REPORT NO. 801

ALGAE

PROFICIENCY TESTING PROGRAM

ROUND 15

APRIL 2013

ACKNOWLEDGMENTS

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Summary of Results

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1. FOREWORD

This report summarises the results of a proficiency testing program covering the identification and enumeration of selected Phytoplankton.

Proficiency Testing Australia conducted the exercise in December 2012/January 2013. The Program Coordinator was Mrs K Weller. This report was authorised by Ms W Fajloun, PTA Quality Coordinator.

The main aim of the program was to assess laboratories' ability to competently perform the tests examined.

2. STATISTICAL DESIGN OF THE PROGRAM

Each participating laboratory was provided with three (3) samples labelled Sample A, Sample B and Sample C, containing a range of algal and Cyanobacterial genera. Samples A and B were duplicates and were examined to identify and enumerate the two dominant Cyanobacterial genera. Sample C was examined to identify 2 Diatoms, 1 Dinoflagellate and 1 Chrysophyte present.

For this round participants also had the option to analyse a sample containing marine phytoplankton (Sample D). Sample D was examined to identify and enumerate the three (3) dominant Dinoflagellates present.

Robust statistical procedures were used to generate the z-scores and summary statistics for each sample – number of results, median, uncertainty of the median, normalised interquartile range, robust co-efficient of variation, minimum, maximum and range.

3. FEATURES OF THE PROGRAM

(a) A total of 22 laboratories received samples. All laboratories submitted results by the due date. Participants included laboratories from Australia, New Zealand, Peru and USA.

(b) Participants were supplied upon request with either three (3) or four (4) samples, in amber glass bottles.

(c) The results for each test as reported by participants are presented in Appendix A, together with summary statistics, calculated z-scores and graphical presentations of the data.

(d) Participating laboratories were requested to perform the tests according to the "Instructions to Participants", and to record their results on the accompanying "Results Sheets", all of which were distributed to laboratories with the test samples.

Copies of the "Instructions to Participants" and "Results Sheets" are included in Appendix C of this report.

(e) Each laboratory was randomly allocated a unique code number for the program to ensure confidentiality of results. Reference to each laboratory in this report is by its code number. Where a laboratory has reported more than one set of results, their code number will appear with a corresponding letter for each set of results.

4. FORMAT OF APPENDICES

Appendix A

Identification: The dominant genera identified as present by each laboratory are tabulated.

Enumeration: For Sample A and B, and Sample D, the following is given for each of the genera enumerated.

(i) The results of the enumeration (in cells mL^{-1}) as reported by participating laboratories;

(ii) the transformed results, calculated z-scores and table of summary statistics;

(iii) ordered z-score charts and youden diagrams (Sample A and B only).

A description of the statistics and graphical displays used can be found at the beginning of Appendix A. Further details about the statistics and graphical displays, including guidance on their interpretation, may be found in the *Guide to Proficiency Testing Australia (2012)* [1].

Appendix B

(i) Sample Preparation and Distribution.

(ii) Homogeneity, Stability Testing and Trip Control.

Appendix C

(i) Instructions to Participants.

(ii) Results Sheets.

5. OUTLIER RESULTS

Identification

Any genera reported other than the listed verified genera are considered 'identification outlier' results and are marked in Appendix A by the symbol ♦. Outliers in identification were restricted to those genera not observed by the Technical Advisors and Supplier during sample preparation and those that are clearly incorrect with respect to presence/absence of key classification criteria and characteristics for identification. Alternative names (synonyms), due to recent changes/revision in taxonomic classification of the organisms, were not deemed identification outliers. Tables D and E lists those genera in each classification group that are deemed to be valid identifications by the Technical Advisors and Supplier for each sample.

Enumeration

Robust z-scores have been used to assess each laboratory's testing performance. When calculated from single results, z-scores are used to detect excessively large or excessively small results in comparison to the consensus value (the median). Any result with an absolute z-score greater than or equal to 3.0 (i.e. ≤-3.0 or ≥3.0) is classified as a 'statistical outlier' and is marked in Appendix A by the symbol **§**. Participants are also encouraged to review any results which have an absolute z-score between two and three (i.e. 2.0 < |z-score| < 3.0). Any results deemed 'mis-identifications' are marked by ♦, however were included in the analysis as only two (or three) organisms were present in the samples for enumeration. These are counted as 'identification outlier' results.

Z-SCORE CALCULATION PARAMETERS

These parameters were used in the calculation of the z-scores (between-laboratories and within-laboratory) for Samples A and B.

Test $\log_{10}$ (cells mL^{-1})	Standardised Sums (*S*)		Standardised Differences (*D*)	
	Median (*X*)	Norm. IQR (*Y*)	Median (*V*)	Norm. IQR (*W*)
Arthrospira	6.859	0.187	0.004	0.064
Anabaena	6.696	0.171	0.005	0.068

Calculation

The following procedure is used to calculate a laboratory's z-scores for a particular test/sample pair, e.g. Samples A and B.

Let *ZB* denote the between-laboratories z-score and *ZW* denote the within-laboratory z-score.

Using the laboratory's results for Samples A and B, denoted by A and B respectively, calculate the standardised sum (*S*) and standardised difference (*D*) as follows:

$S = (A + B) / \sqrt{2}$ and $D = (B - A) / \sqrt{2}$ [*median*(B) > *median*(A)].

Then $ZB = (S - X) / Y$ and $ZW = (D - V) / W$ where *X*, *Y*, *V* and *W* are values from the table.

For further details on the calculation and interpretation of robust z-scores, please see the *Guide to Proficiency Testing Australia (2012)* [1].

TABLE A: OUTLIER RESULTS –
SAMPLE A and B IDENTIFICATION AND ENUMERATION
(by laboratory code number)

Dominant Cyanobacteria in order of abundance	Sample A and B		
	Identification Outlier	Between-Laboratories Z-Score Outlier	Within-Laboratory Z-Score Outlier
ORGANISM 1 (*Arthrospira)*	2, 13	-	2, 13
ORGANISM 2 (*Anabaena)*	18	13	2

TABLE B: OUTLIER RESULTS – SAMPLE C IDENTIFICATION
(by laboratory code number)

Classification Group	Sample C Identification Outlier
Diatom - pennate (*Synedra)*	1, 2, 7A, 7B, 11, 13, 15, 22, 23
Diatom - centric (*Cyclotella*)	1, 8, 13, 15
Dinoflagellate (*Ceratium*)	-
Chrysophyte (*Dinobryon*)	-

TABLE C: OUTLIER RESULTS –
SAMPLE D IDENTIFICATION AND ENUMERATION
(by laboratory code number)

Dominant Dinoflagellate in order of abundance	Sample D	
	Identification Outlier	Robust Z-Score Outlier
ORGANISM 1 (*Gymnodinium catenatum)*	2	-
ORGANISM 2 (*Prorocentrum lima)*	-	6, 17
ORGANISM 3 (*Alexandrium catenella)*	2, 6, 9, 11, 14, 16, 22	2, 11

6. PTA AND TECHNICAL ADVISORS' COMMENTS

Overall Performance

Round 15 of the PTA Algae Proficiency Testing Program has been successful in terms of response from the participating laboratories. The Phytoplankton samples provided were selected to be representative of the kind of samples received for analysis in the course of routine activity in a laboratory.

The level of difficulty of testing with respect to identification and enumeration of Phytoplankton was deemed to be moderate. Overall, the majority of participating laboratories performed satisfactorily on both identification and enumeration, although a large range in cells mL^{-1} results was evident.

Homogeneity, stability and trip control test results of the samples indicated that the procedures for sample preparation and dispatch were satisfactory.

Samples A and B were prepared from Phytoplankton cultures placed in freshly distilled water containing no other Phytoplankton cells. Sample C was prepared from an environmental sample and Sample D was prepared from marine Phytoplankton cultures placed in seawater preserved with Lugol's iodine solution. All samples were preserved with Lugol's iodine solution. The samples contained several Phytoplankton genera from different classification groups (refer Tables D and E). These samples were considered representative of those that would normally be encountered by an analyst in routine work. Participants were asked to identify and/or enumerate genera from various nominated groups that were commonly present or dominant in the test samples. These included Cyanobacteria, Diatoms, Dinoflagellates and Chrysophytes for the freshwater samples and Dinoflagellates for the marine sample. This required knowledge of the major Phytoplankton groups and their morphological characteristics.

As in previous rounds, participants were invited to choose their own method for enumeration, rather than adhere strictly to a prescribed method. Individual judgements could be made on suitable magnification, type of counting chamber, the proportion of chamber to be counted, the number of cells or filaments to count and the appropriate methods to estimate cells in colonies or trichomes.

Verified and Consensus Results

Verified results were used for the identification component of the proficiency test and were determined by the Technical Advisors and Supplier at the time of sample preparation. These may include alternative names (synonyms), due to changes/revision in taxonomic classification of the organism.

The "Instructions to Participants" requested identification and enumeration of Cyanobacteria that were present in Sample A and B. Identification to genus level only was required.

For the purposes of testing enumeration proficiency, the consensus value was derived from the median result of all participants that are deemed to have enumerated the same nominated organism, irrespective of verified identification.

Participants were also requested to identify the Phytoplankton in Sample C that fitted the following criteria:

1. Two (2) Diatoms;
2. One (1) Dinoflagellate;
3. One (1) Chrysophyte

Identification was required to genus level only.

Participants that requested the marine sample were instructed to identify and enumerate the Dinoflagellates that were present in Sample D. Identification to species level was required.

The verified identifications for all samples are listed in Tables D and E.

TABLE D: VALID VERIFIED TAXA PRESENT IN SAMPLE A, B and C

Sample A and B	**Sample C**		
Cyanobacteria	**Diatoms**	**Dinoflagellate**	**Chrysophyte**
Arthrospira	Pennate: *Synedra*	*Ceratium*	*Dinobryon*
Anabaena	Centric: *Cyclotella*		

Identification – Samples A and B

- Organism 1 (most abundant Cyanobacteria) - *Arthrospira*
- Organism 2 (second most abundant Cyanobacteria) - *Anabaena*

Twenty two participants correctly identified *Arthrospira*. Two participants identified *Spirulina*. Although *Spirulina* is a synonym for *Arthrospira* the separation of these two genera has been widely accepted and has been documented in literature for many years. Significant morphological difference are evident between these two genera, namely the difference in spiral width ratios and the fact the cross walls of *Arthrospira* are visible under light microscopy but are not visible in *Spirulina* species. Thus, the identification of *Spirulina* is classified as an outlier.

Twenty three participants correctly identified *Anabaena/Dolichospermum*. One participant identified *Anabaenopsis*.

Identification – Sample C

Pennate Diatom
Fifteen participants correctly identified *Synedra*. Five participants identified *Fragilaria* and one participant *Synedra/Fragilaria*. Three participants identified *Nitzschia*.

Centric Diatom
Nineteen participants correctly identified *Cyclotella*. Four participants identified *Aulocoseira*. One participant identified only to centric diatom level as required by their laboratory procedures.

Dinoflagellate
All participants correctly identified *Ceratium.*

Chrysophyte
All participants correctly identified *Dinobryon*.

TABLE E: VALID VERIFIED TAXA PRESENT IN SAMPLE D

Sample D
Dinoflagellates
Gymnodinium catenatum
Prorocentrum lima
Alexandrium catenella

Identification – Sample D

- Organism 1 (most abundant Dinoflagellate) - *Gymnodinium catenatum*
- Organism 2 (second most abundant Dinoflagellate) - *Prorocentrum lima*
- Organism 3 (third most abundant Dinoflagellate) - *Alexandrium catenella*

Eleven participants correctly identified *Gymnodinium catenatum* or *Gymnodinium* as required by their scope of accreditation. One participant identified *Alexandrium catenella*.

All participants correctly identified *Prorocentrum lima* or *Prorocentrum* as required by their laboratory procedures.

Five participants correctly identified *Alexandrium catenella* or *Alexandrium* as required by their laboratory procedures. Four participants identified *Alexandrium tamarense*. A few clear hypotheca, where the diagnostically important 1' plate is, were clearly visible in the sample. Its shape and the absence of a ventral pore (the presence of which is diagnostic for *Alexandrium tamarense,* for example) led to the identification of *Alexandrium catenella*. The culture came from University of Tasmania, Plant Science where identification was also confirmed as *Alexandrium catenella.* Other organisms identified were *Ceratium tripos* - one participant and *Gambierdiscus toxicus* - one participant. One participant was unable to identify a third Dinoflagellate.

Enumeration

For Sample A, B and D the statistical assessment of cell abundance estimates was performed for all participants who reported results, even if the organism was incorrectly identified.

Samples A and B

The majority of participants correctly identified genera in Samples A and B, based on the verified results. The majority of participants who reported results did not report any outliers in the enumeration of the two (2) requested genera in the sample, based upon variability about the consensus median result.

No laboratories had a between-laboratories outlier for the enumeration of *Arthrospira.* However, two laboratories (laboratories 2 and 13) were identified as having within-laboratory outliers. Overall, the results ranged from 14,806 cells mL^{-1} to 180,000 cells mL^{-1}.

One participant (laboratory 13) was identified as having a between-laboratories outlier for the enumeration of *Anabaena* with reported results lower than the median. One laboratory (laboratory 2) was identified as having a within-laboratory outlier. Overall, the results ranged from 11,673 cells mL^{-1} to 100,000 cells mL^{-1}.

There appears to be a large spread of results for enumeration. The results are log-transformed before statistical analysis is performed and the spread of the log-transformed results is not so large. Participants are encouraged to review any results which have an absolute z-score between two and three (i.e. $2.0 < |z\text{-score}| < 3.0$) even though these results are not highlighted as outliers.

The majority of participants (15 of 24) chose to use a Sedgewick-Rafter counting chamber for Round 15. Four participants used an Utermöhl chamber. Another three participants chose to use a Lund Cell and each of these provided the measured volume of sample. One participant used a Haemocytometer, with two Neubauer reticles and one participant used a Nannoplankton counting chamber and Palmer-Maloney style of slide.

Magnification for enumeration of Phytoplankton taxa ranged from 100x to 1000x. The majority of participants used 400x or 200x magnification.

Variation Between Methods

There were a variety of different methodologies employed for the enumeration of Samples A and B. A variety of different counting chambers, magnifications and methods to determine cells/unit were used. The method of counting chamber did not appear to have had a contributing influence on the variation of count results which is a satisfying outcome. However, the magnification used to enumerate cells and the methodologies applied to determine the cells/unit values may have potentially affected results. The cell/unit values, in Sample A, varied from 10 to 176 cells/unit for Organism 1; and 26.4 to 40 cells/unit for Organism 2. The cell/unit values, in Sample B, varied from 10 to 183 cells/unit for Organism 1; and 25.2 to 44 cells/unit for Organism 2.

Sample D

The majority of participants correctly identified genera/species in Sample D, based on the verified results. The majority of participants who reported results did not report any outliers in the enumeration of the two requested genera in the sample, based upon variability about the consensus median result.

No laboratories had an outlier for the enumeration of *Gymnodinium catenatum.* Overall, the results ranged from 102 cells mL^{-1} to 450 cells mL^{-1}.

Two participants (laboratories 6 and 17) were identified as having an outlier for the enumeration of *Prorocentrum lima* with reported results lower than the median. Overall, the results ranged from 97 cells mL^{-1} to 290 cells mL^{-1}.

Two participants (laboratories 2 and 11) were identified as having an outlier for the enumeration of *Alexandrium catenella* with laboratory 2 reporting a result lower than the median and laboratory 11 reporting a result higher than the median. Overall, the results ranged from 3 cells mL^{-1} to 80 cells mL^{-1}.

There appears to be a large spread of results for enumeration. The results are log-transformed before statistical analysis is performed and the spread of the log-transformed results is not so large. Participants are encouraged to review any results which have an absolute z-score between two and three (i.e. $2.0 < |z\text{-score}| < 3.0$) even though these results are not highlighted as outliers.

The majority of participants (7 of 12) chose to use a Sedgewick-Rafter counting chamber for Round 15. Two participants used an Utermöhl chamber. One participant chose to use a Lund Cell and provided the measured volume of sample. One participant used a Haemocytometer, with two Neubauer reticles and one participant used a Nannoplankton counting chamber and Palmer-Maloney style of slide.

Magnification for enumeration of Phytoplankton taxa ranged from 100x to 400x. The majority of participants used 200x magnification.

Variation Between Methods

There were a variety of different methodologies employed for the enumeration of Sample D. A variety of different counting chambers, magnifications and methods to determine cells/unit were used. The method of counting chamber did not appear to have had a contributing influence on the variation of count results which is a satisfying outcome.

Metrological Traceability

For enumeration, consensus values (median) derived from participant's results are used in this program. These values are not metrologically traceable to an external reference.

As the assigned value for this program is the median of the results submitted by the participants, the uncertainty of the median has been calculated for each analysis and is tabulated in the summary statistics tables for each sample in Appendix A.

Samples A, B and D were prepared by Analytical Services Tasmania from cultures obtained from the Department of Science, Information Technology, Innovation and the Arts (QLD) and the University of Tasmania. These cultures were verified by the Technical Advisors before sample preparation. Samples A and B were prepared using deionised water and preserved with Lugol's iodine solution. Sample D was prepared using seawater preserved with Lugol's iodine solution. Sample C was prepared from an environmental sample provided by Port Macquarie Hastings Council and preserved with Lugol's iodine solution.

Analysis of Results by Method Groups

In order for methods to be grouped for analysis, PTA requires more than 10 sets of results from the same method group. As there were less than 10 results submitted for each method (including magnification), reliable conclusions cannot be drawn from analysing grouped methods on this occasion. Therefore, results from all method groups have been pooled for analysis.

Possible Sources of Error

Although there is some inherent variation in enumeration, there are some common or possible sources of error which, if eliminated, would help to raise the accuracy of the final count data. These may include the following:

a) The sample container is upturned a standard number of times (i.e. 20) by gentle movements and not vigorous shaking to ensure homogeneity of mixing. The sub-sample should be withdrawn quickly with a wide bore pipette, not allowing time for the Phytoplankton to settle out of the water column in the container.

b) At the time of sub-sampling, the tip of the pipette must be located in the middle of the homogenised water column in the container i.e. not towards the bottom of the container or closer to the surface of the sample in the container.

c) The volume of the counting chambers used must be taken into account in the calculations.

d) It is important to avoid introducing excess sample into the chamber and then blotting out the excess as this could be a source of error. Blotting carries the risk of drawing Phytoplankton towards the sides thereby destroying the assumed random distribution of Phytoplankton in the chamber.

e) Unless the chamber is clean and dry there is a risk of bias in the distribution of the Phytoplankton in the chamber when the sample is delivered to fill the chamber. Also the chamber must be kept on a flat surface at the time the sample is introduced and then allowed to stand for a minimum of 30 - 60 minutes. These precautions will help to minimise the non-random distribution of counting units. It is also prudent to examine a number of replicate traverses of the chamber to be satisfied of random distribution of the counting units before commencement of counting.

f) Enumerating each cell within a trichome, where possible, and not using a standard predetermined cell/unit figure.

g) Ensuring the methods used and magnifications employed to estimate cell/unit values, where cells are not easily determined, are appropriately and consistently applied.

h) Enumeration is undertaken at 200x or 400x magnification at a minimum, to assist with a more accurate determination of cell size and hence cell/unit values.

Recommendations

A review of the Round 15 Algae Proficiency Testing Program demonstrated that while the enumeration results of Phytoplankton showed a measure of variability, some misidentifications or identification outliers underline the fact that further development in algal taxonomic skills is necessary in some of the participating laboratories.

It is recommended that staff undertaking bench work in a Phytoplankton laboratory are given exposure to algal taxonomic training whenever opportunities arise. Also with the constant development in algal classification systems and revision of names, it is necessary that training is regularly updated.

It is also recommended that all enumeration be undertaken at 200x or 400x magnification, at a minimum, and that laboratories examine their methodologies for determining cell/unit values when cells are not easily determined under this magnification.

7. REFERENCE

[1] "*Guide to Proficiency Testing Australia*" (*2012)*. (This document can be found on the PTA website, www.pta.asn.au).

APPENDIX A

Summary of Results

APPENDIX FORMAT

This appendix consists of all the results submitted by the participating laboratories for both the Identification and Enumeration components of the program, and the calculated z-scores and summary statistics for the enumeration component.

(a) Results Submitted

These tables contain the results returned by each laboratory, enumeration results tables also including MU and the type of chamber used for counting.

(b) Transformed Results and Z-Scores

These tables contain the transformed (log_{10}) results and the calculated z-scores.

Between-laboratories and within-laboratory z-scores have been calculated for the related pair, Samples A and B (based on the sums and differences of the two results).

Outliers are identified in the table by a marker "**§**" next to the relevant z-score.

Please see the *Guide to Proficiency Testing Australia (2012)* [1] for further details on how these z-scores are calculated.

(c) Summary Statistics

The list of summary statistics appears at the bottom of the table of results and consists of:

(i) the number of results for that test/sample (*No. of Results*);

(ii) the median of laboratories' results - i.e. the middle value (*Median*);

(iii) the normalised interquartile range of the results (*Normalised IQR*) - the interquartile range times 0.7413;

(iv) the robust coefficient of variation, expressed as a percentage (*Robust CV*) - i.e. 100 x *Normalised IQR* / *Median*;

(v) the minimum and maximum laboratory results;

(vi) the range (*Maximum* - *Minimum*);

(vii) the uncertainty of the median.

The median is a measure of the centre of the data. The Normalised IQR is a measure of the spread of the results. It is calculated by multiplying the interquartile range (IQR) by 0.7413, a factor which converts the IQR to an estimate of the standard deviation. The IQR is the difference between the upper and lower quartiles (i.e. the values above and below which a quarter of the results lie, respectively).

Please see reference [1] for further details on these robust summary statistics.

(d) Ordered Z-Score Charts

On these charts each laboratory's z-score is shown, in order of magnitude, and is marked with its code number. From these each laboratory can readily compare its performance relative to the other laboratories.

(e) Youden Diagrams

Youden two-sample diagrams are presented to highlight laboratory systematic differences. They are based on a plot of each laboratory's pair of results, sample 'two' versus sample 'one', represented by a black spot •.

These diagrams also feature an approximate 95% confidence ellipse for the bivariate analysis of the results, and dashed lines which mark the median value for each of the samples.

All points which lie outside the ellipse are labelled with the laboratory's code number. Note, however, that these points may not correspond with those identified as outliers. This is because the outlier criterion ($|z\text{-score}| \geq 3.0$) has a confidence level of approximately 99%, whereas the ellipse is an approximate 95% confidence region.

The points outside the ellipse on the Youden diagram will roughly correspond to those with z-scores greater than 2.0 or less than –2.0. The laboratories which are outside the ellipse but have not been identified as outliers (those which have $2.0 < |z\text{-score}| < 3.0$) are encouraged to review their results.

It is important to note, however, that Youden diagrams are an illustration of the data only, and are *not* used to assess the results (this is done by the z-scores).

Further details of the construction and interpretation of these diagrams, and a glossary of terms is given in the *Guide to Proficiency Testing Australia (2012)* [1].

Identification

Sample A and B

Sample C

Sample D

IDENTIFICATION - SAMPLE A and B

Lab Code	SAMPLE A and B **Dominant Cyanobacteria** (most abundant listed first)
1	*Arthrospira* *Anabaena*
2	*Spirulina (Arthrospira)*♦ *Anabaena*
3	*Arthrospira* *Anabaena*
4	*Arthrospira sp.* *Dolichospermum sp.*
5	*Arthrospira* *Anabaena*
7A	*Arthrospira* *Anabaena*
7B	*Arthrospira* *Anabaena*
8	*Arthrospira maxima* *Anabaena flos aquae*
9	*Arthrospira* *Anabaena (now: Dolichospermum)*
10	*Arthrospira* *Anabaena*
11	*Arthrospira* *Dolichospermum (anabaena)*
12	*Arthrospira* *Anabaena*
13	*Spirulina* ♦ *Anabaena*
14	*Arthrospira* *Anabaena*

♦ Denotes an identification outlier result.

IDENTIFICATION - SAMPLE A and B cont.

Lab Code	SAMPLE A and B
	Dominant Cyanobacteria (most abundant listed first)
15	*Arthrospira* *Anabaena*
16	*Arthrospira* *Anabaena (coil)*
17	*Arthrospira sp.* *Anabaena sp.*
18	*Arthrospira* *Anabaenopsis* ♦
19	*Arthrospira* *Anabaena*
20	*Arthrospira* *Anabaena (coiled)*
21A	*Arthrospira* *Anabaena*
21B	*Arthrospira* *Anabaena*
22	*Arthrospira* *Anabaena*
23	*Arthrospira* *Anabaena*

♦ Denotes an identification outlier result.

IDENTIFICATION - SAMPLE C

Lab Code	SAMPLE C		
	Diatoms (2)	Dinoflagellate (1)	Chrysophyte (1)
1	*Fragilaria* ♦ *Aulocoseira* ♦	*Ceratium*	*Dinobryon*
2	*Nitzchia* ♦ *Cyclotella*	*Ceratium*	*Dinobryon*
3	*Synedra* (pennate) *Cyclotella* (centric)	*Ceratium*	*Dinobryon*
4	*Synedra sp.* *Cyclotella sp.*	*Ceratium sp.*	*Dinobryon sp.*
5	*Synedra* (Pennate) *Cyclotella* (centric)	*Ceratium*	*Dinobryon*
7A	*Fragilaria* ♦ *Cyclotella*	*Ceratium*	*Dinobryon*
7B	*Fragilaria* ♦ *Cyclotella*	*Ceratium*	*Dinobryon*
8	*Synedra* *Aulacoseira* ♦	*Ceratium*	*Dinbryon*
9	*Synedra* *Cyclotella*	*Ceratium*	*Dinobryon*
10	*Synedra* *Cyclotella*	*Ceratium*	*Dinobryon*
11	*Nitzschia* ♦ *Cyclotella*	*Ceratium*	*Dinobryon*
12	*Synedra* Centric diatoms	*Ceratium*	*Dinobryon*

♦ Denotes an identification outlier result.

IDENTIFICATION - SAMPLE C cont.

Lab Code	SAMPLE C		
	Diatoms (2)	Dinoflagellate (1)	Chrysophyte (1)
13	one pennate - *Fragilaria* ♦ one centric - *Aulacoseira* ♦	*Ceratium*	*Dinobryon*
14	*Synedra* (pennate) *Cyclotella* (centric)	*Ceratium*	*Dinobryon*
15	*Nitzchia* ♦ *Aulacoseira* ♦	*Ceratium*	*Dinobryon*
16	*Synedra* *Cyclotella*	*Ceratium*	*Dinobryon*
17	*Synedra sp.* *Cyclotella sp.*	*Ceratium sp.*	*Dinbryon sp.*
18	*Synedra* *Cyclotella*	*Ceratium*	*Dinobryon*
19	*Synedra* *Cyclotella*	*Ceratium*	*Dinobryon*
20	pennate*: Synedra spp.* centric: *Cyclotella spp.*	*Ceratium spp.*	*Dinobryon spp.*
21A	*Synedra* *Cyclotella*	*Ceratium*	*Dinobryon*
21B	*Synedra* *Cyclotella*	*Ceratium*	*dinobryon*
22	*Synedra/Fragilaria* ♦ *Cyclotella*	*Ceratium*	*Dinobryon*
23	*Fragilaria* ♦ *Cyclotella*	*Ceratium*	*Dinobryon*

♦ Denotes an identification outlier result.

IDENTIFICATION - SAMPLE D

Lab Code	SAMPLE D **Dominant Dinoflagellates** (most abundant listed first)
2	*Alexandrium catenella* ♦ *Prorocentrum lima* *Ceratium tripos* ♦
5	*Gymnodinium catenatum* *Prorocentrum lima* *Alexandrium catenella*
6	*Gymnodinium cf. catenatum* *Prorocentrum lima* *Alexandrium cf. tamarense* ♦
9	*Gymnodinium catenatum* *Prorocentrum lima* *Alexandrium tamarense* ♦
10	*Gymnodinium catenatum* *Prorocentrum lima* *Alexandrium catenella*
11	*Gymnodinium catenatum* *Prorocentrum lima* *Gambierdiscus toxicus* ♦
12	*Gymnodinium* *Prorocentrum* *Alexandrium*
14	*Gymnodinium catenatum* *Prorocentrum lima* *Alexandrium tamarense* ♦
16	*Gymnodinium catenum* *Prorocentrum lima* ♦
17	*Gymnodinium catenatum* *Prorocentrum lima* *Alexandrium catenella*
19	*Gymnodinium* *Prorocentrum* *Alexandrium*
22	*Gymnodinium catenatum* *Prorocentrum lima* *Alexandrium tamarense* ♦

♦ Denotes an identification outlier result.

Enumeration

Organism 1 - Arthrospira - (Sample Pair A and B)

Organism 2 - Anabaena - (Sample Pair A and B)

Marine Organisms - (Sample D)

ORGANISM 1 - *ARTHROSPIRA* (Sample Pair A and B)
RESULTS SUBMITTED (Cells/mL)

Lab Code	Genus Enumerated	Sample A		Sample B		Chamber Used
		Cells/mL	MU	Cells/mL	MU	
1	*Arthrospira*	80560	±48336	70480	±8457	Sedgewick Rafter
2	*Spirulina (Arthrospira)*	27778	10%	100000	10%	Heamocytometer
3	*Arthrospira*	66275	±0.05 Log_{10} Cells/mL	45950	±0.05 Log_{10} Cells/mL	Sedgewick Rafter
4	*Arthrospira sp.*	180000		61000		Sedgewick Rafter
5	*Arthrospira*	55504	10%	41690	10%	Sedgewick Rafter
7A	*Arthrospira*	104300	95000-110000	36219	33000-40000	Utermöhl
7B	*Arthrospira*	110000	100000-120000	81000	74000-89000	Utermöhl
8	*Arthrospira maxima*	40000	7900	28000	5600	Sedgewick Rafter
9	*Arthrospira*	75777	±5%	40538	±5%	Utermöhl
10	*Arthrospira*	82429	±20%	53284	±20%	Sedgewick rafter
11	*Arthrospira*	75956	±7846	52309	±9030	Nannoplankton (Palmer-Maloney)
12	*Arthrospira*	99520	8.06%	67100	8.06%	Sedgewick Rafter
13	*Spirulina*	58474		14806		Sedgewick - Rafter
14	*Arthrospira*	83311	11%	58807	15%	Lund Cell
15	*Arthrospira*	62160		36650		Sedgewick Rafter
16	*Arthrospira*	64300	±15000	58100	±19000	Sedgewick Rafter
17	*Arthrospira sp.*	92000	33.8%	73000	33.8%	Utermöhl
18	*Arthrospira*	45000		45000		Lund Cell
19	*Arthrospira*	44050	33900-57300	36300	27900-47200	Sedgewick rafter
20	*Arthrospria*	45325		43600		Sedgewick Rafter
21A	*Arthrospira*	76448		53533		Sedgewick Rafter
21B	*Arthrospira*	87091		54278		Sedgewick Rafter
22	*Arthrospira*	109000		59000		Sedgewick - rafter
23	*Arthrospira*	58961	±22405	52811	±20068	Lund Cell

ORGANISM 1 - *ARTHROSPIRA* (Sample Pair A and B)
TRANSFORMED RESULTS (log_{10}Cells/mL) AND Z-SCORES

Lab Code	log_{10}(Cells/mL)		Between-Labs z-score	Within-Lab z-score
	Sample A	Sample B		
1	4.91	4.72	-0.28	2.15
2	4.44	5.22	-0.13	-8.59 **§**
3	4.82	4.71	-0.63	1.27
4	5.26	5.08	2.40	2.03
5	4.74	4.75	-0.78	0.02
7A	5.02	4.76	0.28	2.99
7B	5.04	5.08	1.59	-0.35
8	4.60	4.73	-1.38	-1.38
9	4.88	4.84	0.07	0.52
10	4.92	4.93	0.53	-0.03
11	4.88	4.95	0.51	-0.76
12	5.00	5.00	1.11	0.10
13	4.77	4.33	-2.29	4.96 **§**
14	4.92	4.87	0.34	0.63
15	4.79	4.89	-0.07	-0.99
16	4.81	4.76	-0.51	0.63
17	4.96	4.88	0.52	1.06
18	4.65	4.69	-1.35	-0.34
19	4.64	4.68	-1.43	-0.31
20	4.66	4.68	-1.38	-0.19
21A	4.88	4.96	0.54	-0.77
21B	4.94	4.95	0.72	-0.04
22	5.04	5.05	1.45	-0.02
23	4.77	4.69	-0.91	0.99

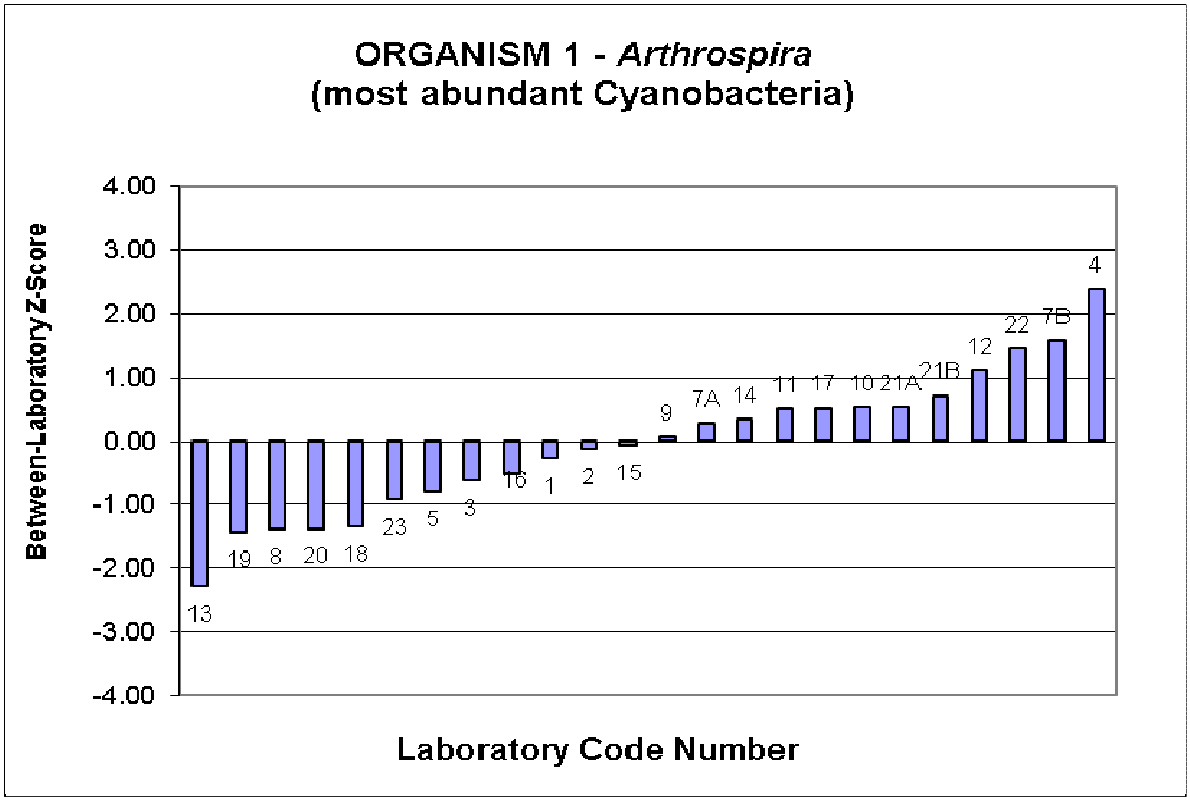
Note:

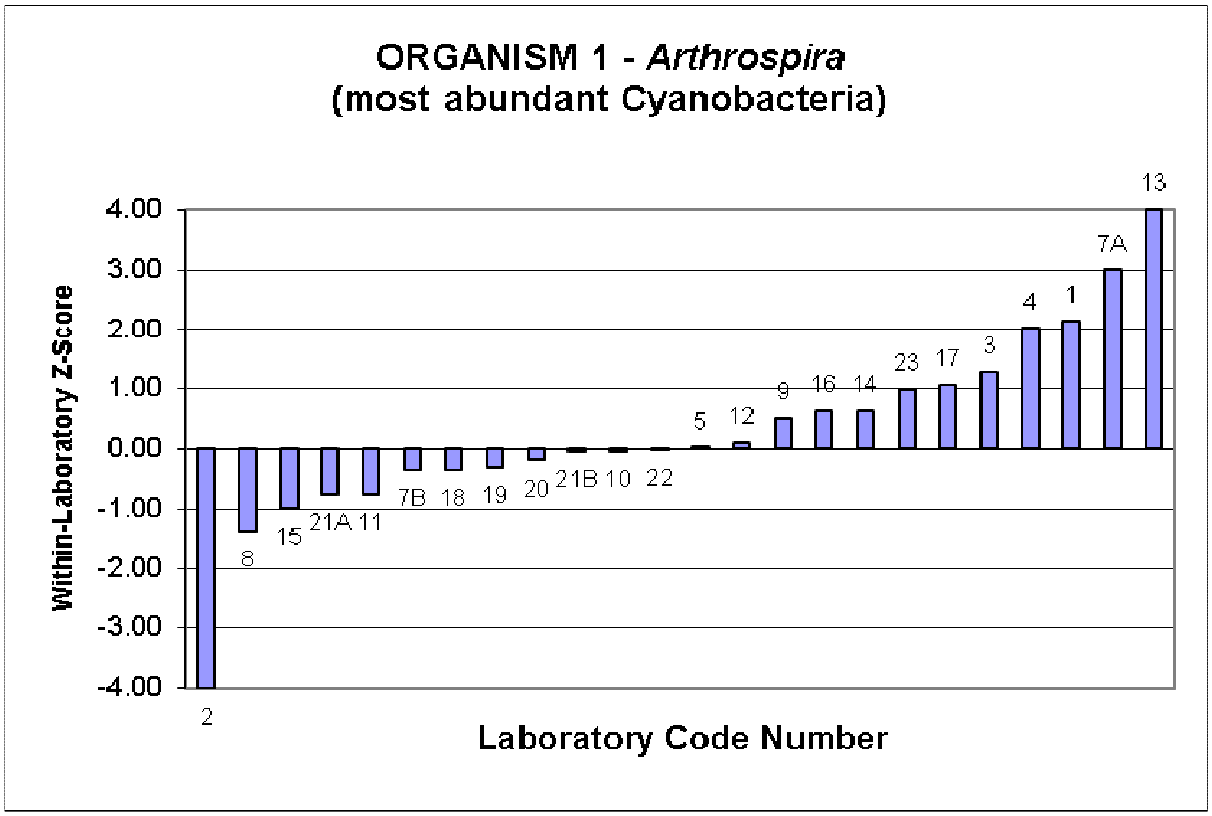
1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).

SUMMARY STATISTICS

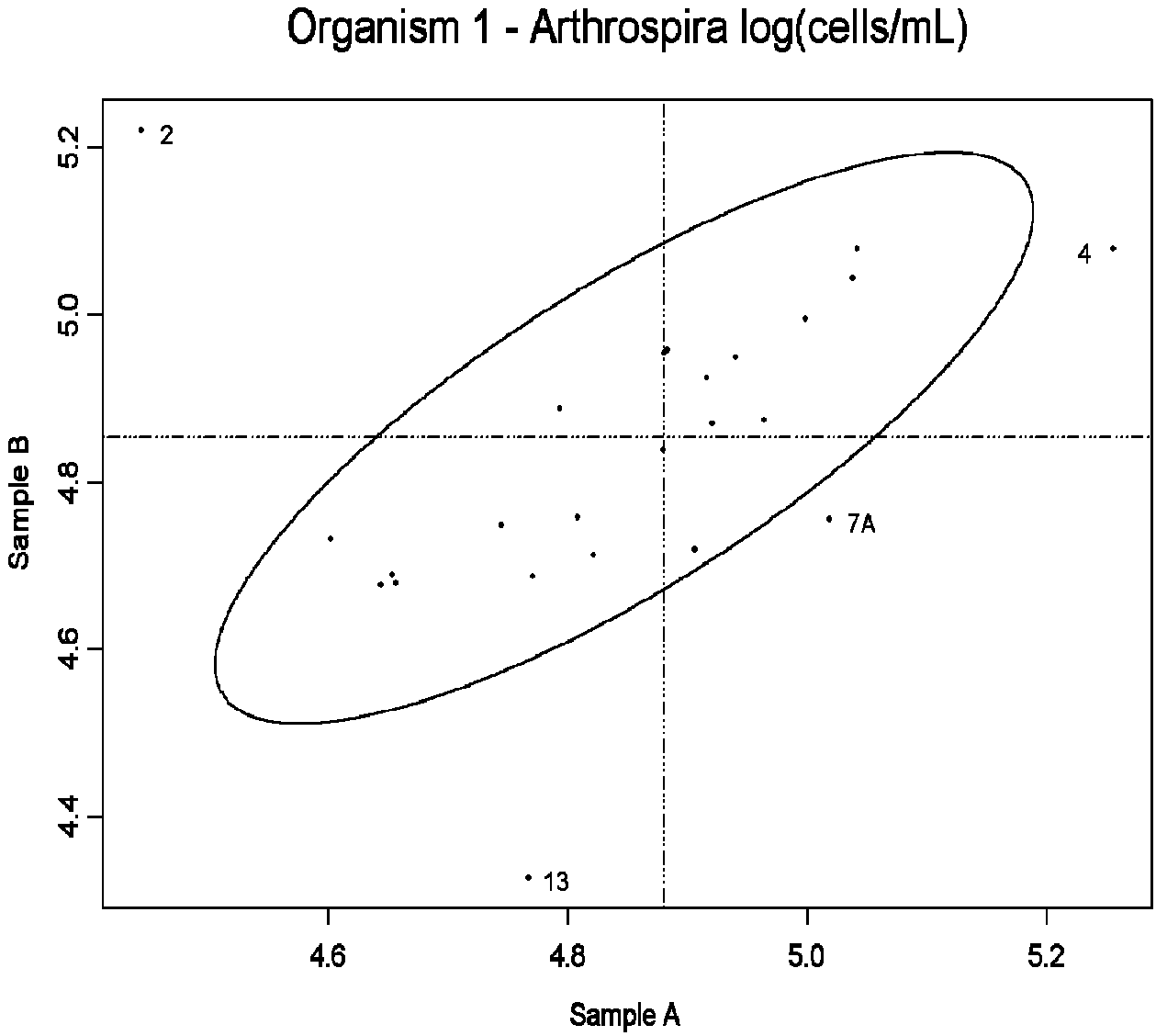
Statistic	*Sample A*	*Sample B*
No. of results	24	24
Median	4.880	4.855
Normalised IQR	0.137	0.176
Robust CV	2.8%	3.6%
Minimum	4.44	4.33
Maximum	5.26	5.22
Range	0.81	0.89
Uncertainty (Median)	0.035	0.045

ORGANISM 1 - *ARTHROSPIRA* (Sample Pair A and B)
BETWEEN-LABORATORY and WITHIN-LABORATORY ORDERED Z-SCORE CHARTS





YOUDEN DIAGRAM



ORGANISM 2 - *ANABAENA* (Sample Pair A and B)
RESULTS SUBMITTED (Cells/mL)

Lab Code	Genus Enumerated	Sample A		Sample B		Chamber Used
		Cells/mL	MU	Cells/mL	MU	
1	*Anabaena*	70480	±8457	58240	±6989	Sedgewick Rafter
2	*Anabaena*	100000	10%	22222	10%	Haemocytometer
3	*Anabaena*	45950	±0.05 Log_{10} Cells/mL	41975	±0.05 Log_{10} Cells/mL	Sedgewick Rafter
4	*Dolichospermum sp.*	61000		62000		Sedgewick Rafter
5	*Anabaena*	41690	10%	40720	10%	Sedgewick Rafter
7A	*Anabaena*	36219	33000-40000	29000	26000-32000	Utermöhl
7B	*Anabaena*	81000	74000-89000	56000	51000-61000	Utermöhl
8	*Anabaena flos aquae*	28000	5600	31000	6200	Sedgewick Rafter
9	*Anabaena (now: Dolichospermum)*	40538	±5%	43185	±5%	Utermöhl
10	*Anabaena*	53284	±20%	64180	±20%	Sedgewick rafter
11	*Dolichospermum (anabaena)*	52309	±9030	62579	±6654	Nannoplankton (Palmer-Maloney)
12	*Anabaena*	67100	8.06%	56000	8.06%	Sedgewick Rafter
13	*Anabaena*	14806		11673		Sedgewick - Rafter
14	*Anabaena*	58807	15%	52104	14%	Lund Cell
15	*Anabaena*	36650		28600		Sedgewick Rafter
16	*Anabaena (coil)*	58100	±19000	69300	±19000	Sedgewick Rafter
17	*Anabaena sp.*	73000	33.8%	51000	33.8%	Utermöhl
18	*Anabaenopsis*	45000		48000		Lund Cell
19	*Anabaena*	36300	27900-47200	40000	30800-52000	Sedgewick rafter
20	*Anabaena (coiled)*	43600		55950		Sedgewick Rafter
21A	*Anabaena*	53533		62833		Sedgewick Rafter
21B	*Anabaena*	54278		56556		Sedgewick Rafter
22	*Anabaena*	59000		62000		Sedgewick - rafter
23	*Anabaena*	52811	±20068	53769	±20432	Lund Cell

ORGANISM 2 - *ANABAENA* (Sample Pair A and B)
TRANSFORMED RESULTS (log_{10}Cells/mL) AND Z-SCORES

Lab Code	log_{10}(Cells/mL)		Between-Labs z-score	Within-Lab z-score
	Sample A	Sample B		
1	4.85	4.77	0.60	0.94
2	5.00	4.35	-0.51	6.91 **§**
3	4.66	4.62	-0.77	0.49
4	4.79	4.79	0.45	0.00
5	4.62	4.61	-0.99	0.18
7A	4.56	4.46	-1.86	1.09
7B	4.91	4.75	0.78	1.75
8	4.45	4.49	-2.20	-0.38
9	4.61	4.64	-0.94	-0.21
10	4.73	4.81	0.27	-0.77
11	4.72	4.80	0.19	-0.74
12	4.83	4.75	0.44	0.90
13	4.17	4.07	-5.11 **§**	1.16
14	4.77	4.72	0.07	0.63
15	4.56	4.46	-1.86	1.20
16	4.76	4.84	0.56	-0.72
17	4.86	4.71	0.42	1.71
18	4.65	4.68	-0.56	-0.22
19	4.56	4.60	-1.28	-0.36
20	4.64	4.75	-0.34	-1.06
21A	4.73	4.80	0.24	-0.65
21B	4.73	4.75	0.07	-0.11
22	4.77	4.79	0.39	-0.15
23	4.72	4.73	-0.07	0.00

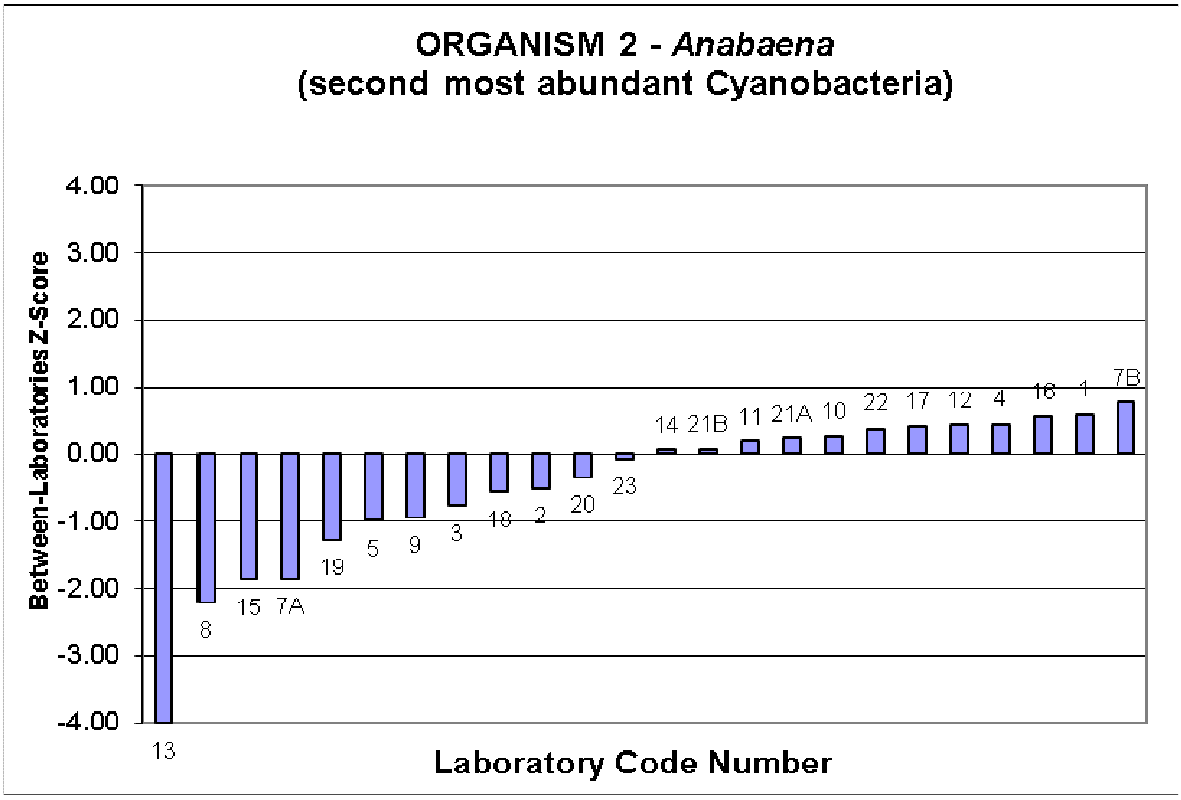
Note:

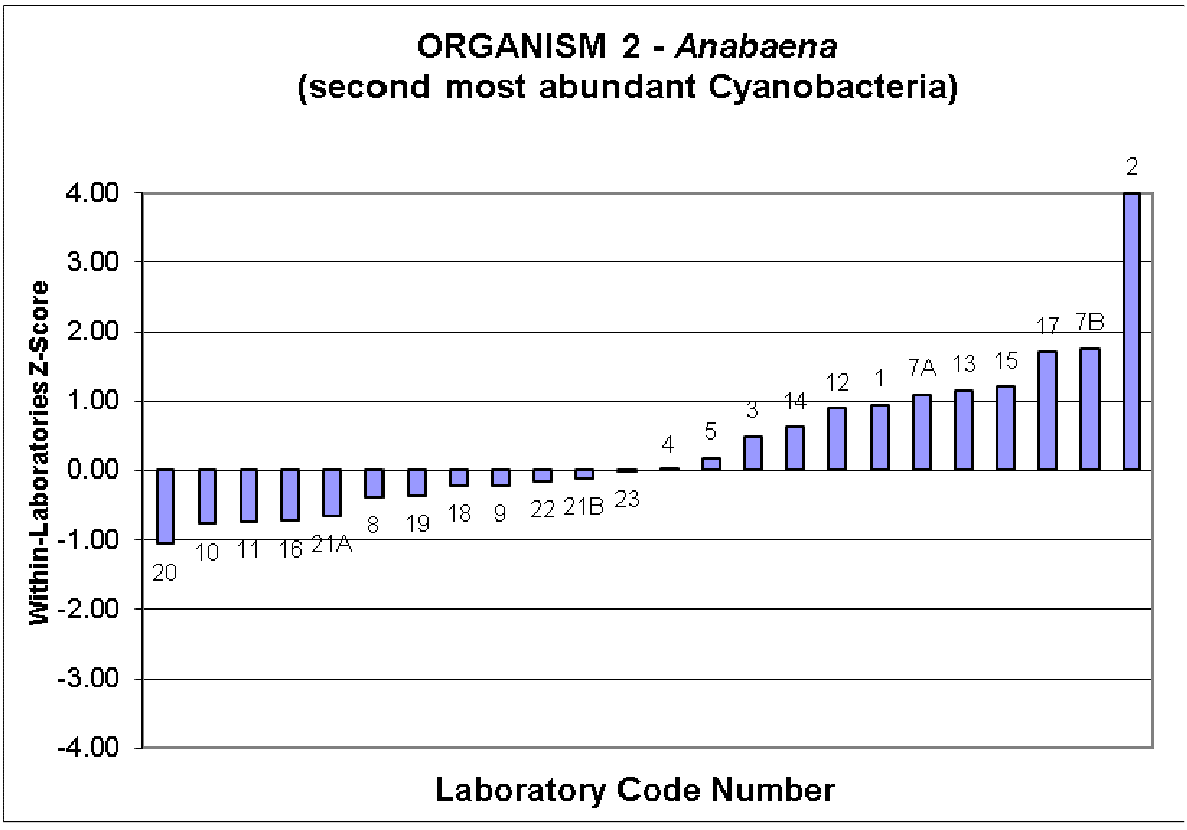
1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).

SUMMARY STATISTICS

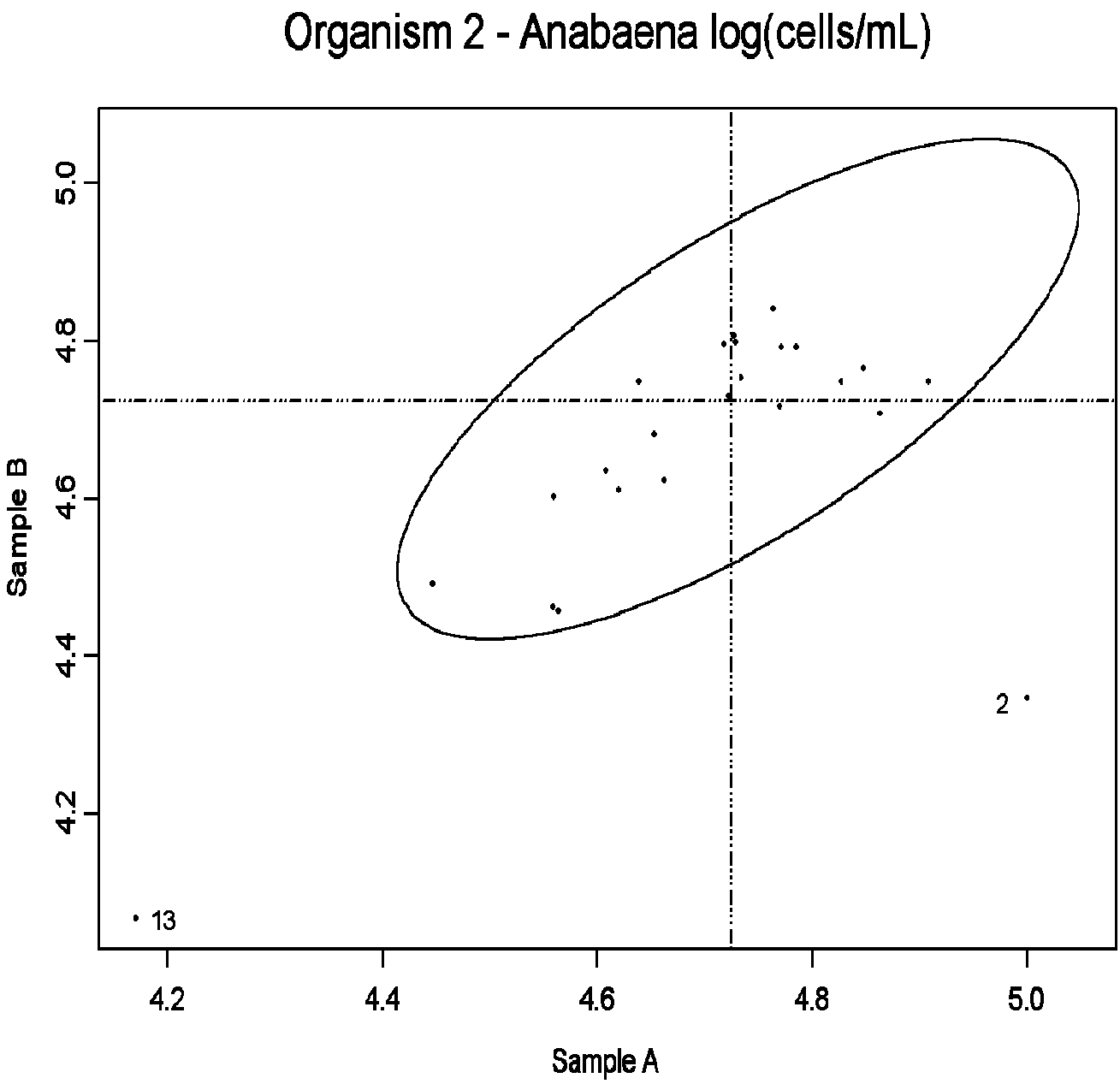
Statistic	*Sample A*	*Sample B*
No. of results	24	24
Median	4.725	4.724
Normalised IQR	0.117	0.122
Robust CV	2.5%	2.6%
Minimum	4.17	4.07
Maximum	5.00	4.84
Range	0.83	0.77
Uncertainty (Median)	0.030	0.031

ORGANISM 2 - *ANABAENA* (Sample Pair A and B)
BETWEEN-LABORATORY and WITHIN-LABORATORY ORDERED Z-SCORE CHARTS





YOUDEN DIAGRAM



ORGANISM 1 - *GYMNODINIUM CATENATUM* (Sample D)
RESULTS SUBMITTED (Cells/mL)

Lab Code	Genus Enumerated	Sample D		Chamber Used
		Cells/mL	MU	
2	*Alexandrium catenella*	175	5%	Haemocytometer
5	*Gymnodinium catenatum*	185	10%	Sedgewick Rafter
6	*Gymnodinium cf. catenatum*	102		Sedgewick Rafter
9	*Gymnodinium catenatum*	311	±5%	Utermöhl
10	*Gymnodinium catenatum*	346.25	±20%	Sedgewick rafter
11	*Gymnodinium catenatum*	450	±225	Nannoplankton (Palmer-Maloney)
12	*Gymnodinium*	310	8.06%	Sedgewick Rafter
14	*Gymnodinium catenatum*	243	25%	Lund Cell
16	*Gymnodinium catenum*	450		Sedgewick Rafter
17	*Gymnodium catenatum*	161	38.5%	Utermöhl
19	*Gymnodinium*	180	142-229	Sedgewick rafter
22	*Gymnodinium catenatum*	250		Sedgewick rafter

ORGANISM 2 - *PROROCENTRUM LIMA* (Sample D)
RESULTS SUBMITTED (Cells/mL)

Lab Code	Genus Enumerated	Sample D		Chamber Used
		Cells/mL	MU	
2	*Prorocentrum lima*	212	5%	Haemocytometer
5	*Prorocentrum lima*	288	10%	Sedgewick Rafter
6	*Prorocentrum lima*	97		Sedgewick Rafter
9	*Prorocentrum*	226	±5%	Utermöhl
10	*Prorocentrum lima*	254.75	±20%	Sedgewick rafter
11	*Prorocentrum lima*	250	±81	Nannoplankton (Palmer-Maloney)
12	*Prorocentrum lima*	228	8.06%	Sedgewick Rafter
14	*Prorocentrum*	208	23%	Lund Cell
16	*Prorocentrum lima*	290		Sedgewick Rafter
17	*Prorocentrum lima*	141	38.5%	Utermöhl
19	*Prorocentrum lima*	230	182-292	Sedgewick rafter
22	*Prorocentrum lima*	250		Sedgewick rafter

ORGANISM 3 - *ALEXANDRIUM CATENELLA* (Sample D)
RESULTS SUBMITTED (Cells/mL)

Lab Code	Genus Enumerated	Sample D		Chamber Used
		Cells/mL	MU	
2	*Ceratium tripos*	3	5%	Haemocytometer
5	*Alexandrium catenella*	26		Sedgewick Rafter
6	*Alexandrium cf. tamarense*	14		Sedgewick Rafter
9	*Alexandrium tamarense*	30	±5%	Utermöhl
10	*Alexandrium catenella*	38.75	±20%	Sedgewick rafter
11	*Gambierdiscus toxicus*	80	±31	Nannoplankton (Palmer-Maloney)
12	*Alexandrium*	30	8.07%	Sedgewick Rafter
14	*Alexandrium tamarense*	17	32%	Lund Cell
17	*Alexandrium catenella*	25	38.5%	Utermöhl
19	*Alexandrium*	29	23-37	Sedgewick rafter
22	*Alexandrium tamarense*	32		Sedgewick rafter

MARINE ORGANISMS (Sample D)
TRANSFORMED RESULTS (log_{10}Cells/mL) AND Z-SCORES

Lab Code	log_{10} (Cells/mL)			Organism 1 *(Gymnodinium catenatum)* Robust z-score	Organism 2 *(Prorocentrum lima)* Robust z-score	Organism 3 *(Alexandrium catenella)* Robust z-score
	Organism 1 *(Gymnodinium catenatum)*	Organism 2 *(Prorocentrum lima)*	Organism 3 *(Alexandrium catenella)*			
2	2.24	2.33	0.48	-0.80	-0.60	-7.51 **§**
5	2.27	2.46	1.41	-0.67	1.77	-0.36
6	2.01	1.99	1.15	-2.05	-6.65 **§**	-2.41
9	2.49	2.35	1.48	0.54	-0.10	0.11
10	2.54	2.41	1.59	0.79	0.82	0.96
11	2.65	2.40	1.90	1.40	0.68	3.36 **§**
12	2.49	2.36	1.48	0.53	-0.03	0.11
14	2.39	2.32	1.23	-0.03	-0.74	-1.77
16	2.65	2.46	Not Detected	1.40	1.83	
17	2.21	2.15	1.40	-0.99	-3.75 **§**	-0.49
19	2.26	2.36	1.46	-0.73	0.03	0.00
22	2.40	2.40	1.51	0.03	0.68	0.33

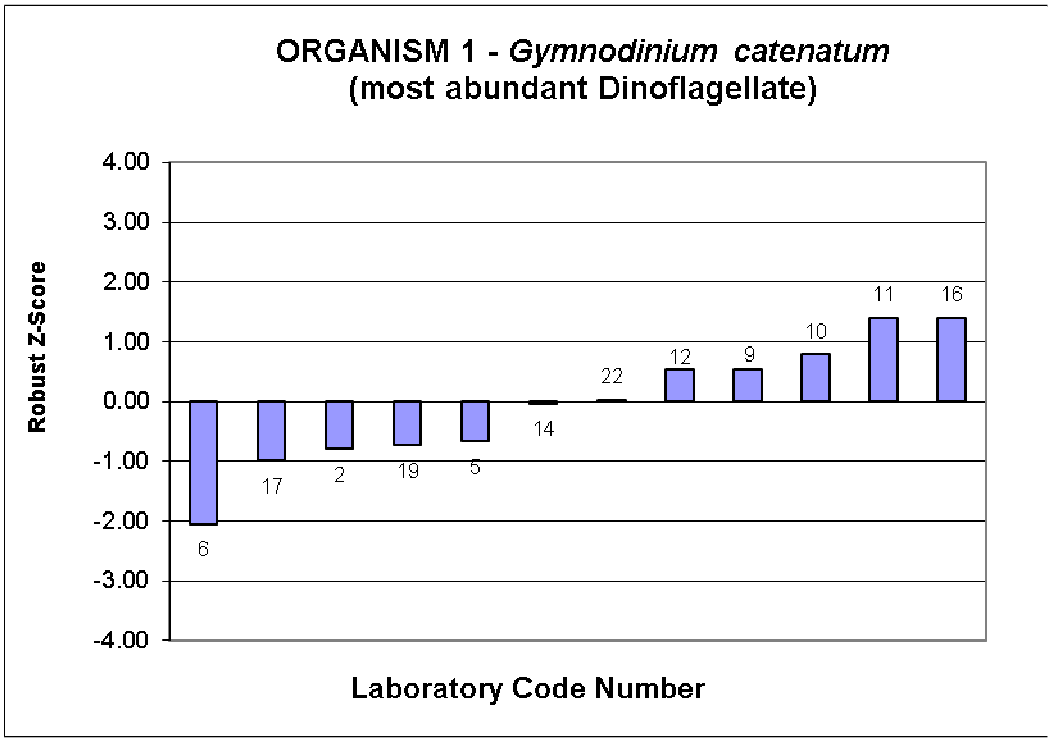
Note:

1. **§** denotes an outlier (i.e. |z-score| ≥ 3.0).

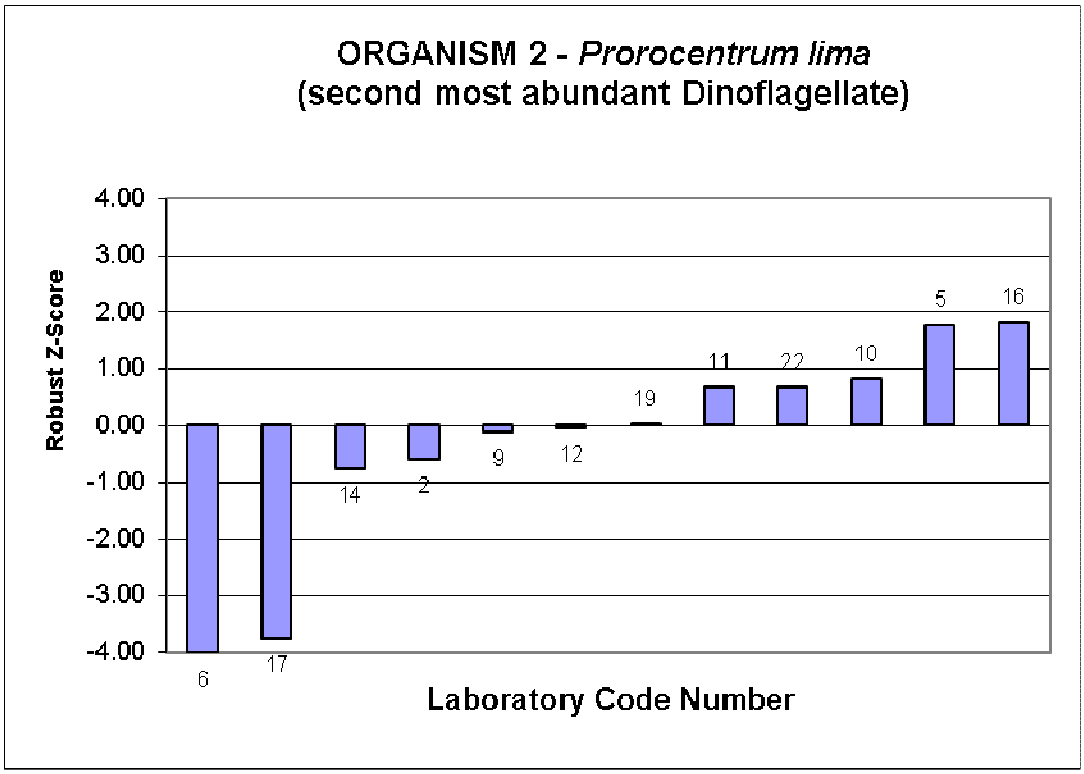
SUMMARY STATISTICS

Statistic	Organism 1 *(Gymnodinium catenatum)*	Organism 2 *(Prorocentrum lima)*	Organism 3 *(Alexandrium catenella)*
No. of results	12	12	11
Median	2.392	2.360	1.462
Normalised IQR	0.187	0.056	0.131
Robust CV	7.8%	2.4%	9.0%
Minimum	2.01	1.99	0.48
Maximum	2.65	2.46	1.90
Range	0.64	0.48	1.43
Uncertainty (Median)	0.068	0.020	0.050

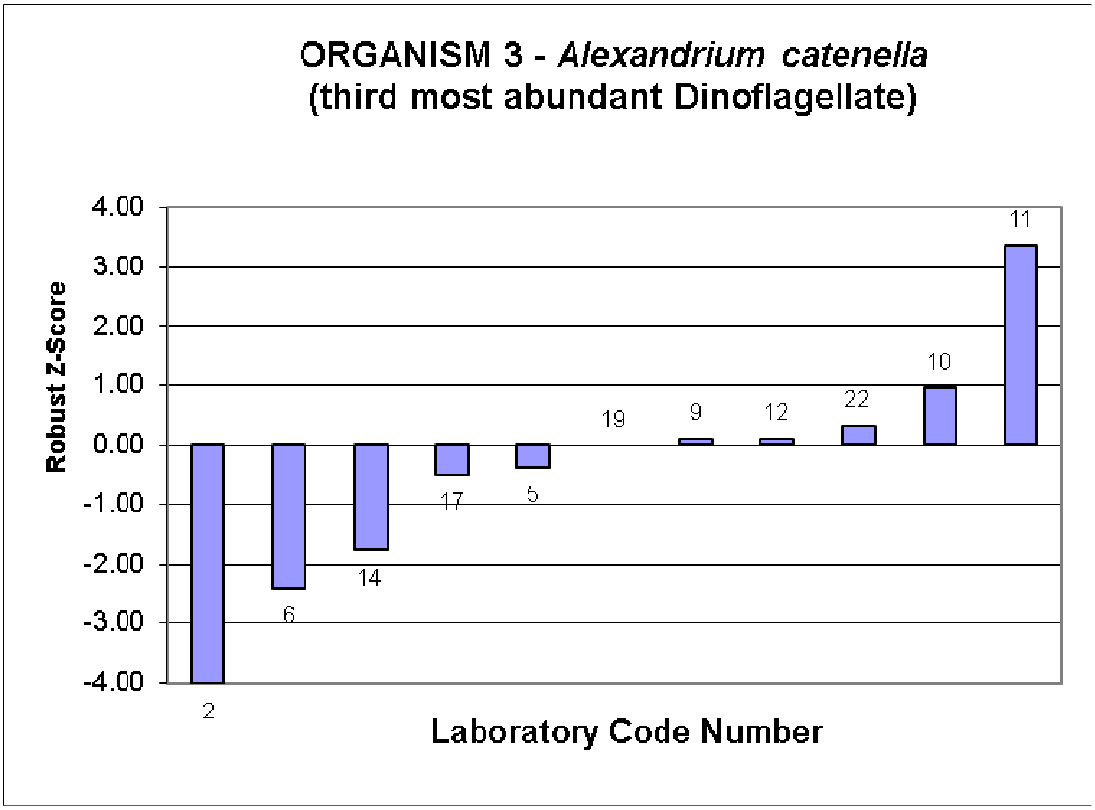
ORGANISM 1 - *GYMNODINIUM CATENATUM* (Sample D)
ORDERED ROBUST Z-SCORE CHART



ORGANISM 2 - *PROROCENTRUM LIMA* (Sample D)
ORDERED ROBUST Z-SCORE CHART



ORGANISM 3 - *ALEXANDRIUM CATENELLA* (Sample D)
ORDERED ROBUST Z-SCORE CHART



APPENDIX B

Sample Preparation and Distribution

Homogeneity, Stability Testing and Trip Control

SAMPLE PREPARATION AND DISTRIBUTION

The samples utilised in this program were prepared by Analytical Services Tasmania – Department of Primary Industries, Parks, Water and the Environment.

Samples A and B were prepared from Phytoplankton cultures placed in freshly distilled water, which was checked to ensure there were no other algal cells present. Samples were preserved in Lugol's iodine solution.

Sample C was prepared from an environmental sample preserved with Lugol's iodine solution.

Sample D was prepared from marine Phytoplankton cultures placed in seawater preserved with Lugol's iodine solution.

Each participant was provided with three or four samples in amber glass bottles, labelled Sample A, Sample B, Sample C and Sample D. Samples A and B were duplicates. The samples were dispatched to participants on 11 December 2012 using Australian Air Express for Australian laboratories and by courier using TOLL for international participants.

HOMOGENEITY TESTING

For this program, 10 bottles of the Sample A/B, Sample C and Sample D mixture were randomly selected and tested for homogeneity.

For Sample C the following were identified in all 10 homogeneity samples, hence the samples are considered to be homogeneous:

Diatoms: *Synedra, Cyclotella*

Dinoflagellate: *Ceratium*

Chrysophyte: *Dinobryon*

For Sample A/B the following results were reported for homogeneity testing:

	Organism 1: *Arthrospira* (Cells/mL)	Log_{10}	Organism 2: *Anabaena* (Cells/mL)	Log_{10}
1	190000	5.279	57000	4.756
2	200000	5.301	53000	4.724
3	170000	5.230	59000	4.771
4	160000	5.204	50000	4.699
5	170000	5.230	53000	4.724
6	200000	5.301	52000	4.716
7	200000	5.301	55000	4.740
8	170000	5.230	54000	4.732
9	170000	5.230	51000	4.708
10	190000	5.279	57000	4.756
Mean		5.259		4.733
SD		0.039		0.023
%CV		0.733		0.483

The % CV for both organisms in the samples analysed was < 1%. This is within the acceptance CV limit of 5%. Hence the samples are considered to be homogeneous.

For Sample D the following results were reported for homogeneity testing:

	Organism 1: *Gymnodinium catenatum* (Cells/mL)	Log_{10}	Organism 2: *Prorocentrum lima* (Cells/mL)	Log_{10}	Organism 3: *Alexandrium catenella* (Cells/mL)	Log_{10}
1	330	2.519	250	2.398	54	1.732
2	330	2.519	250	2.398	47	1.672
3	330	2.519	270	2.431	59	1.771
4	290	2.462	250	2.398	59	1.771
5	370	2.568	230	2.362	58	1.763
6	320	2.505	290	2.462	73	1.863
7	340	2.531	250	2.398	53	1.724
8	300	2.477	240	2.380	61	1.785
9	350	2.544	320	2.505	48	1.681
10	310	2.491	260	2.415	54	1.732
Mean		2.514		2.415		1.750
SD		0.032		0.045		0.058
%CV		1.279		1.843		3.313

The % CV for all organisms in the sample analysed was < 4%. This is within the acceptance CV limit of 5%. Hence the samples are considered to be homogeneous.

STABILITY TESTING

Three samples each of the Sample A/B, C and D mixture were randomly selected and tested for stability, seven days after dispatch.

For Sample C the following were identified in all three stability samples, hence establishing stability:

Diatoms: *Synedra, Cyclotella*

Dinoflagellate: *Ceratium*

Chrysophyte: *Dinobryon*

For Sample A/B the following results were reported for stability testing:

	Organism 1: *Arthrospira* (Cells/mL)	Log_{10}	Organism 2: *Anabaena* (Cells/mL)	Log_{10}
1	200000	5.301	53000	4.724
2	200000	5.301	54000	4.732
3	200000	5.301	54000	4.732
Mean		5.301		4.730
SD		0.000		0.022
%CV		0.000		0.463

For Sample D the following results were reported for stability testing:

	Organism 1: *Gymnodinium catenatum* (Cells/mL)	Log_{10}	Organism 2: *Prorocentrum lima* (Cells/mL)	Log_{10}	Organism 3: *Alexandrium catenella* (Cells/mL)	Log_{10}
1	330	2.519	210	2.322	55	1.740
2	290	2.462	220	2.342	56	1.748
3	330	2.519	230	2.362	51	1.708
Mean		2.500		2.342		1.732
SD		0.032		0.020		0.022
%CV		1.296		0.844		1.244

Hence all samples were considered to be stable during the testing period.

TRIP CONTROL

One sample set consisting of Samples A/B and C was sent to Queensland to be tested by a Technical Advisor.

For Sample C the following were identified:

Diatoms: *Cyclotella, Synedra*

Dinoflagellate: *Ceratium*

Chrysophyte: *Dinobryon*

For Sample A/B the following results were reported

Organism 1 *Arthrospira* (Cells/mL)				Organism 2 *Anabaena* (Cells/mL)			
Sample A	Log_{10}	Sample B	Log_{10}	Sample A	Log_{10}	Sample B	Log_{10}
56150	4.75	64200	4.81	35375	4.55	41025	4.61

All samples were considered to be stable after transport.

APPENDIX C

Instructions to Participants
and
Results Sheets

pt:a proficiency testing australia

ALGAE PROFICIENCY TESTING PROGRAM - ROUND 15

INSTRUCTIONS TO PARTICIPANTS

Participants are asked to carefully note the following **BEFORE** commencing the analysis of the samples.

1. Samples

Three samples (labelled Sample A, Sample B and Sample C) have been provided, containing a range of algal and cyanobacterial genera, representing 4 major groups: Bacillariophytes (Diatoms), Dinophytes (Dinoflagellates), Chrysophytes (Golden Brown) and Cyanophytes (Cyanobacteria).

Note: The samples A and B have been prepared from laboratory cultures. Sample C is an environmental sample.

Also provided upon request is a Marine sample (Sample D) that has been prepared from laboratory cultures.

2. Analysis – Samples A, B and C

The analysis consists of two parts; (i) Identification and Enumeration, (ii) Identification.

(i) Identification and Enumeration

Examine Sample A and B and identify and enumerate the two (2) dominant Cyanobacterial genera.

An identification to genus level and an estimate of cell abundance (reported as cells mL^{-1}) for each is required.

Samples are NOT to be concentrated prior to enumeration.

For each sample, mix and pipette a sub-sample from the bottle and place into a counting chamber. The sample is to be enumerated using the counting chamber of choice in each laboratory.

Participants are requested to perform the analysis according to their routine method. Information on the method used to enumerate each genus should be written in the spaces provided on the Results Sheet.

Please note that cells can be counted in either transects (strips), squares or fields of view, whichever is more appropriate, and at a magnification which is appropriate to the cell size and abundance of each genus. An estimate of cells per trichome may be determined if deemed appropriate.

The concentration of each cyanobacteria is to be given as **cells per mL** in the space provided on the Results Sheet.

Laboratories are requested to calculate and report an estimate of measurement uncertainty (MU) for each reported measurement result. All estimates of MU must be given as a 95% confidence interval (coverage factor k ≈ 2). Submitted MU information will not form part of the evaluation of performance, and is for information purposes only.

(ii) Identification

Examine Sample C and identify the algal genera that are present, fitting the following criteria:

1. Two (2) dominant Diatoms (one pennate and one centric).
2. One (1) dominant Dinoflagellate.
3. One (1) dominant Chrysophyte.

Please note: Identification to genus level only is required.

Analysis – Sample D (optional Marine sample)

Identification and Enumeration

Examine Sample D and identify and enumerate the three dominant dinoflagellates

An identification to species level and an estimate of cell abundance (reported as cells mL^{-1}) for each is required.

Samples are NOT to be concentrated prior to enumeration.

For each sample mix, pipette a sub-sample from the bottle and place into a counting chamber. The sample is to be enumerated using the counting chamber of choice in each laboratory.

Participants are requested to perform the analysis according to their routine method. Information on the method used to enumerate each genus or species should be written in the spaces provided on the Results Sheet.

Please note that cells can be counted in either transects (strips), squares or fields of view, whichever is more appropriate, and at a magnification which is appropriate to the cell size and abundance of each genus. An estimate of cells per trichome may be determined if deemed appropriate.

The concentration of each dinoflagellate is to be given as **cells per mL** in the space provided on the Results Sheet.

Laboratories are requested to calculate and report an estimate of measurement uncertainty (MU) for each reported measurement result. All estimates of MU must be given as a 95% confidence interval (coverage factor $k \approx 2$). Submitted MU information will not form part of the evaluation of performance, and is for information purposes only.

3. Reporting

(i) Please submit results on the Results Sheet provided.

(ii) The following information must be recorded on the results sheet:

(a) The genera or species identified.

(b) The total magnification used for enumeration of each designated Cyanobacteria.

(c) The number of cells enumerated for each genus/species.

(d) The number of transects, squares or fields of view examined.

(e) The type of counting chamber used and its total volume.

(f) The method used (if applicable) to estimate cells in trichomes.

(g) Any additional information you may wish to provide regarding the method / technique used.

4. Testing should commence as soon as possible after receiving samples, and results reported NO LATER THAN **MONDAY 14 JANUARY 2013** to:

Ms Kathy Weller
Proficiency Testing Australia
PO Box 1122
ARCHERFIELD BC QLD 4108

Email: Kathy.Weller@pta.asn.au
Phone: +61 7 3721 7373
Fax: +61 7 3217 1844

C5

PROFICIENCY TESTING AUSTRALIA
ALGAE PROFICIENCY PROGRAM ROUND 15 - NOVEMBER 2012
RESULTS SHEET

Laboratory Code

(i) IDENTIFICATION AND ENUMERATION – SAMPLE A and B

SAMPLE A (Table for the identification and enumeration of the two most dominant cyanobacteria)

Organism	Name of Genus enumerated[1]	Magnification	Total no. of units/cells counted		No. of replicate counts[3]			Estimate of cells/filaments[2]	Cells mL^{-1}	MU
			cells	filaments[2]	transects	squares	fields of view			
1										
2										

SAMPLE B (Table for the identification and enumeration of the two most dominant cyanobacteria)

Organism	Name of Genus enumerated[1]	Magnification	Total no. of units/cells counted		No. of replicate counts[3]			Estimate of cells/filaments[2]	Cells mL^{-1}	MU
			cells	filaments[2]	transects	squares	fields of view			
1										
2										

[1] Identification to genus level is required.

[2] Only complete this column if the method used included an estimation of cells per filament

[3] Enter result for only one column (number of complete transects, squares or fields of view), whichever is appropriate

C6

PROFICIENCY TESTING AUSTRALIA
ALGAE PROFICIENCY PROGRAM ROUND 15 - NOVEMBER 2012
RESULTS SHEET

Laboratory Code

(ii) IDENTIFICATION – SAMPLE C

Sample C – Please report to genus level only

Diatoms (2)	
Dinoflagellate (1)	
Chrysophyte (1)	

C7

PROFICIENCY TESTING AUSTRALIA
ALGAE PROFICIENCY PROGRAM ROUND 15 - NOVEMBER 2012
RESULTS SHEET

Laboratory Code

IDENTIFICATION AND ENUMERATION – SAMPLE D (Marine sample)

SAMPLE D **(Table for the identification and enumeration of the three most dominant dinoflagellates)**

Organism	**Name of Species enumerated[1]**	**Magnification**	**Total no. of units/cells counted**		**No. of replicate counts[3]**			**Estimate of cells/filaments[2]**	**Cells mL^{-1}**	**MU**
			cells	filaments[2]	transects	squares	fields of view			
1										
2										
3										

[1] Identification to species level is required.

[2] Only complete this column if the method used included an estimation of cells per filament

[3] Enter result for only one column (number of complete transects, squares or fields of view), whichever is appropriate

C8

PROFICIENCY TESTING AUSTRALIA
ALGAE PROFICIENCY PROGRAM ROUND 15 - NOVEMBER 2012
RESULTS SHEET

Laboratory Code

IDENTIFICATION

Please provide any necessary comments relating to identification:

ENUMERATION

Please confirm the type of chamber used and its volume (mL)

Please provide details of method used (if applicable) to estimate cells in trichomes

Any comments relating specifically to the method used

PROFICIENCY TESTING AUSTRALIA
ALGAE PROFICIENCY PROGRAM ROUND 15 - NOVEMBER 2012
RESULTS SHEET

Laboratory Code

Date of sample receipt:

Date of Analysis:

Analysts name: (please print)

Signature:

Please return results NO LATER THAN **Monday 14 January 2013** to:

Ms Kathy Weller
Proficiency Testing Australia
PO Box 1122
ARCHERFIELD BC QLD 4108

Email: Kathy.Weller@pta.asn.au
Phone: +61 7 3721 7373
Fax: +61 7 3217 1844

---------- End of report ---------