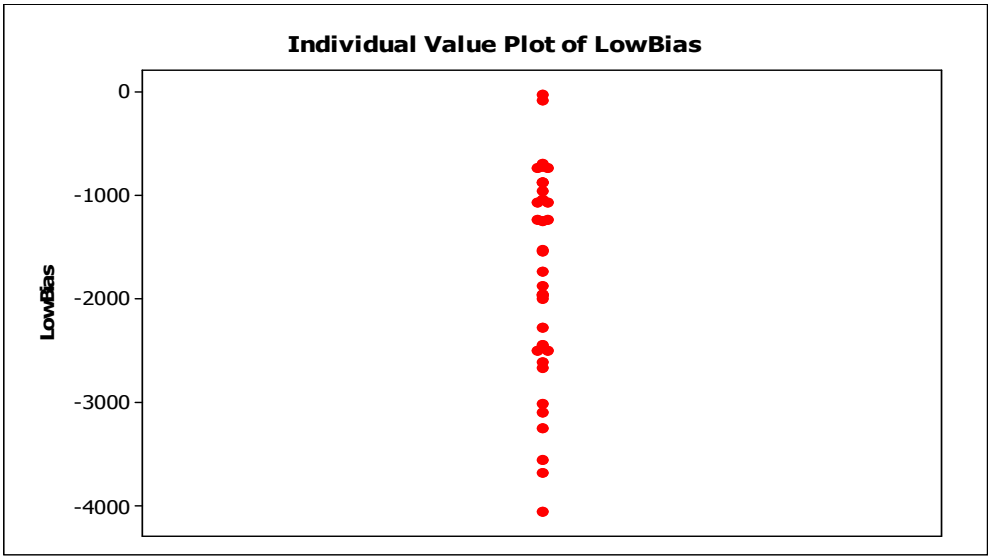


Graph 18: Individual value plot by method and counting strategy

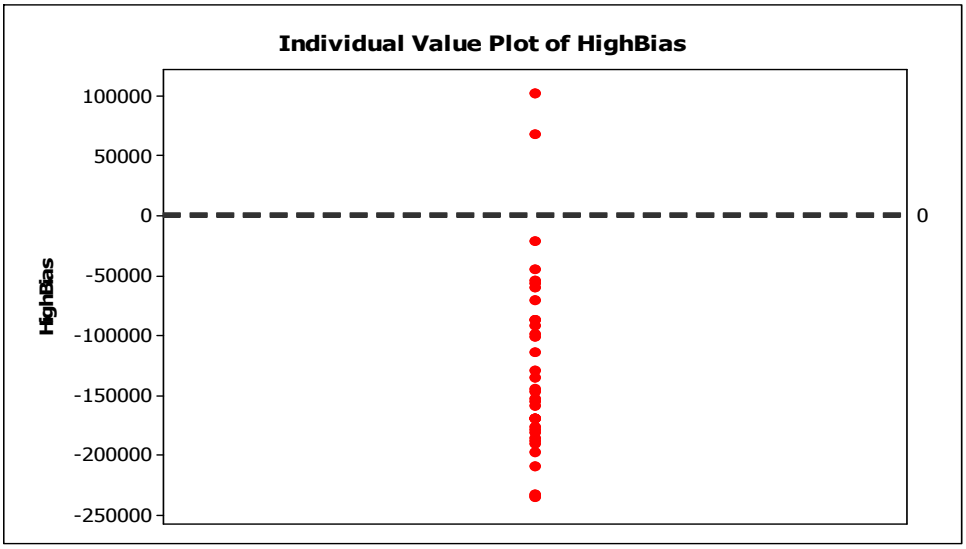


Graphs 19 and 20 are the Bias plots for Low and High concentrations against the Hypothesised means. These graphs clearly illustrate that the bias is always negative which suggests underestimation of cell counts and that the bias is larger at high cell densities.

Graph 19: Individual value plot low bias

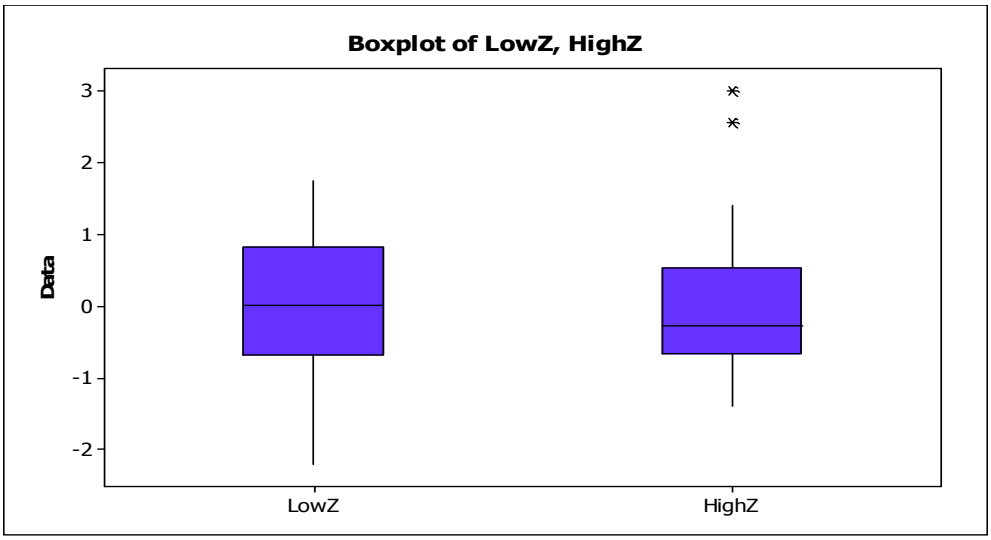


Graph 20: Individual value plot high bias



Graph 21 indicates, however that when you convert the bias for low and high density against the hypothesised means into Z-score box plots, both the low and high Z-scores are comparable.

Graph 21: Box plot of Lows versus High Z



5.2.4 Z-scores

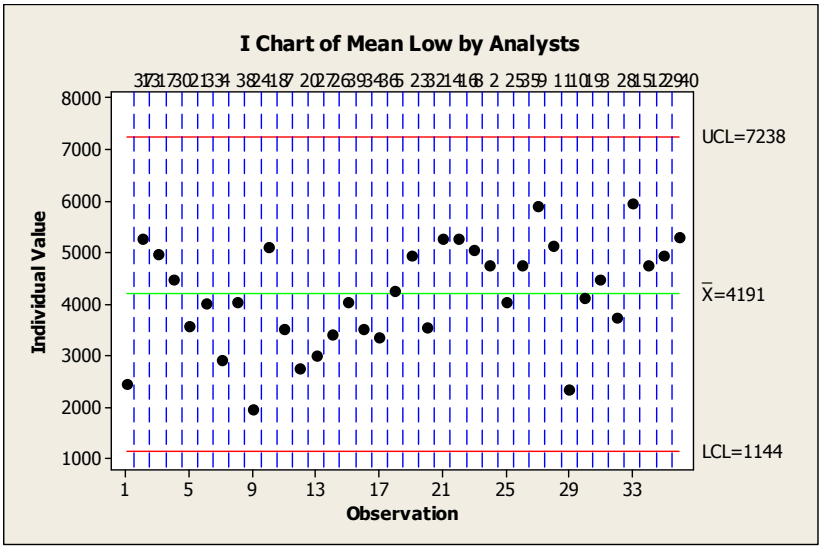
All analysts' results have been used in this exercise to calculate the mean and 3 Standard deviations of the mean. Z-scores have been calculated for each analyst and lab for the low density and high density samples.

Graph 22 shows an 'I chart' of low density samples by lab in cells per litre with the upper and lower confidence limits. Graph 23 illustrates the same but as a Z-score.

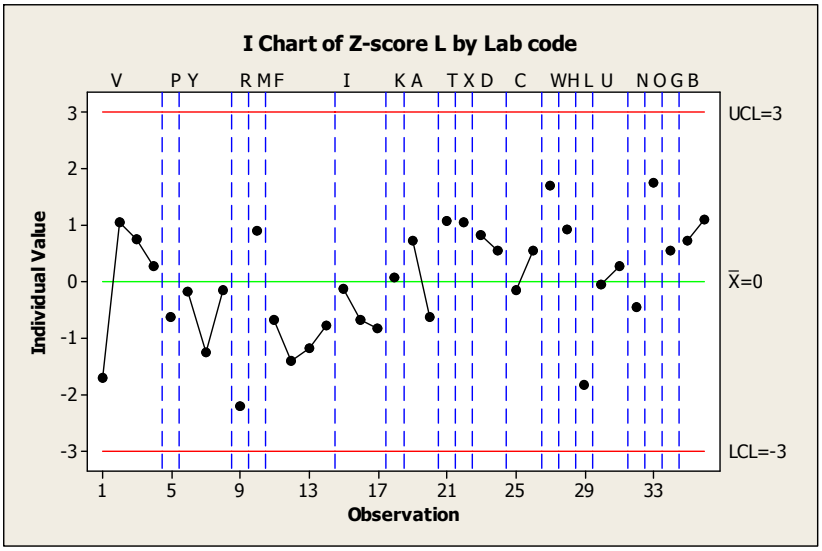
These graphs demonstrate that all analysts have performed within the 3 sigma limits or standard deviations of the mean of the population.

It also shows that there are variations between and within labs and between analysts.

Graph 22: I chart of mean (Low) by analysts

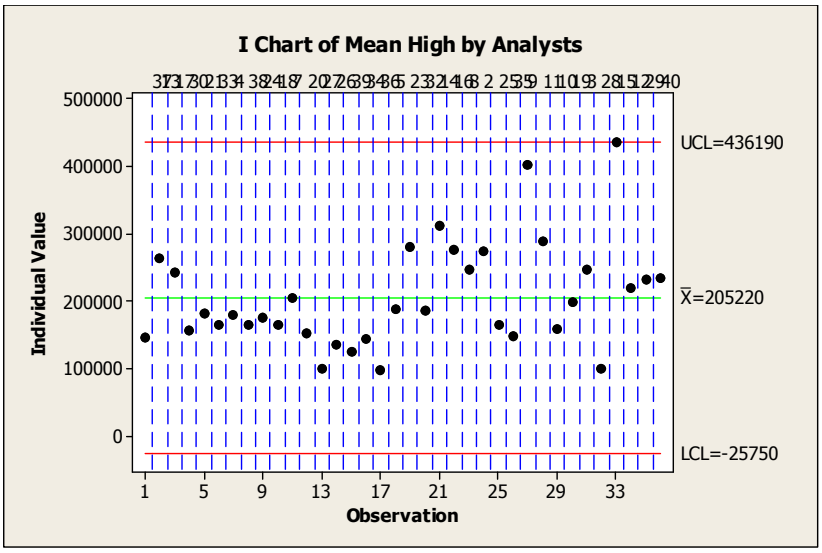


Graph 23: Z-score (Low) by lab code

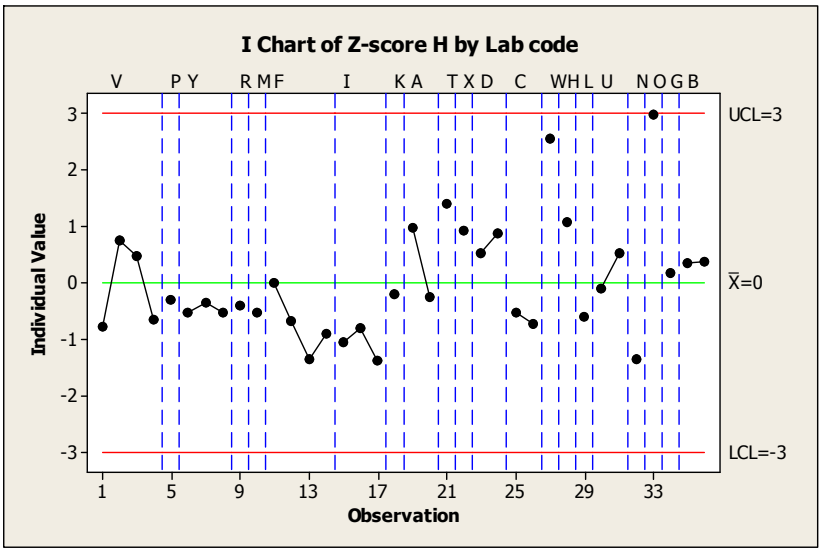


Graphs 24 and 25 are the results in cells per litre and Z-scores of the high density samples. The analysts performed again within the required parameters for the Intercomparison exercise. Also, there is variability between analysts and between and within labs.

Graph 24: I chart of mean (High) by analysts



Graph 25: Z-score (high) by lab code



Graphs 22 to 25 demonstrate that results in general seem to be mirrored at both densities, that is, analysts tend to perform similarly at both sample concentrations compare to other analysts within and between labs. For example Labs W and O with single analysts in each lab have a positive Z-score in both counts (low and High) compare with labs N and L which consistently have a negative Z-score for their labs, in labs with more than one analyst patterns also emerge where one analyst tend to score higher in relation to the other/s in both counts. This is the case for example in lab A or Lab V where one of the analysts tends to score lower than the other/s.

5.3 Performance evaluation

On the identification exercise, most analysts exceeded the 70% overall pass mark. All analysts performed above 90%, 2 analysts achieved full marks (100%), 5 analysts 99%, 3 analysts 98% and 9 analysts between 97 and 90%.

Overall, the standard on the identification exercise was very high by all of the participating labs and analysts.

On the enumeration exercise all analysts performed within the mean and +/-3 standard deviations prescribed for this Intercomparison exercise.

While the analysts and labs performed within the parameters for the exercise there is evidence of lack of reproducibility of results between and within labs and also between analysts.

6. Conclusions and recommendations

6.1 Identification exercise

The identification exercise Bequalm 2010 was a repeat of the exercise from 2008 and the reasons for doing this were various. One of the recommendations from the workshop in the previous year was to shorten the Intercomparison exercise that the exercise was becoming very long and cumbersome and that labs felt they did not have the amount of time required to carry out the exercise accordingly. Also, this year we had a great amount of new entrants for the exercise, 19 analysts or roughly 50% of the total and it was decided that a repeat of the exercise in 2008 was a good idea because it is a basic exercise and it would not be as difficult as the quiz in 2009 for new entrants.

The identification exercise is a good basic exercise it is however purposely biased towards toxic species and also towards certain groups of marine Phytoplankton, namely Diatoms and Dinoflagellates. The exercise is a good exercise for new entrants of the scheme and tests their basic knowledge of Phytoplankton taxonomy.

All analysts completing the exercise did exceedingly well this year with an overall score of over 90% of correct answers for all analysts.

There were no significant differences in the way analysts answered certain questions compared to others, they seem to answer equally well the questions on Diatoms or questions on Dinoflagellates. Analysts did slightly better in the questions on Diatoms compare to Dinoflagellates but not significantly.

Question 8 was the best scored question of the exercise with full marks for all analysts.

There were a number of images which were found to be more difficult to identify than others. These were question 4 image A and question 5 images A and B.

There were various suggestions and recommendations at the workshop with regards to the taxonomy quiz for future ring trials.

Should the pass mark be raised for the exercise? This question arose because of the scores achieved in the exercise. All analysts seem to be well above the 70% pass mark, perhaps for this type of exercise the mark should be raised slightly from here, maybe to 80 or even 90%.

Should graphics and illustrations be used in these exercises? Most participants agreed that images should be used at all times if possible and that we should introduce other type of images like Scanning Electron Microscopy (SEM) or Transmission Electro Microscopy (TEM) images to show taxonomical details.

It was also recommended that a panel of experts or advisory group should in collaboration with the organizing lab review and to some extend certify or validate the exercise to make sure that the exercise is of a good standard.

6.2 Enumeration exercise

Analysts in this Intercomparison exercise had to count 6 samples at two cell concentrations. There were 3 replicate counts for each cell concentration. One cell concentration was low and the other high.

The analysed data demonstrates that there is no evidence of learning effects between replicates at low or high cell concentrations.

Most analysts chose different sub-sample volumes to analyse their low density samples, these were either 10ml or 25ml aliquots. Independently of the volume used, most analysts decided to use a Whole Chamber (WC) counting strategy for the low density samples

The data suggests that there are differences in the mean concentration between 10 and 25ml sub-sample volumes but that this difference is not significant. The Standard deviation of results for the 25ml sub-sample is smaller suggesting that the variation is smaller in 25ml sample volumes at this cell concentration.

On the high density samples analysts as well as using 10 or 25ml sub-samples, they also chose different counting strategies. The main counting strategies used at these volumes were Whole Chamber (WC) and Transect (TR) counts.

The results obtained suggest that there are significant differences in the mean concentration between 10 and 25ml sub-sample volumes and between TR and WC counting strategies. This difference is significantly larger in 10ml than in 25ml sub-samples and larger again in TR than in WC counting strategies.

However, all analysts performed within the mean +/- 3 SD of all the results, both for the low and high density samples. The variability is larger at the higher cell concentration. Is this variability acceptable? What is the desirable variability for a given cell concentration? Should we have set values based on experience?

Results of the high concentration samples by volume sub-sampled or counting strategies are significantly different. Should protocols based on experience be set in terms of the volume sub-sample and the cell counting strategy depending on the concentration of the samples?

The bias test using the hypothesised values against the Low and High cell concentrations show that all the values are below zero. This indicates that method effects tend to underestimate the final cell concentration on all samples independently of the volume and the counting strategy used. This would suggest that there are other methodology effects that potentially have an influence in this underestimation.

While the bias is larger at the high cell concentration compare to the bias at the low concentration both bias are still comparable as a Z-score.

Since the test method tends to undererestimate by as much as 30% the hypothesized values, should correction factors be introduced for this method? or How could we minimised these method effects? Should an expert group set up reference values for the samples?

Several recommendations were made at the workshop on future enumeration exercises:

Concentration ranges on samples should work around trigger levels depending on the toxic species studied. For example: *Dinophysis spp.* are more likely to be found in small cell densities in samples and they are known to cause toxicity even at these quantities where *Pseudo-nitzschia spp.* cell densities are only relevant when they reach higher cell concentrations (50000 c/L). When using concentration ranges in the samples this should be taken into account.

To avoid methodology effects caused by factors like counting strategies, guidelines should be given as to which counting strategy should be used depending on cell concentrations. Perhaps for high cell concentrations fields of view or transect counts should be used instead of whole chamber cell counts.

Samples sent to the participants should contain a larger volume to sub-sample, 100ml was suggested as a good volume size.

Overall, participants thought it was vital that Bequalm exercises continue into the future.

Annex I: Participating labs in Bequalm Phytoplankton Intercomparison 2010

Appendix 1: BEQUALM 10 LABS	
Marine Institute Phytoplankton lab Rinville, Oranmore Co. Galway Ireland	**IRTA** Carretera del Poblenou km 5,5 Sant Carles de la Ràpita 43540 Spain
AFBI HQ, Newforge Lane Belfast Northern Ireland BT9 5PX United Kingdom	**DLGE** Ballakermeen Road Douglas, Isle of Man IM1 4BR United Kingdom
FRS Marina Laboratory Victoria Road Aberdeen Scotland AB11 9DB United Kingdom	**INTECMAR NIF- Q3600376B** Peirao de Vilaxoán s/n. Vilagarcía de Arousa Pontevedra, Galicia 36611 Spain
SAMS Research Services Ltd Dunstaffnage Marine Laboratory Oban Argyll PA37 1QA United Kingdom	**WEAQ AB** Doktorsgatan 9 d Angelholm SE-26252 Sweden
L.C.C.RR.PP. Ctra. PUNTA UMBRIA - CARTAYA km 12 CARTAYA HUELVA 21459 Spain	**Marine Institute** Phytoplankton lab Gortalassa Bantry Co.Cork Ireland
CEFAS Barrack Road, The Nothe Weymouth Dorset DT4 8UB United Kingdom	**IZOR** Setaliste I. Mestrovica 63 P.O. Box 500 Split 21000 Croatia
The Water Management Unit Northern Ireland Environment Agency 17 Antrim road, Lisburn Down BT283AL United Kingdom	**SEPA** Clearwater House, Heriot Watt Research Park Avenue North, Riccarton EDINBURGH EH14 4AP
CEFAS Laboratory Pakefield Rd Lowestoft NR33 0HT United Kingdom	**SAHFOS, The Laboratory** Citadel Hill Plymouth Devon PL1 2PB United Kingdom
IRB G. Paliaga 5 Rovinj 52210 Croatia	**Departamento Científico Ficología** Facultad de Ciencias Naturales y Museo Paseo del Bosque s/n 1900 La Plata Argentina
Certificaciones del Perú S.A. Av. Santa Rosa No. 601 La Perla Callao Callao 4 Peru	**IMARES** Haringkade 1 Ijmuiden NH 1976 CP Netherlands
Apem Ltd Riverview A17 Embankment Business Park Heaton Mersey, Stockport Cheshire SK4 3GN United Kingdom	**AquaEcology GmbH & Co. KG** Marie-Curie-Str. 1 Oldenburg 26129 Germany

Annex II: Instructions for phytoplankton Intercomparison PHY-ICN-10-MI1

Marine Institute BEQUALM Phytoplankton Proficiency Test PHY-ICN-10-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 ISO 17025 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 any inter-laboratory and inter-analyst variability in the enumeration of Marine Phytoplankton species within and between labs from a number of samples spiked with cultured material.

A taxonomic quiz has been designed to test analysts' knowledge on phytoplankton species and in important morphological and structural characteristics that could help in their identification. This quiz is an updated version of the quiz sent in the 2008 round. Analysts which have previously completed this exercise are exempted from doing it again. Only analysts that have not completed this quiz before should do the taxonomy quiz.

This year we are also including a wild sample as part of the exercise. The results of this sample won't be published in the final report and won't count for the final mark in the individual certificates. The results however will be analysed as part of the exercise and they could be discussed later on at the workshop.

The wild sample will consist of one 30ml sample sent to each analyst. This sample is not compulsory work and analysts will decide whether they want to carry it out or not. A total Phytoplankton species count with results given in cells/litre should be reported. Analysts are also asked to take as many images as possible of phytoplankton species found in the wild sample to discuss the identification later on.

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 Taxonomic quiz (Form 3) and the Enumeration Hard copy results sheet (Form 2). Once you are happy that you have received everything you need to complete this exercise and samples are in working order. Complete form 1: Return slip and checklist form and send it by Fax or 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 hardcopy of enumeration results (Form 2) and the Taxonomic quiz (Form 3) **must be received** by the Marine Institute by **April 16th, 2010**.

Please note: Hardcopy results and Taxonomic quiz results received after the April 16th, 2010 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 since 2005.

In previous years, we have advised others labs taking part in the exercise to use the Utermöhl cell counting method to analyse the samples. This year, we are asking labs to use their own in house cell counting methodologies to carry out the analysis of the samples.

Those labs using the Utermöhl cell counting method can if they wish sub-sample to analyse different sample volumes.

Those labs using methodologies other than the Utermöhl method should describe briefly, which method they use, how it works and how they carry out their calculations to obtain the final density in cells per litre. These labs should send their Standard Operating Procedures along with their results to us in order to understand better how they analysed their samples and compare fairly the results at the workshop.

4. Equipment

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

- 7 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.

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). 7 sedimentation chambers will be required.

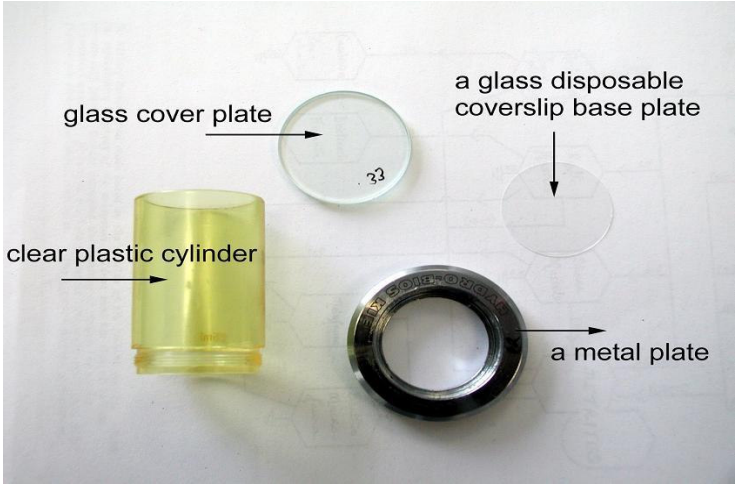


Fig 1: Sedimentation counting chamber

If using the Utermöhl method follow the following instructions:

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 and proceed with the next step.

5.4 To set up a sample for analysis or sub-sample. Firmly invert the sample at least 20 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 an 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 results for each sample are to be entered on **Form 2 Enumeration Hardcopy Results 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 the cell counting according to their own lab procedures. This could be in the case of the Utermöhl method a whole chamber cell count (WC), a half chamber count (HC), a transect count (TR) or a field of view count (FV). If counting transects or field of view on a sedimentation chamber the analyst should average at least three counts.

If the analysts are using a different methodology to the Utermöhl method, these should provide information on their counting strategy and calculations to obtain cells/Litre counts.

7. Sample types

This Intercomparison exercise comprises 6 + 1 samples. The one sample is a marine seawater sample collected from the natural population and preserved in lugol's iodine. This sample should be analysed for a total Phytoplankton cell count, that is the identification and enumeration of all species found in the sample. The sample is not compulsory work for the exercise and it is left up to the individual analysts to carry out the analysis in a voluntary basis.

The analysts wishing to do this sample are asked to provide their own tally sheet with the results of this analysis. The final tally should be given in cells/litre. Analysts are also asked to provide digital images for their identifications.

This sample results will be a discussion item for the workshop and I hope that as many analysts as possible take on the opportunity to contribute by analyzing this sample.

The other six samples for this Intercomparison have been spiked with cell culture material kept in the Marine Institute Phytoplankton culture collection. 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.

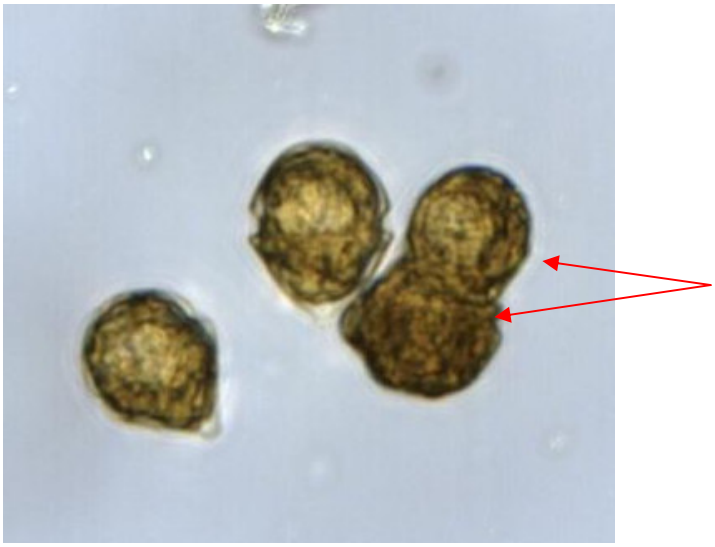


Figure 1: Two Cells fusing

Also note that cells empty theca may appear in the sample (see figure 2),

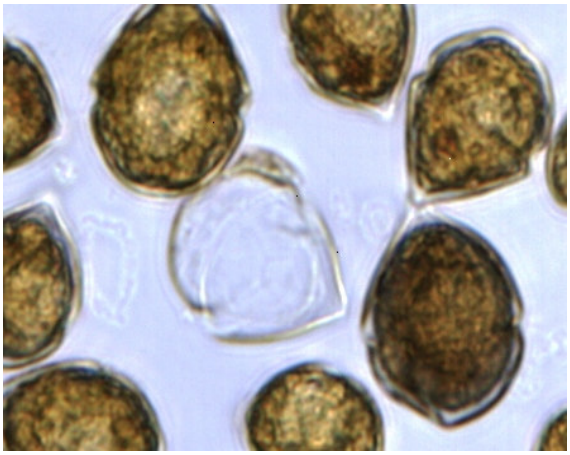


Figure 2: Empty theca

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.

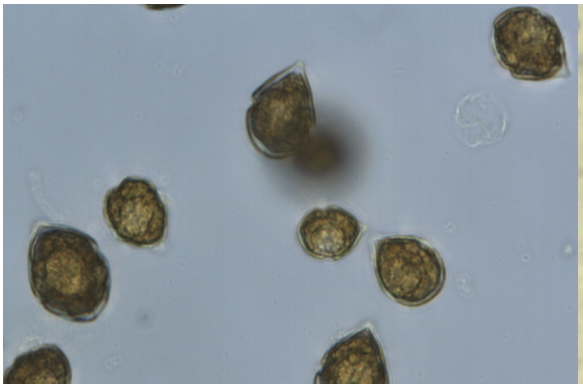


Figure 3: Big versus small cells

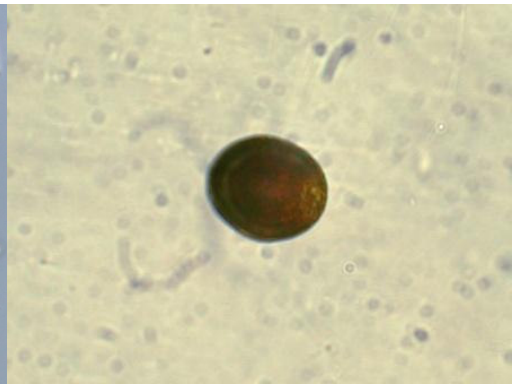


Figure 4: Plasmolised cell

The following rules should be applied for cell counting 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

d) Plasmolised cells should be counted

e) Aberrant forms should be counted

f) There is no need to identify the cultured organism in the set of 6 samples as this is purely an enumeration exercise.

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: Hardcopy enumeration results sheet

9. Identification

The Taxonomic quiz for the exercise in 2010 is an updated version of the exercise completed in the Bequalm exercise 2008. Analysts which had already completed this exercise back in 2008 won't have to take part on the identification part on this years exercise.

Analysts that have not participated before or analysts from labs which have participated before but did not take part on this particular exercise should complete the taxonomy quiz.

The quiz has been designed to test the general taxonomic skills of the participants. The quiz comprises the use of images, figures and diagrams in various ways to test participants' knowledge of species morphological characteristics and identification.

Please identify and include your results on the Taxonomic quiz (**Form 3**).
The identification exercise carries a total of 300 marks. Make sure you keep a copy of your results before you send the original form in the post.

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.

10. Points to Remember

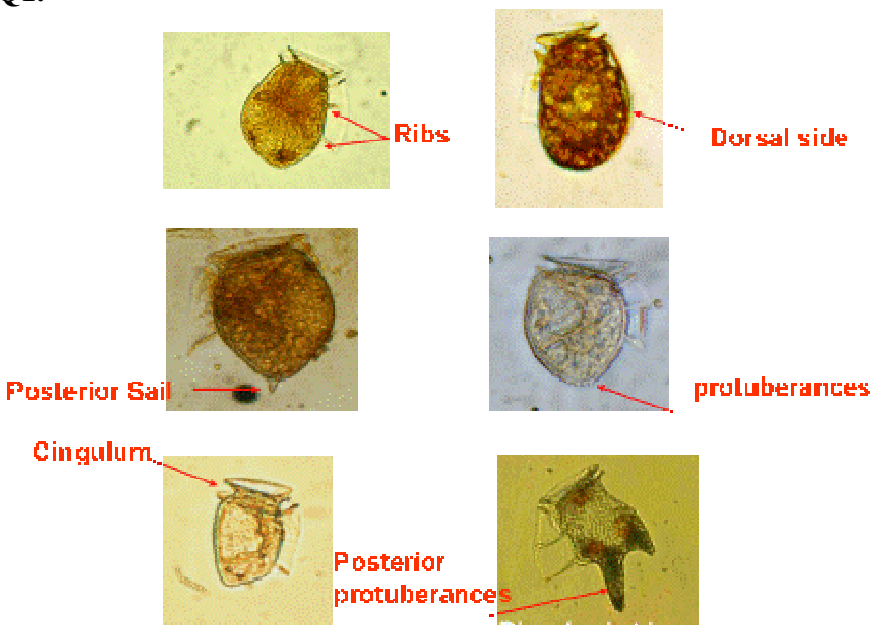
1. **All results must be the analysts own work. Conferring with other analysts is not allowed.**

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 2: Enumeration Hardcopy Results Sheet and Form 3: taxonomic quiz must be received by the Marine Institute, Phytoplankton unit by **Friday April 16th** 2010.

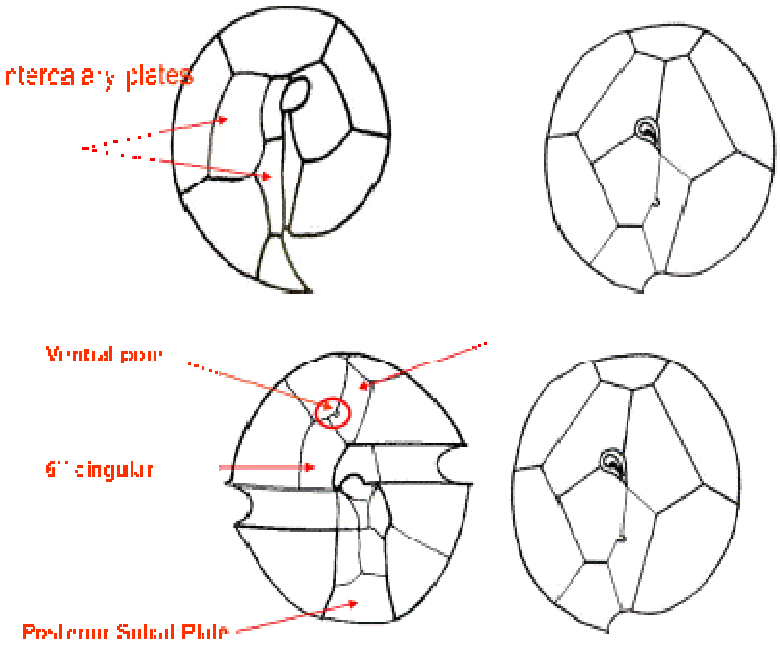
Annex III: Detailed results of the identification test PHY-ICN-10-MI1

Q1.

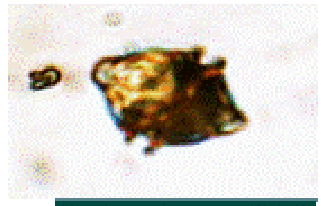


Q2 & Q3

Gonyaulax ***Alexandrium***

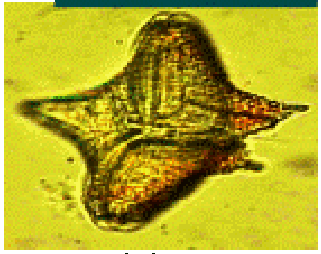


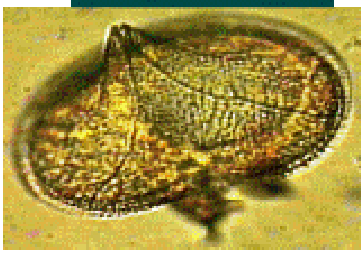
Q4.



Heterocapsa triquetra

Prorocentrum micans





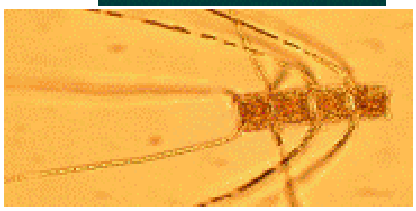
Protoperidinium crassipes

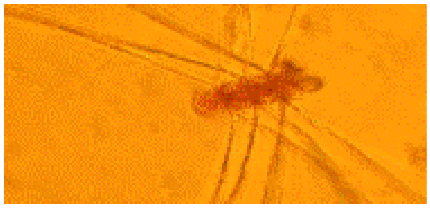
Prorocentrum lima

Dinophysis hastata

Q5.

Chaetoceros peruvianus



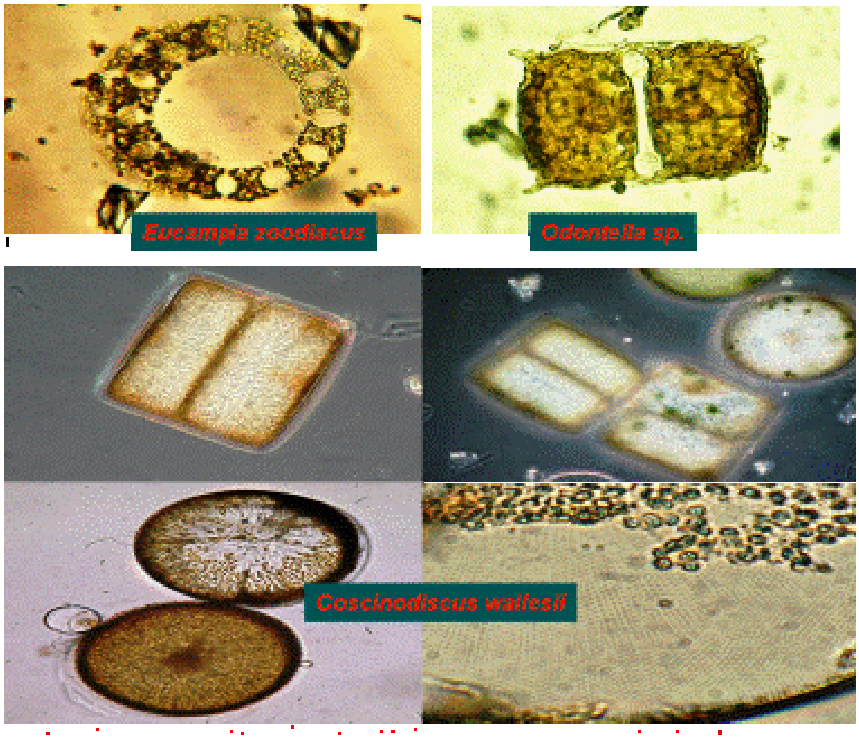


Chaetoceros densus

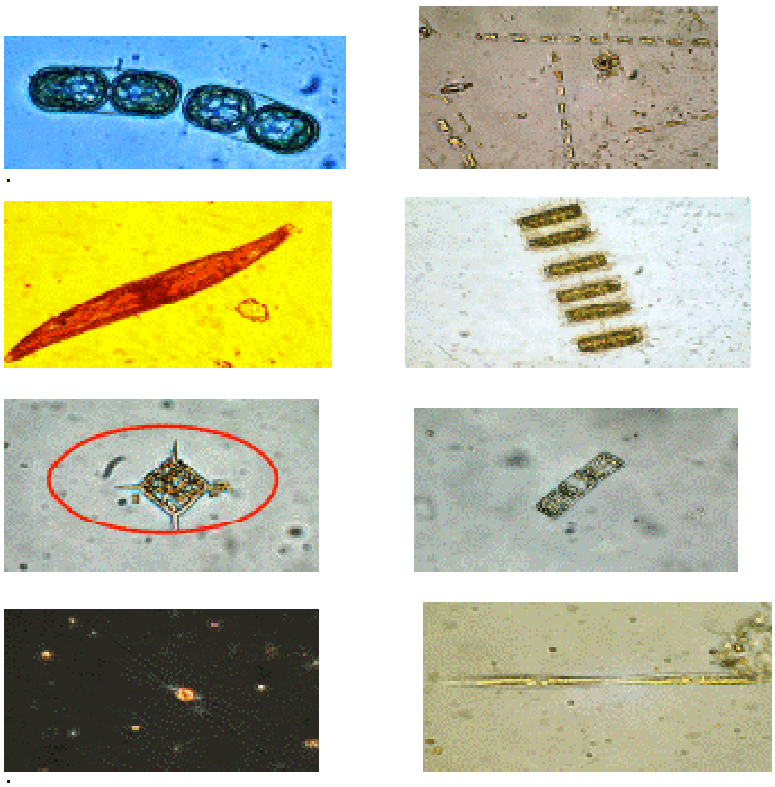
Chaetoceros decipiens

Neocaliptrella robusta

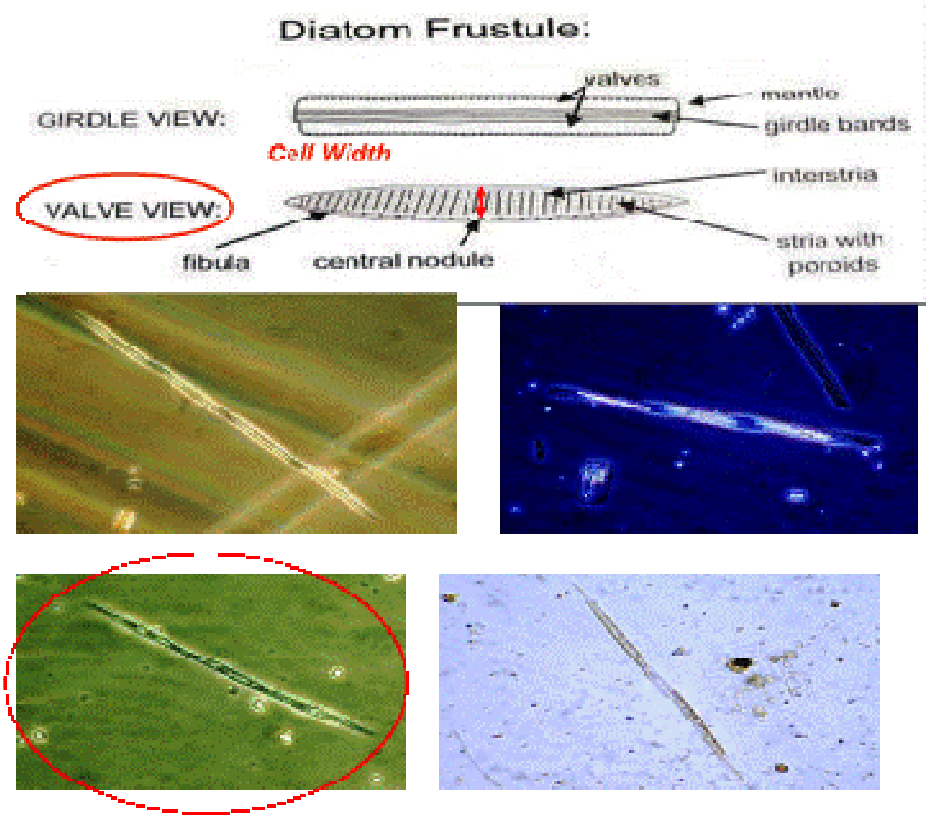
Q5.



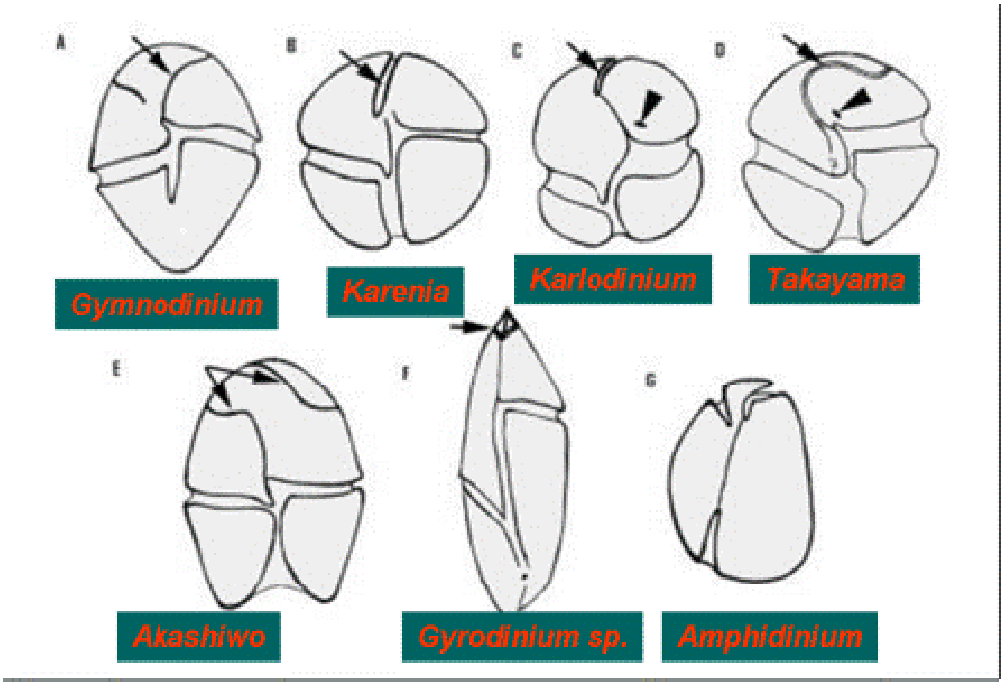
Q6.



Q7.


Diatom Frustule:
GIRDLE VIEW:
valves
mantle
girdle bands
Cell Width
VALVE VIEW:
interstria
fibula
central nodule
stria with poroids

Q8.


A
B
C
D
Gymnodinium
Karenia
Karlodinium
Takayama
E
F
G
Akashiwo
Gyrodinium sp.
Amphidinium

Annex IV: Workshop Agenda

BEQUALM / National Marine Biological Analytical Quality Control Scheme
Phytoplankton ring test PHY-ICN-010-MI1

Workshop

Thursday, 27th May 2010
Instituto Tecnolóxico para o Control do Medio Mariño de Galicia : INTECMAR
Vilaxoán, Pontevedra, Spain

Agenda

09:30	**Arrival to INTECMAR** **Meeting in Oceanography and Phytoplankton Laboratory**
10:00	**Galician Monitoring Programme of the Marine Environment** **Covadonga Salgado, Director INTECMAR** **Auditorium** **Visit to Biotoxin laboratories** **Fabiola Arévalo, INTECMAR**
11:30	**Coffee Break**
12:00	**Intercalibration exercise BEQUALM 2010** **Preparation of materials and methodology** **Identification exercise results/Enumeration exercise results** **Statistical analysis/Conclusions** **Rafael Gallardo. Marine Institute. Ireland**
13:30	**Lunch**
14:30	**Species concept on HABs monitoring** **Santiago Fraga, Instituto Español de Oceanografía: IEOVigo, Spain**
15:30	**FP7 201724 PROJECT presentation** **Microarrays for the Detection of Toxic Algae: MIDTAL** **Francisco Rodríguez - Beatriz Reguera, IEO; Yolanda Pazos, INTECMAR**
16:00	**Open Discussion: Future developments Bequalm ICN 2011.** **All participants**

Agenda Intercomparison 2010 workshop

Annex V: FORM 1_Checklist to Fax bequalm PHY-ICN-10 MI1.pdf

BEQUALM / NATIONAL MARINE BIOLOGICAL ANALYTICAL QUALITY CONTROL SCHEME

Bequalm Intercomparison PHY-ICN-10-MI1
FORM 1: RETURN SLIP AND CHECKLIST

Please ensure to complete the table below upon receipt of samples, and fax or e-mail immediately to the Marine Institute. + 353 91 387237 or rafael.salas@marine.ie		
Analyst Name:		
Laboratory Name:		
Analyst Code Assigned :		
Contact Tel. No. / e-mail		
CHECKLIST OF ITEMS RECEIVED	**(Please circle the relevant answer)**	
Sample numbers _____	YES	NO
Wild Sample number _____	YES	NO
Set of Instructions	YES	NO
Enumeration Result Sheet **(Form 2)**	YES	NO
Taxonomic Quiz **(Form 3)**	YES	NO

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

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

SIGNED: _____

DATE: _____

Form 1: Return Slip and Checklist

Annex VI: FORM 2_Enumeration Hardcopy results

Marine Institute
Foras na Mara

BEQUALM

BEQUALM / NATIONAL MARINE BIOLOGICAL ANALYTICAL QUALITY CONTROL SCHEME

Analyst Name:	
Laboratory Name:	
Analyst Code :	

Enumeration exercise

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

Describe briefly methodology used:_____

_____

_____

_____

_____

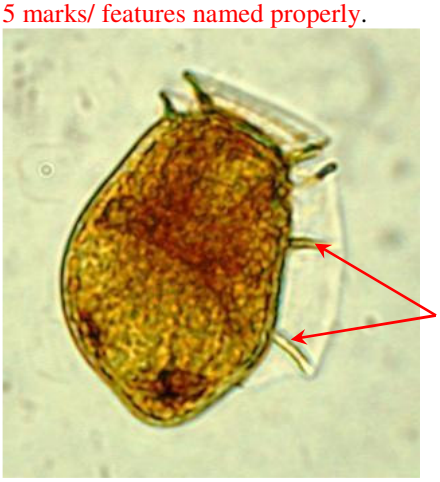
Signed: _____

Date: _____

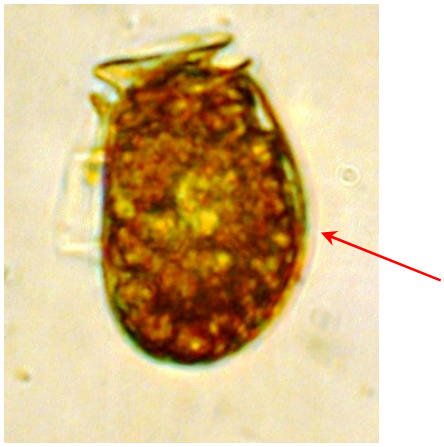
Annex VII: Form 3: Taxonomic quiz

Form 3: TAXONOMIC QUIZ BEQUALM PHY-ICN-10-MI1

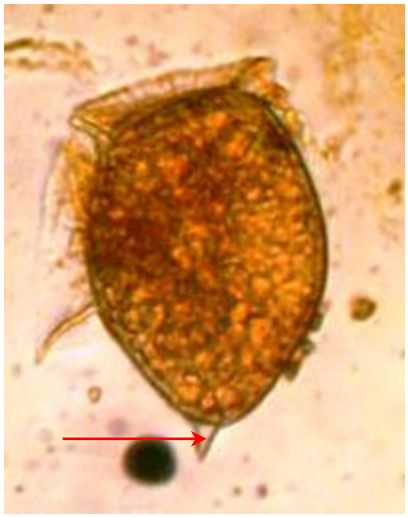
QUESTION 1: The following photographs belong to the genus *Dinophysis*. Participants are asked to name the species and the morphological features that the arrows are pointing at. This question is worth 60 marks. 5 marks/ species named correctly and 5 marks/ features named properly.



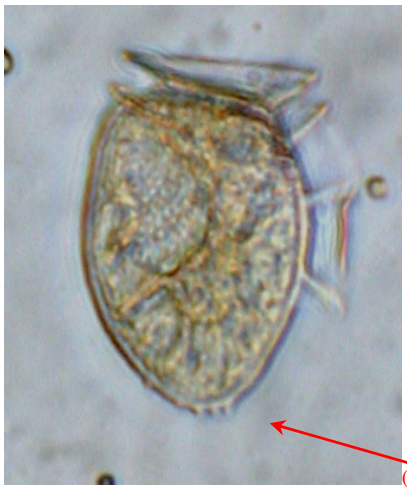
A. ***Dinophysis***_____
Size: L: 85.0, W: 55.0 µm



B. ***Dinophysis***_____
Size: L: 65.0, W: 43.0 µm

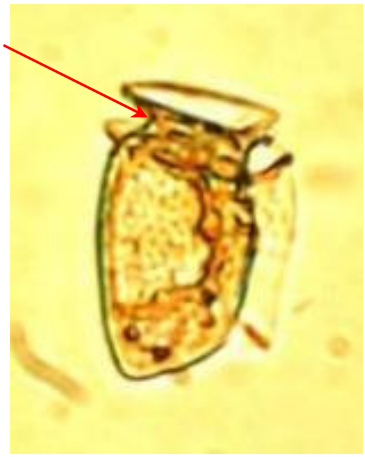


C. ***Dinophysis***_____
Size: L: 74, W: 58 µm

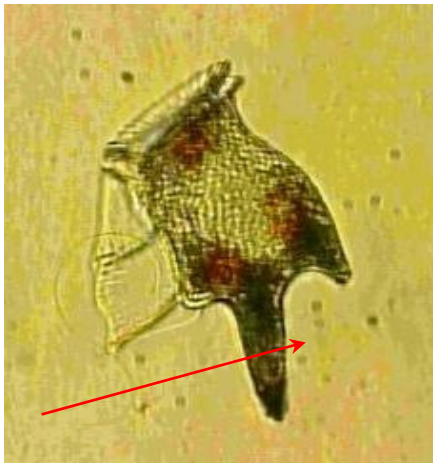


(the small bulgy things)

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

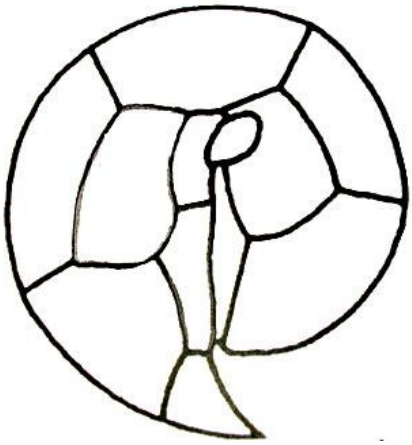
QUESTION 2: The following diagrams show the **Kofoidean tabulation** of two different armoured dinoflagellates in apical view.

This question is worth 20 marks. 10 marks/question

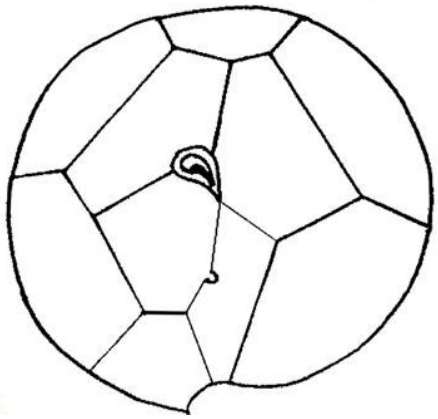
You are asked:

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

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



Answer:_____



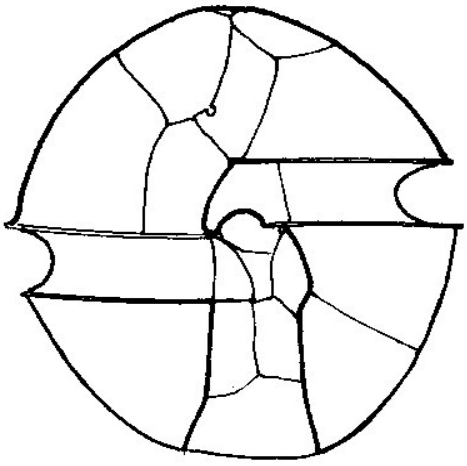
Answer:_____

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:

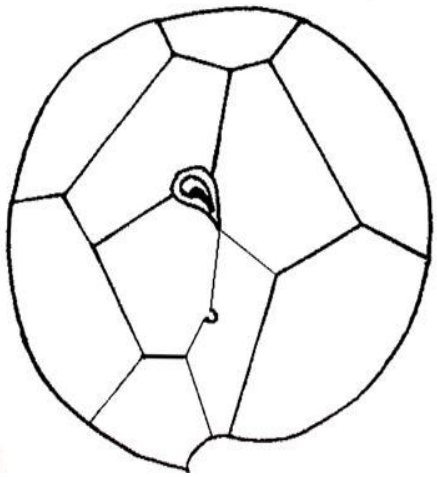
a) the 1' (apical) plate
b) the 6'' (pre-cingular) plate
c) the ventral pore (vp)
d) the sulcal plate (sp)

Use either diagram to point to the features

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



Ventral view

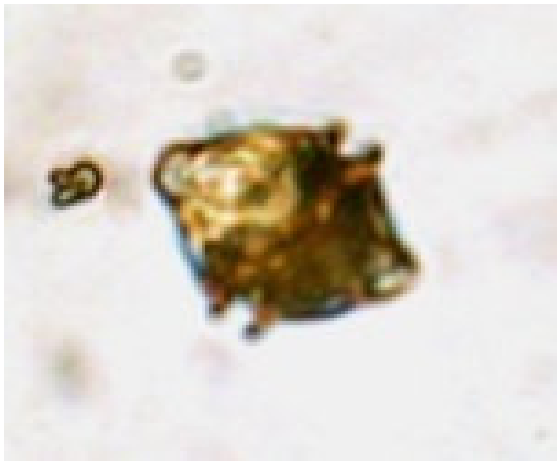


Apical view

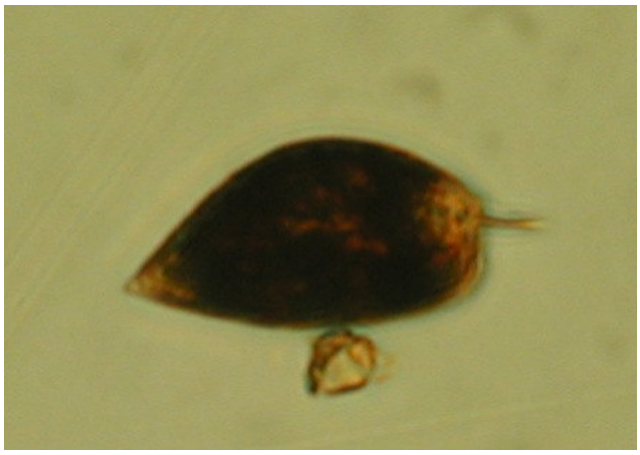
QUESTION 4: **Identify to species level** the following pictures of armoured dinoflagellates.

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
Name:

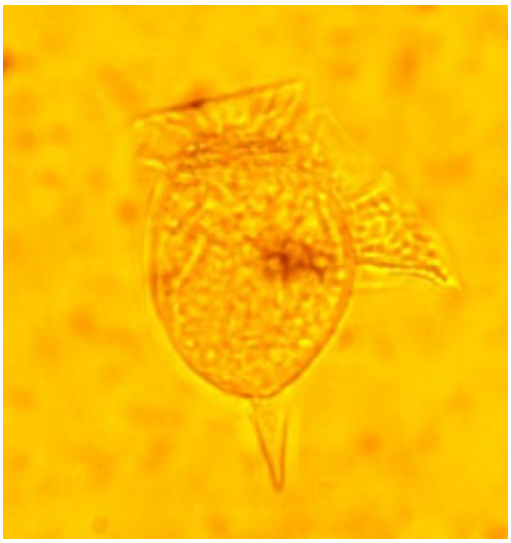


B. Size: L: 65, W: 30 µm
Name:

C. Size: L: 100, W: 105 µm
Name:

second image showing plate structure

D. Size: L: 47.5, W: 32.5 µm
Name:

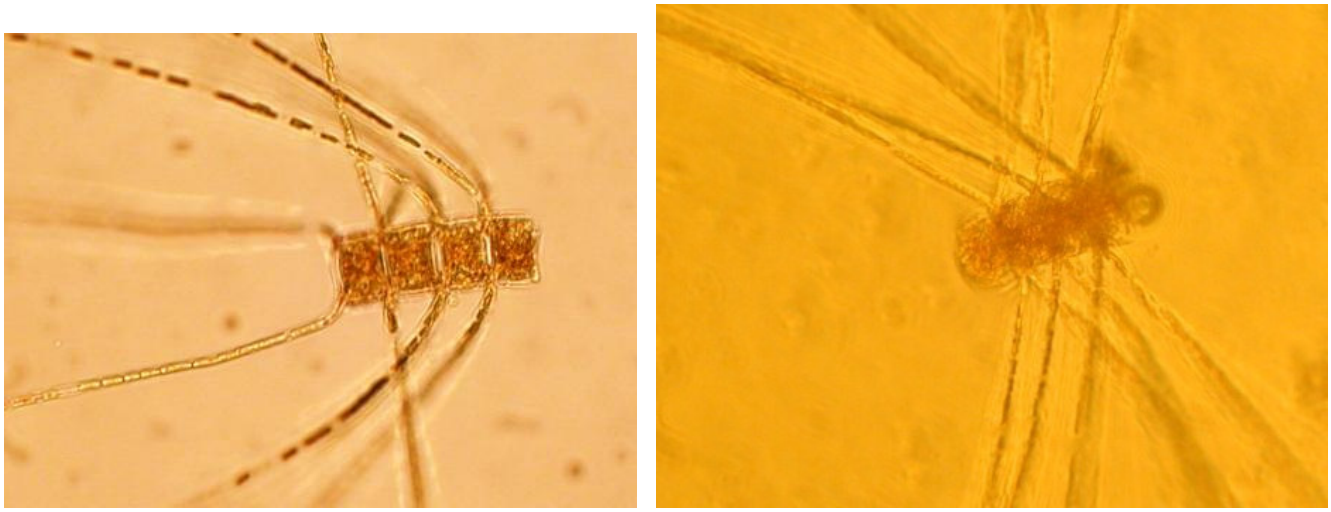


E. Size: L: 64, W: 38 µm
Name:

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.

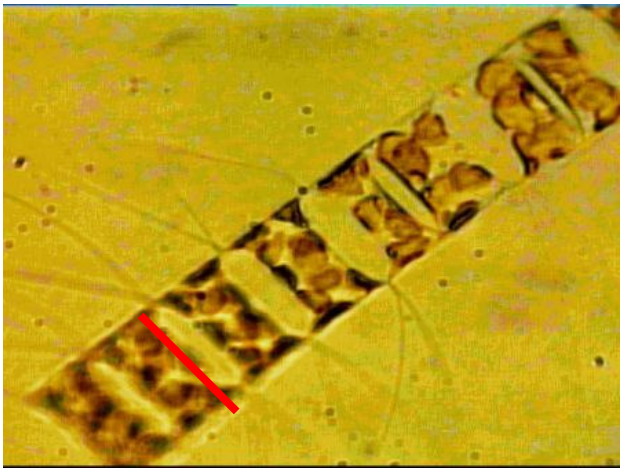
2 images of the same organism (This organism doesn't form chains, Images show organism undergoing division): Size: 35µm length of valve in girdle view Transapical plane

A. Name:



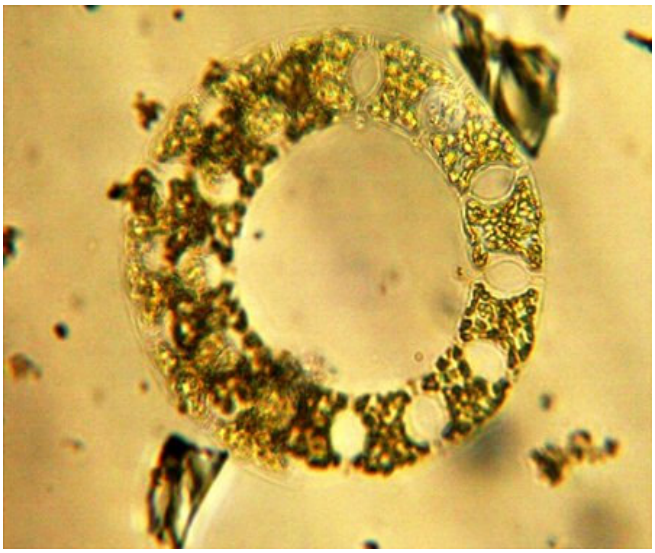
2 images of the same organism. Setae diverge equally from the apical plane. Size: 45µm Length of valve in girdle view transapical plane

B. Name:

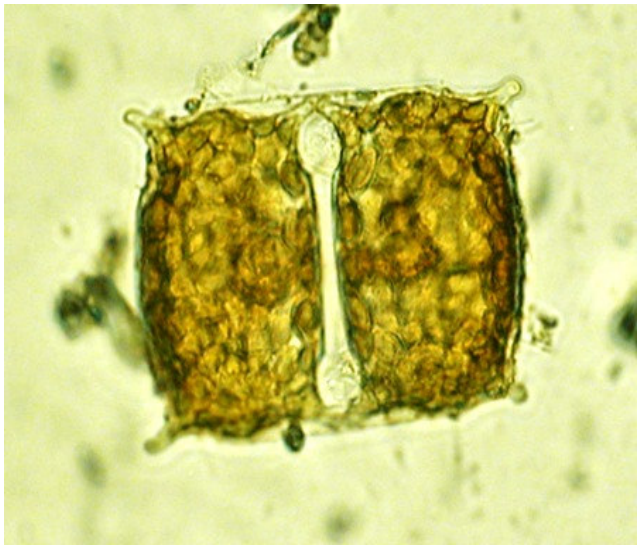


C. Name:
Size: 56µm

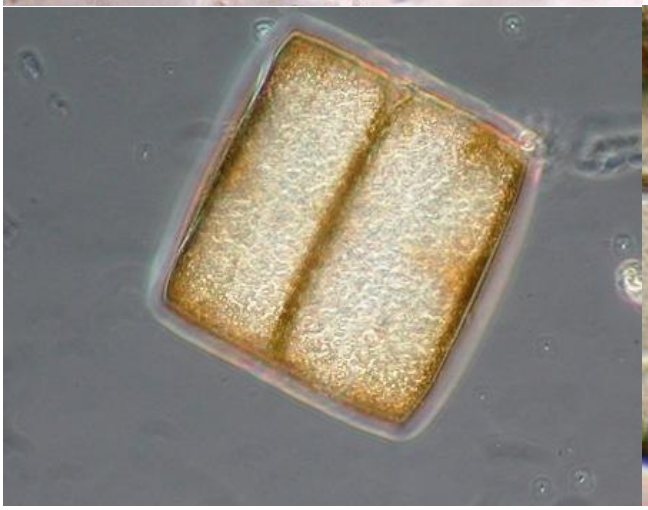
D. Name:
Size: L: 650, W: 100 µm

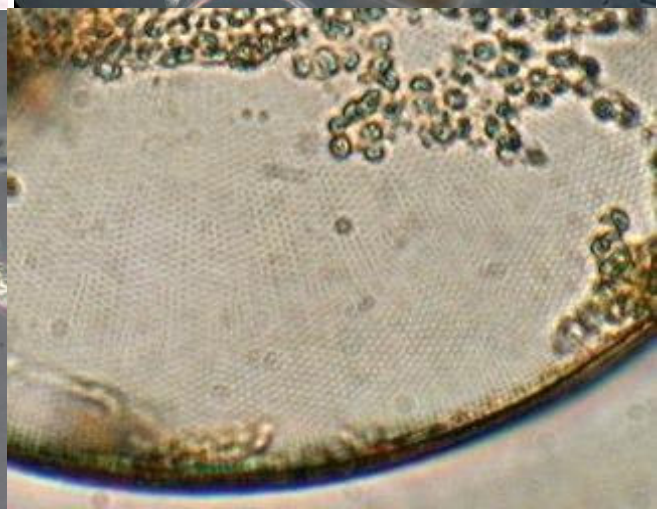


E. Name:
Size: 45µm Apical axis



F. Name:
Size: 65µm wide

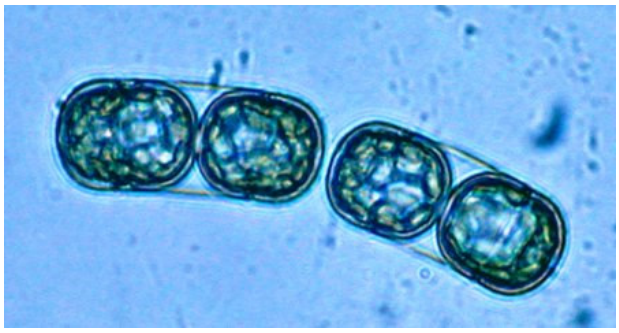




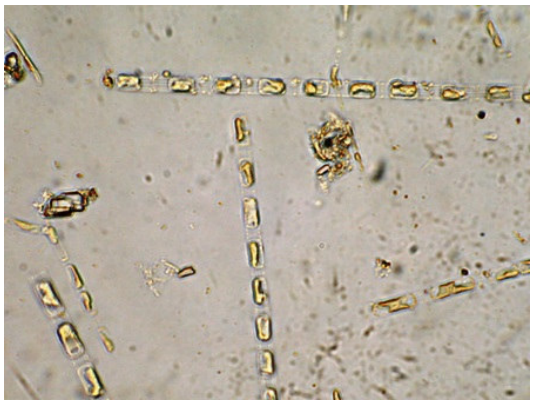
4 images of the same organism. (300 µm diameter) **Areolae details**

G. Name:

QUESTION 6: Could you circle the odd one out?
This question is worth 15 marks

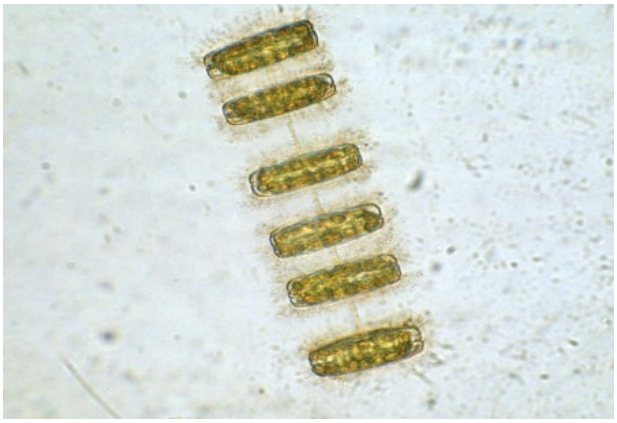


A

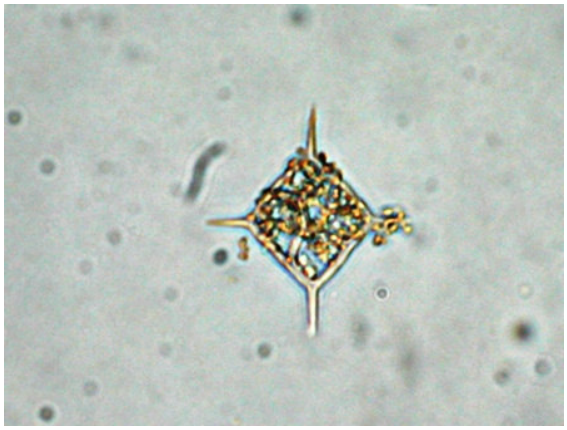


B

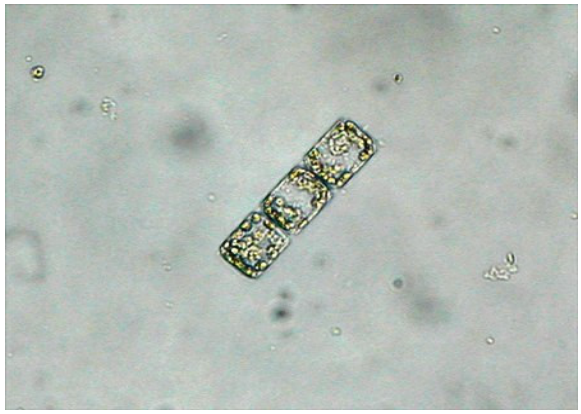
C



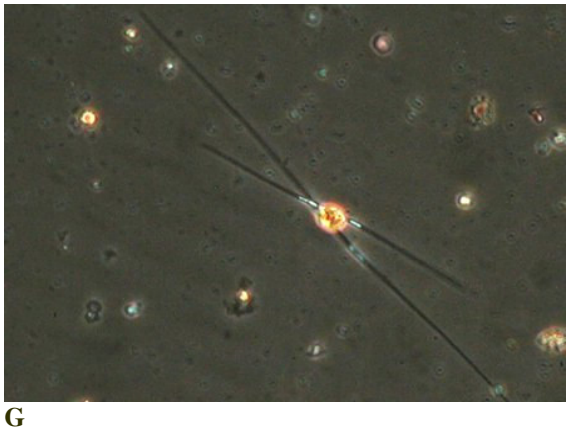
D



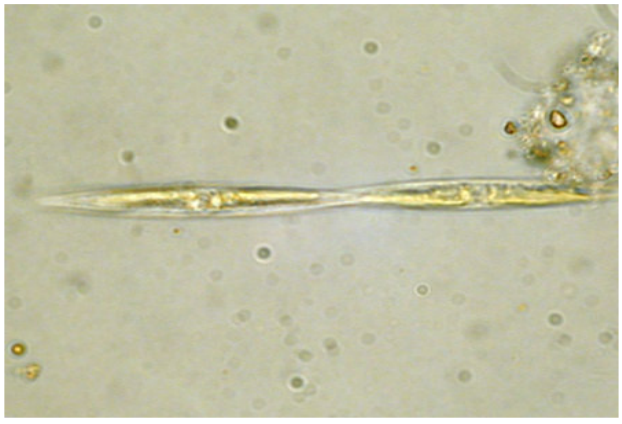
E



F



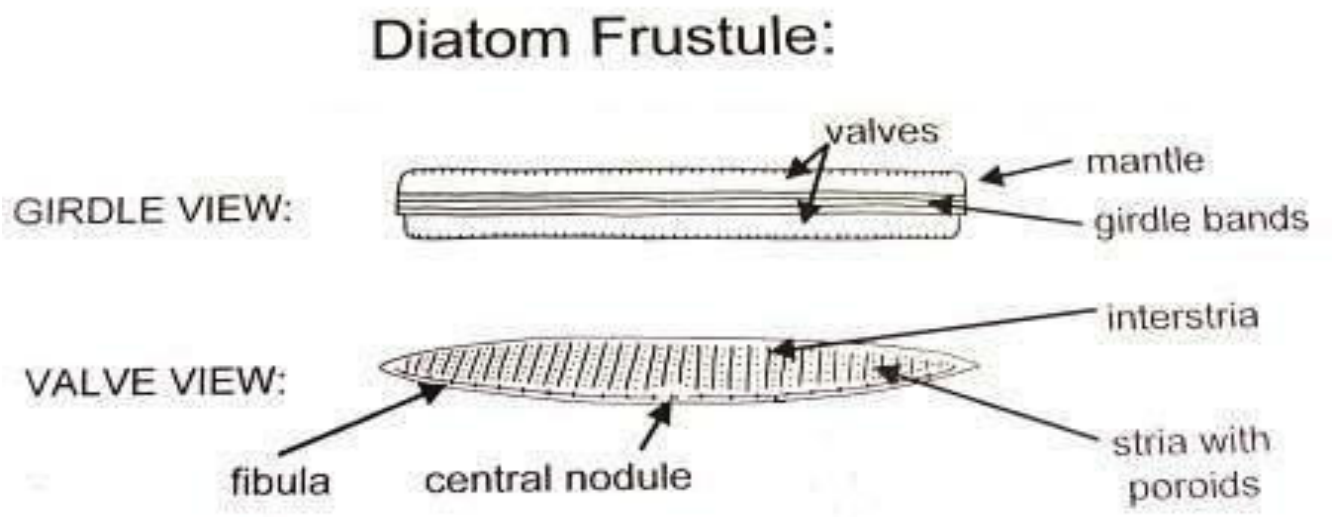
G



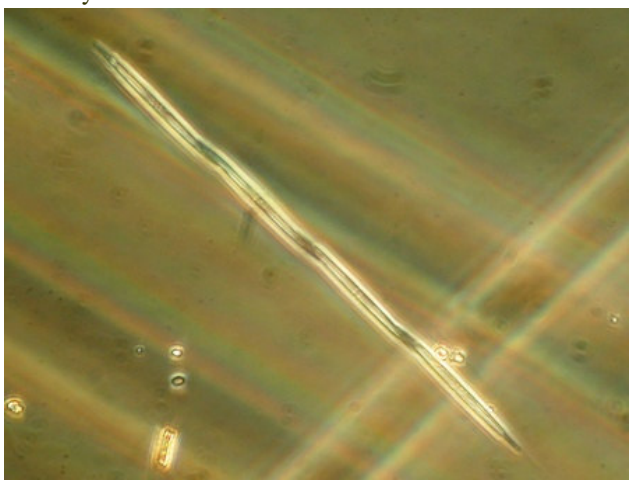
H

QUESTION 7: The following diagrams show a schematic picture of a *Pseudo-nitzschia* cell in valve and girdle view.
A) If you were to measure the 'width' of a *pseudo-nitzschia* cell, which **view** would you choose to do this? (Draw a line showing where you would measure the cell's 'width')

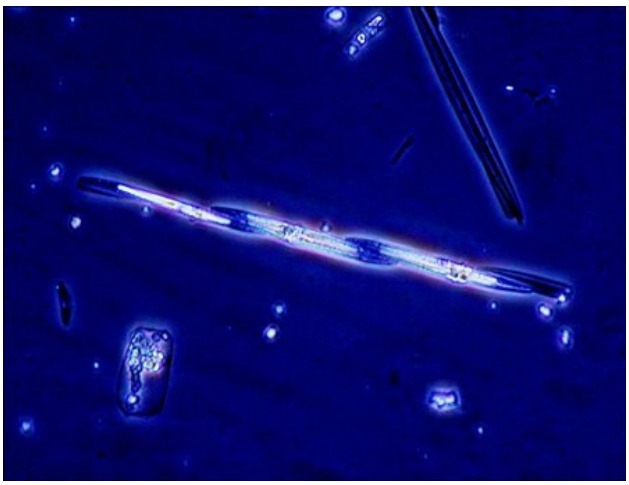
B) And give a reason why you would choose that particular view to measure the width of the cell?
This question is worth 30 marks. 10 marks/correct answer.



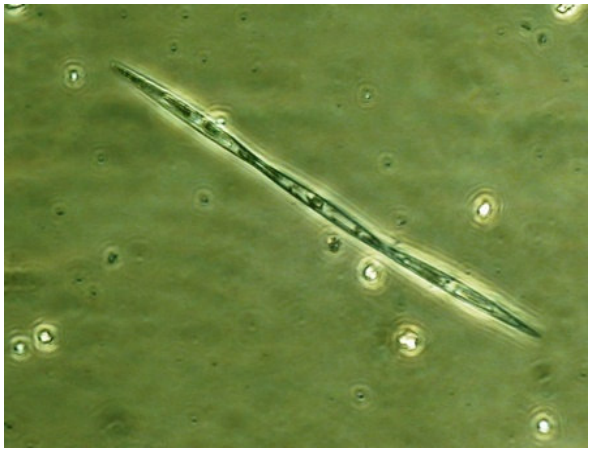
C) Taking into account the answers to **A** and **B**. which of the following photographs of *pseudonitzschia* cells would you choose to carry out a width measurement?



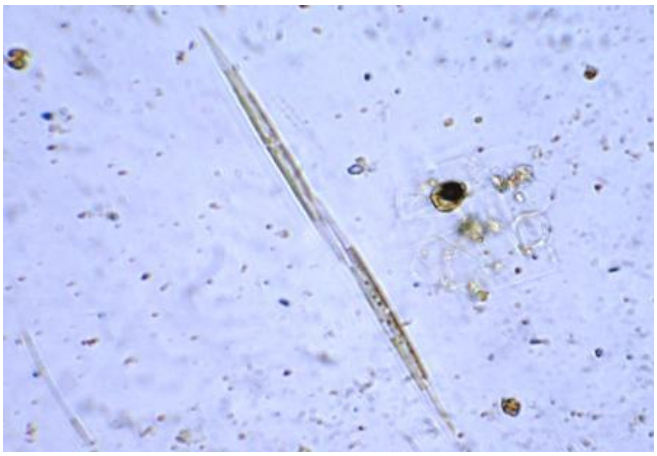
A



B



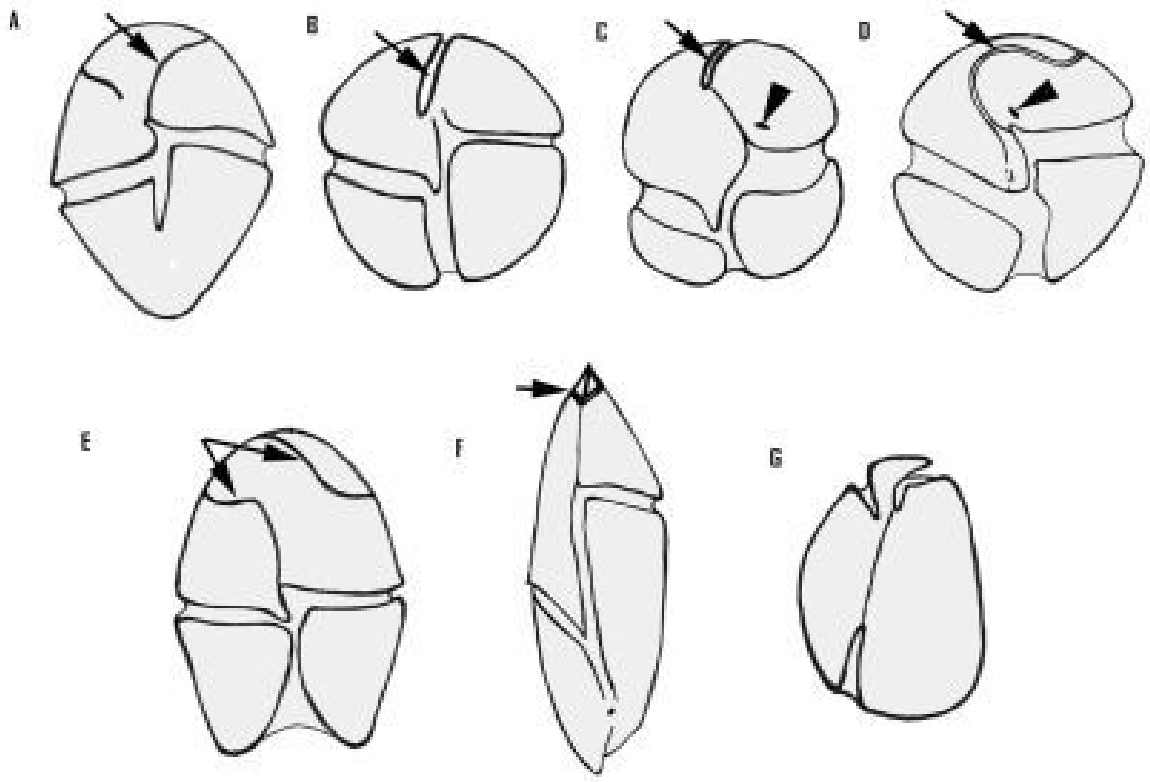
C



D

QUESTION 8: Which **Genera** do these diagrams of naked dinoflagellates represent?
This question is worth 35 marks. 5 marks/correct answer

A:
B:
C:
D:
E:
F:
G:



Analyst code:_____

Date:_____

Annex VIII: Statement of performance certificate

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 2010

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

Statement Issued:
Statement Number: MI-BQM-10-

Summary of results:

Component Name	Exercise	Subcontracted	Results Z-score (+/- 3 Sigma limits)	
Phytoplankton Enumeration	PHY-ICN-10-MI1	Marine Institute	**Low density samples**	**High density samples**
			Results Pass Mark 70% (over 90% proficient)	
toplankton Identification	PHY-ICN-10-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:

Details certified by:

Section Manager
Joe Silke (MI)

Senior Lab Analyst
Rafael Salas (MI)

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 when undertaking analysis of a prepared sample/s of Seawater preserved in Lugol's iodine and spiked using biological or synthetic subjects using the Utermöhl 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 by the participating laboratories
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 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, and results below 70% are reported as "Participated". There are no standards for phytoplankton identification. These exercises are unique and made from scratch.