The National Marine Biological
Analytical Quality Control Scheme
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Macroalgae/Angiosperms Component
Macroalgae and Seagrass % Cover Module
Report – OMC RT05 2014

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MACROALGAE/ANGIOSPERMS COMPONENT REPORT FROM THE CONTRACTOR SCHEME OPERATION –2013-14

1 Introduction

To enable correct water quality classification and good management decision-making, quality control of biological data is a high priority. This extends through all biological elements including macroalgae and seagrass. Good quality control ensures consistency of data being reported for management purposes, and for macroalgae and marine angiosperms this has been driven primarily by the requirements of the Water Framework Directive. This QC scheme aims to facilitate improvements in biological assessment whilst maintaining the standard of marine biological data. The scheme should help to ensure consistency between analysts with improved confidence in ecological quality status.

The National Marine Biological Analytical Quality Control (NMBAQC) Scheme addresses several issues relating to macroalgae and seagrass data collection, this report focuses on two of these:

- The estimation of % cover
- The comparison of methodologies

This is the fifth year in which % cover estimations of macroalgae have been included as an element of the NMBAQC scheme and the third year for which seagrass has been assessed as a separate entity. This included a single exercise for macroalgae and one for seagrass both of which were split into three smaller exercises based on methodology. The format followed that of previous years (RT01 – RT04). Test material was distributed to participating laboratories from which data forms were completed with macroalgae and seagrass % cover results and returned for analysis.

Thirteen laboratories were issued test material. Twelve laboratories completed the % cover macroalgae/seagrass component of the NMBAQC scheme with a total of 24 participants, one laboratory failed to submit any results. Of those laboratories submitting results, ten were government organisations and one was a private consultancy. To ensure consistency between scheme years, each participating laboratory was assigned the same laboratory code as in previous years, except where a laboratory was new to the scheme. Individual codes may, however, change slightly due to variations in individual participants. Due to the nature of the exercise there was no limit on the number of participants per lab.

Laboratories were able to complete the % cover test that best represented the methodology used within their laboratory to allow comparisons of methodology. However, the laboratories were encouraged to complete all three variations of both the macroalgae and seagrass exercise in order to facilitate comparisons of the methods.

Currently this scheme does not specify a definite qualifying performance level, and NMBAQC ring tests may be treated as training exercises. However, certain indicative targets have been applied to the assessment of the results based on Z-scores allowing “Pass” or “Fail” flags to be assigned accordingly; these may be used by competent monitoring authorities for internal monitoring of performance. These flags have no current bearing on the acceptability of data from such participating laboratories. Ring tests offer a means of assessing personal and laboratory performance from which continued training requirements may be identified or from which improvements in current field and laboratory procedures may be addressed.

1.1 Summary of Performance

This report presents the findings of the macroalgae/seagrass component for the fifth year of operation within the National Marine Biological Analytical Quality Control (NMBAQC) Scheme. This component consisted of one macroalgae and one seagrass exercise which was subsequently split into three alternative means of assessment which may be considered as separate modules from which laboratories could complete one or more module.

The analytical procedures of the exercise remained consistent with previous rounds of the scheme (OMC RT01 – RT04). The results for the exercise are presented and discussed with comments provided on the overall participant performance.

Two sets of fifteen quadrat photographs showing various % covers of opportunist macroalgae and seagrass were used for the exercise. These sets of photographs were duplicated to produce the three separate modules incorporating the different assessment methods utilised by the various participating laboratories. The set of quadrat photos differed by the use of grid squares of varying quantities; open quadrat, 10 x 10 square grid and 5 x 5 square grid. Each photo represented natural levels of opportunist macroalgae and seagrass cover.

Results for % cover of both opportunist macroalgae and seagrass varied between participants and between the different methods used. A number of results deviated from the sample mean and from the % cover as calculated by image analysis. However deviation from the latter was more noticeable. There was a much higher range of results submitted for seagrass which appears to be more difficult to estimate % cover and may be attributed, in part, to its patchy nature. Although there was a slight preference for using method B (10 x 10 square grid) for the macroalgae the results using this method was less accurate as seen in previous years. It was noticed that method B for both macroalgae and seagrass resulted in the greatest number of 'Fails' when compared against ImageJ % cover analysis due to overestimation of % cover.

2 Summary of Macroalgae Exercise

2.1 Introduction

There was one exercise for the assessment of % cover of macroalgae and one for seagrass, which took the form of three methodology options. This exercise is described in full below to include details of distribution and logistics, procedures for estimation of % cover, completion of test result forms and full analysis and comparison of final submitted results.

2.2 Description

This exercise examined the participants' ability to estimate accurately various levels of opportunist macroalgae and seagrass percentage cover. The exercise is able to determine the level of inter-laboratory variation and the degree of deviation from % cover estimations as calculated using image analysis software. It identifies areas of significant error, problematic coverage or mis-use of grid squares for aiding with estimations.

Three sets of 15 representative macroalgae and seagrass quadrat photos were distributed to each participating laboratory in January 2013. Participating laboratories were required to estimate the % cover of the opportunist macroalgae and seagrass using one or more of the methodologies provided. The nature of the photos was consistent with those provided for RT04 with the two overlying grid systems. Opportunist algae consisted of species of *Ulva* (previous known as *Enteromorpha*) and seagrass was identified as *Zostera noltii*.

2.3 Logistics

The test material was distributed on CD to each laboratory. Each disc contained the six tests, description of methods and data submission forms. Participants were given six weeks to complete the test and return the results; this was later extended to two months due to the severe weather conditions affecting several laboratories. There were no restrictions on the number of participants per laboratory.

Email has been the primary means of communication for all participating laboratories subsequent to the initial postal distribution of test material.

2.4 Preparation of the Samples

In order to assess the accuracy of determining % cover of opportunist macroalgae and seagrass, photographs were taken of quadrats overlying varying degrees of algae or seagrass cover. In total 15 representative photographs each of macroalgae and seagrass were taken by Wells Marine for the purpose of this exercise. Each photograph was ground-truthed at the time of collection with additional drawings of areal coverage produced on a grid scale to ensure % cover could be determined accurately subsequent to field analysis and during image analysis.

The two sets of 15 photographs were adapted to produce three tests of each component that utilised different methods of % cover estimation.

2.4.1 Method A

Method A was an open quadrat, this allowed the analyst to estimate the percent cover in a $0.25m^2$ quadrat without visual obstruction or assistance from gridlines. A general estimation is conducted looking solely at the total area within the quadrat that is clearly covered by the opportunist macroalgae or seagrass.

2.4.2 Method B

Method B consisted of a 9 x 9 crosshair quadrat. This method splits the quadrat into 100 squares. The crosshair referred to the point at which the gridlines cross and within a 9 x 9 grid amounts to a total of 81 crosshairs. The method of cover estimation was achieved by recording the presence or absence of algae/seagrass under each of the crosshair points. Where present this was recorded as 1 and absence was recorded as 0. The number of cross hairs with algae/seagrass present was divided by 81, and then multiplied by 100 to give a percentage.

2.4.3 Method C

Method C split the $0.25m^2$ quadrat into 25 squares with each square representing 4% of the total quadrat. The percent cover was estimated by counting the number of squares, to the nearest half square, that were covered by macroalgae/seagrass. Completely covered squares were counted as one each. Between 50% and 100% cover in individual squares was estimated to the nearest quarter and these portions were summed. For quadrats with sparse macroalgae cover (i.e. always < 50% cover per square) the participants accumulated the small portions of algal coverage (totalling to the nearest half square). The number of squares was divided by 25 and then multiplied by 100 to give a percentage.

2.5 Quadrat image analysis

An image analysis programme called ImageJ was used to achieve a more precise measurement of % cover which could be compared with the traditional means of assessment. The photographs were opened within the ImageJ program which distinguishes contrasts in colour/tone and is therefore able to compare the colour of the macroalgae against the background substrate. Prior to analysis the images were modified within photoshop to ensure a substantial colour contrast and enable the program to pick up the differences. ImageJ converts the colour image to a greyscale which is later changed into binary form to highlight the thresholds. The entire quadrat is calibrated against a known measurement scale from which the highlighted area can be spatially analysed. A percent cover is calculated using the area of macroalgae cover against the area of the quadrat as calibrated in ImageJ. These percentages were used as a comparison against the skilled eye estimations as submitted by the participants.

A full, impartial image analysis comparison was sought as part of the QC exercise. This was previously attempted using GIS but it was thought that this method did not provide a fully independent analysis of % cover. ImageJ is thought to be less subjective providing a more accurate analysis based on colour/tone contrast. Image analysis has been conducted to demonstrate how the comparisons would work, but may require further modification and discussion as to its applicability and accuracy, therefore cannot be taken as a finite measure of % cover.

2.6 *Analysis and Data Submissions*

A prepared results sheet was distributed with the exercise instructions to standardise the format in which the results were submitted. Each participant had the option of completing the test which most represented their own procedures but all participants were encouraged to complete all three tests of both macroalgae and seagrass to enable a comparison of methodologies and levels of accuracy achieved within each.

For each test the participant had to estimate the % cover of opportunist macroalgae/seagrass species only, excluding any additional species that were present within the quadrat and that were not considered to be either of these types of species. The assessment included a large degree of variation in % cover to represent the full range experienced within the field.

Spreadsheet based forms were distributed with the test material to standardise the format in which the results were submitted. These results will be retained and stored appropriately.

2.7 *Confidentiality*

To preserve the confidentiality of participating laboratories, each participant is allocated a four digit laboratory code from which they can identify their results. These codes are randomly assigned. The initial letters (MA) refer to the scheme this is followed by the scheme year which refers to the year in which the NMBAQC scheme original commenced, the final two digits represent the laboratory. For those laboratories where multiple submissions were provided the four digit code is followed by a letter allocated to each participant of that laboratory. For example, participant c from laboratory twelve in scheme year twenty one will be recorded as MA2112c.

2.8 *Results*

The results have been analysed using a number of different approaches to compare the results between participants, between the three different methods of estimation and to compare against ImageJ calculated % cover estimations for both macroalgae and seagrass.

2.8.1 General Comments

In total thirteen laboratories signed up for the % cover component of the macroalgae/seagrass element for RT05. Twelve laboratories returned data; one laboratory did not supply data or communicate their abstention. Of those laboratories that did submit data 17 completed method A, 22 completed method B and 13 completed method C for the macroalgae component. For the seagrass component 16 completed method A, 20 completed method B and 11 completed method C. Eleven participants completed all three macroalgae and ten completed all three seagrass methods. The results have been collated and represented in various formats to enable full comparisons between participants and against % cover as calculated by the image analysis.

Details of each participating laboratory performance were distributed in the macroalgae OMC RT05 Bulletin Report and the seagrass OMC RT05 Bulletin Report, which represent a summary of the results for RT05. The Bulletin provides ‘Pass’ and ‘Fail’ flags to each data set to highlight deviation from sample mean and actual results. Values of Z-scores were used to apply the ‘Pass’ and ‘Fail’ assessment.

Z-scores, calculated to indicate the level of deviation of % cover, used the following formula:

$$Z = \frac{X - \mu}{\delta}$$

X is a raw score to be standardized;

μ is the mean of the population;

σ is the standard deviation of the population.

Z-scores were calculated using the mean % cover and the image analysis % cover. A Z-score value of greater than +/- 2.0 was considered to be outside an acceptable limit of deviation from the mean. This value is considered standard practice and was used assign a ‘Fail’ or ‘Pass’ flag on the data.

2.8.2 Macroalgae Results from Participating Laboratories

2.8.2.1 Test A Results (open quadrat)

Test A consisted of 17 participants and was the second most popular of the three methods. The range of results per quadrat varied considerably with the largest range of results produced for quadrat 2, with a range of 38% from 32% to 70%. Quadrats 1, 4, 11, 12 and 13 all displayed a range of between 30% and 35%. The smallest range was for quadrat 14 from 90% to 100%, the remaining quadrats had % cover ranges of between 12 and 25. Z-scores calculated against the population mean resulted in seven laboratories failing just one quadrat and three laboratories failing between 2 and 5 quadrats. In total there was a 94% pass rate for test A when using Z-scores derived from the mean.

The deviation from % cover as calculated using ImageJ was much greater than seen when using the population mean. Participants showed an average % cover deviation from image analysis % cover ranging between 3.29% and 13.02%. The pass rate was equally much lower using Z-scores derived from image analysis estimates of % cover with 14 out of 17 labs failing at least one quadrat. The overall pass rate was lower at 85%. These results are consistent with those from RT04 with similar pass rates.

2.8.2.2 Test B Results (9 x 9 crosshairs quadrat)

Test B had the greatest number of participants with 22. As with test A there was a greater degree of correlation of % cover against population mean compared with the image analysis. A total of 68% of participants (15 out of the 22) consistently produced Z-scores of less than 2.0, which is regarded as a ‘pass’. The remaining 9 labs failed between 1 and 5 quadrats. The largest range of % covers per quadrat was a range of 85% cover recorded in quadrat 15 (it is unclear at this stage if this was a typing error or misuse of the methodology). The lowest range of % cover estimates was for quadrat 4 differing considerably from the results seen in test A.

Consistent with test A, test B also showed a higher degree of deviation from the image analysis results compared with the population mean, with all 24 participants failing at least one quadrat and an overall pass rate of only 68% compared with a pass rate of 96% using Z-score from the population mean. Laboratory MA2103c had the greatest number of 'fails', a total of 12 for comparisons against % cover from ImageJ. Method B also resulted in the highest level of deviation from % cover as calculated by ImageJ.

2.8.2.3 Test C Results (5 x 5 gridded quadrat)

A total of 13 participants opted to complete Test C using the 25 square method with varying levels of deviation from the population mean. This was the least popular of the estimation methods. The results verified that as with the other two test methods there was a higher degree of deviation when comparing results against the image analysis % cover as opposed to the population mean.

The average range of percentage covers per quadrat was 25.6%, much higher than in RT04 (18%), with quadrat 2 producing the highest range of 41%. Only four laboratories failed at least one quadrat using Z-scores from the mean with between 1 and 2 failures and an overall pass rate of 97%. There were also more 'Fails' using Z-scores from image analysis with between 1 and 9 'Fails' per person with six people receiving no 'Fails', and an overall pass rate of 85%.

2.8.3 Seagrass Results from Participating Laboratories

2.8.3.1 Test A Results (open quadrat)

Test A consisted of 16 participants. The range of results submitted per quadrat varied more considerably than with the macroalgae test. The largest range was for quadrat 1 with % cover estimates ranging from 35% to 80%, with most quadrats having a % cover range of between 30% and 40%. The smallest range was for quadrat 6 from 75% to 90%. Z-scores calculated against the population mean resulted in four people failing between 1 and 8 quadrats. In total there was a 94% pass rate for test A when using Z-scores derived from the mean.

When comparing results against % cover as calculated using ImageJ, the number of 'Fails' per laboratory was greater with a total number of 49 'Fails' (79.6 pass rate) with all labs failing at least one quadrat. The average deviation of results from ImageJ % cover per lab ranged from 5.56 to 20.54, similar to previous years. Quadrat 6 appeared to be the most problematic, with all participants 'Failing'. Most participants failed between 0 and 5 quadrats with one lab (MA2103b) failing 8 and 12 of the 15 quadrats when compared against the mean and ImageJ respectively.

2.8.3.2 Test B Results (9 x 9 crosshairs quadrat)

Test B had the greatest number of participants with a total of 20 participants opting to complete the open quadrat method, resulting in varying levels of deviation from the population mean. This test followed the same trend as the other tests for both macroalgae and seagrass with comparisons against image analysis resulting in a greater number of failures using the Z-score than when comparing against mean % cover. The range of % cover values showed a similar level of variation as described for test A with most quadrats having % cover ranges in the order of between 20% and 45% indicating a high level of discrepancy between participants. Quadrat 9 had the largest range of between 40% and 86%. Comparing against mean % covers resulted in a total of 9 'Fails' distributed between 4 labs with a pass rate of 97%. In comparison, the total number of 'Fails' using ImageJ was much higher at 57 and was distributed among 18 of the 20 participants. The overall pass rates using ImageJ was 81%. These results are consistent with RT04 with similar numbers of 'Fails'.

2.8.3.3 Test C Results (5 x 5 gridded quadrat)

Test C had the least number of participants with only 11 choosing to use the 25 squared quadrat method to estimate % cover. Unlike the previous years' results which were very consistent between the two methods of comparison with population mean and ImageJ analysis both resulting in a pass rate of 95% this year the results were less consistent between tests. However the range of results, between 21% and 34%, for each quadrat was not as high as seen with the other two methods.

Only one person failed one quadrat using Z-scores from the mean which is a much higher pass rate than seen with the other two methods. There were also more 'Fails' using Z-score from image analysis with a total of 24 'Fails', which were relatively evenly distributed between all labs. Only one person had a 100% pass rate.

2.9 Discussion

The % cover of opportunist algae in a 0.25 m^2 quadrat is usually estimated based on a skilled eye observation using either an open quadrat or gridded quadrat with +/- 5% agreement between surveyors. It is highly unlikely that this method of % cover estimation is 100% accurate due to the subjectivity of individuals, although over time people can become highly skilled. OMC RT04 used the population mean and an image analysis method to calculate a more precise % cover for comparison with individual participants' records. There are difficulties in obtaining 100% accuracy for % cover of opportunist algae or seagrass; however using the image analysis method should provide a lesser degree of subjectivity than skilled eye estimation. The ImageJ program is able to select areas of cover based on the colouration, identifying by depth of colour. Each of the quadrat photographs is enhanced prior to analysis using Photoshop to ensure maximum contrast between algae and substrate by selecting the areas of algal coverage and in this instance converting to a black and white scale. Once the two distinct colours have been identified within the ImageJ program it is able to calculate the total area covered thus reducing the degree of subjectivity experienced with skilled eye evaluations. During this fourth round of the macroalgae scheme photographs were also ground-truthed against actual presence of algae within the field to ensure the area of algae could be identified accurately within each quadrat thereby ensuring full calibration of the photographs.

Z-scores were used to establish a level of acceptance for results submitted by participants. These Z-scores used both the mean % cover per quadrat and the % cover as calculated by ImageJ. The results could then be compared between participants, and between method of cover estimation for both macroalgae and seagrass. The results generally show a higher level of consistency between participants when comparing with the population mean. This was apparent across all tests for both macroalgae and seagrass. In conjunction with this there were a greater number of Z-scores failures when comparing the image analysis % cover with the population mean of the quadrats. This is consistent with previous years. This indicates either a lack of accuracy in % cover estimations or inaccurate % cover results produced using ImageJ. The benefit of comparing participants' results against the mean is that it fully represents the range of results submitted and this is not the case for the ImageJ results. However the image analysis represents a less subjective % cover value.

The overall range of results submitted is still highly variable with some quadrat results ranging in excess of 85% indicating a high degree of participant error. For some participants this was more noticeable than others. Within the macroalgae test the high levels of deviation could be attributed to only a few people who had significantly greater number of 'Fails' than others. However this was less obvious with the seagrass test which showed a greater range of results and higher degree of variability. MA2102a recorded a particularly low % cover for quadrat 15 compared with others on Test B but not on Test A. Since the % cover estimation was so far from the actual cover it is questionable whether this was an unintentional mistake especially as other results from this participant showed little deviation. The level of success rate for individuals was not completely consistent between tests with the greatest number of 'Fails' for each test being attributed to different people, however some people regularly produced a higher deviation from the mean and ImageJ results than other labs (MA2103)). As with previous years this provides some evidence that different methods of % cover estimation provide varying levels of success for the different participants, making it difficult to conclude which method is the best in terms of producing the most accurate result. It seems this is highly dependent upon the person.

The degree of deviation from the image analysis % cover value depended significantly upon the quadrat. Some quadrats were more problematic than others; this was consistent with the range of % cover and could be partly attributed to the more patchy coverage of opportunist algae in some quadrats which is much harder to estimate accurately. It is evident, as in previous years, that those quadrats with a mid percent cover range generally resulted in a higher level of deviation (Figures 1 and 2) with less consistency between estimates. Those quadrats with either a very high or low percent cover appeared much easier to estimate accurately total cover. This was consistent across both seagrass and macroalgae. This trend was most significant within the seagrass tests with the exception of seagrass quadrat 6 which was consistently problematic for all participants, however with an estimated % cover of 64.36 this is consistent with the mid range problem area.

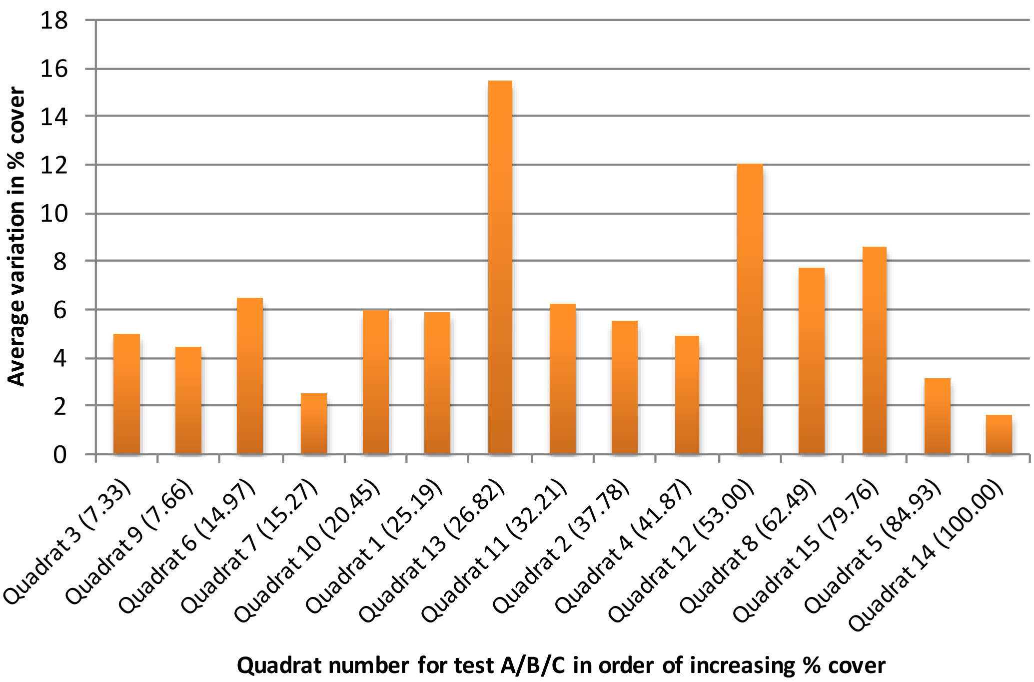

Figure 1: Average deviation from % cover of macroalgae as estimated using ImageJ

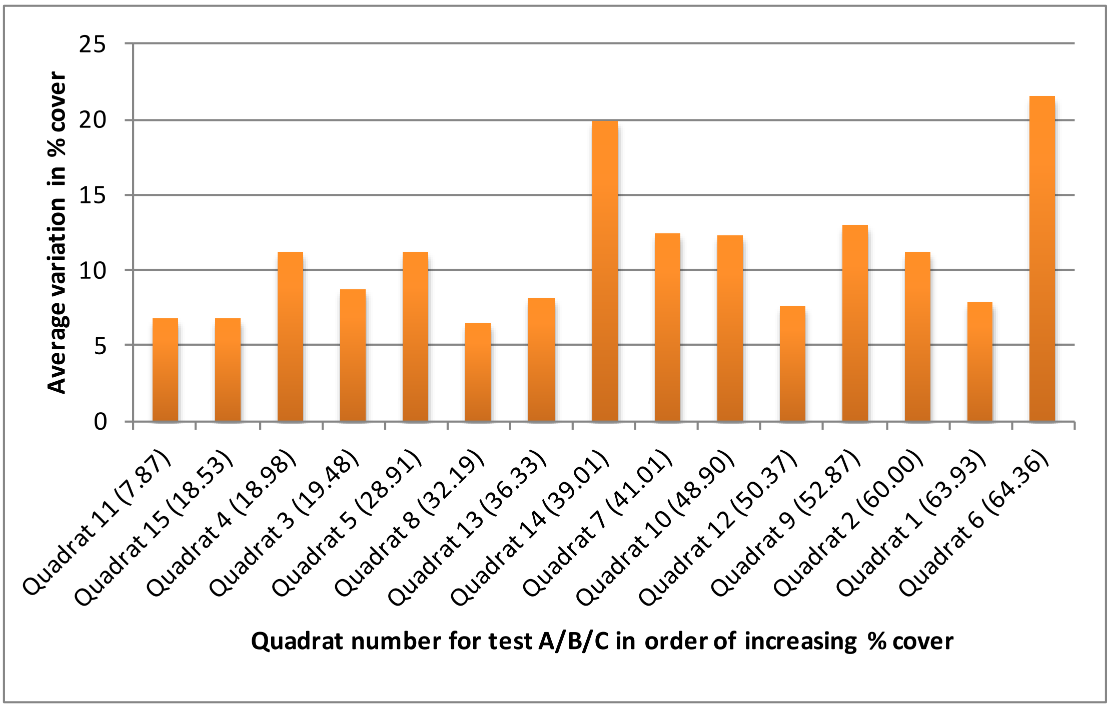

Figure 2: Average deviation from % cover of seagrass as estimated using ImageJ

There were some differences between the methods of estimation used. Both the macroalgae and seagrass tests showed fewer 'Fails' in test C (25 squares) when comparing against Z-scores from ImageJ and when comparing Z-scores from the population mean, for which seagrass only recorded 1 'Fail'. Test B (100 squares) produced the least favourable results for both tests when comparing Z-scores from ImageJ and in contrast produced the most favourable results when comparing against the sample mean. In general the pass rate using Z-scores against image analysis showed a much higher number of 'Fails', in total this amounted to 171 and 130 within the macroalgae and seagrass tests respectively. This was significantly higher than when results were compared against the sample mean producing a total of 34 and 24 'Fails' for the macroalgae and seagrass respectively. Comparing the average % covers across all quadrats for each of the tests (Table 1) shows that test B (100 squares) results in a higher % cover than for the other two tests. This suggests that this method is over estimating the actual % cover. This is also seen in the level of deviation from the image analysis results which again is much higher compared with the other methods of % cover estimation; this is also consistent with previous years.

Table 1: Mean % cover and deviation from image analysis % cover for tests A, B and C for Macroalgae and Seagrass.

	Test A		**Test B**		**Test C**	
	Mean % cover	Deviation from image analysis	Mean % cover	Deviation from image analysis	Mean % cover	Deviation from image analysis
Macroalgae	44.6	-3.9	46.9	-6.3	44.4	-3.8
Seagrass	42.68	7.37	48.67	10.39	44.7	7.7

The preferred test method is unclear although a greater number of participants completed test B with both macroalgae and seagrass. This is also consistent with previous years and suggests this is the method most used by laboratories in the field. Most noticeable was the much higher number of 'Fails associated with test B when comparing against the ImageJ analysis compared with all other tests. There is no definite explanation for this at present; it is possible ImageJ responds better to the seagrass cover then macroalgae cover. However, the range of results was also much higher for seagrass than for macroalgae, indicating a higher degree of variability between participants. Seagrass is a lot patchier than macroalgae and can be much harder to estimate % cover, therefore the higher range of results contributing to an overall higher standard deviation would lessen the risk of achieving a 'Fail', based on the Z-scores. It is clear that these results along with those from previous years require further examination in order to improve the methodologies employed and the means in which the % cover is calculated both by field method and image analysis.

3 Conclusions and Recommendations

1. There is evidently still a high degree of difference between tests as well as between participants and this may prompt the need for a specific workshop whereby methods can be discussed and possibly % cover estimations compared in the field. It is not possible from the current ring test to conclude which % cover estimation method provides the most accurate results, however it is evident through the number of participants that during RT05 Test B 32 was the most favoured method for macroalgae and seagrass.

2. The image analysis method used during RT05 is considered more objective than skilled eye estimation and likely to produce a more accurate result; RT05 also incorporated ground truthing to pick up subtleties of variations in cover within the defined affected area. However, this method is still under development and will continue to undergo improvements prior to the next round of tests. Despite this round incorporating a fully classified and ground truthed image analysis method with more accurate results it is recommended at this time that participants should use the Z-scores derived from comparisons with the mean if they are required for internal quality reports.

3. During this fifth cycle of the macroalgae % cover exercise an extension of time was allowed to account for the unpredictable weather affecting some laboratories, however, one laboratory still failed to submit results. All laboratories should continue to submit results within the requested deadlines as detailed at the beginning of the exercise. This is in both their own interests, and brings greater benefit to all participants in the scheme by increasing the dataset. In subsequent years reminders will continue to be distributed two weeks prior to the completion of the exercise to ensure the deadline is met, with a further reminder one week prior to the deadline. Any results submitted outside of this deadline will not be accepted and will not be included in the analysis.

4. Following consultation with current participants, it has been agreed that the tests are being distributed at the most appropriate time of year for the majority of labs, with a longer time scale within which to complete the exercises. Therefore tests will continue to be distributed early in the New Year with a time limit of 6 weeks. It will remain the responsibility of the laboratory to ensure all results are submitted within the time provided.

5. It is accepted that during field sampling it may be possible to estimate % cover of opportunist algae with a higher degree of accuracy than when using photos. The nature of the photographs can produce difficulties when assessing the density of the algae and the presence of some shadows and the grids can hinder this further. This point has been highlighted by a couple of labs and in subsequent tests further efforts will be made to ensure this doesn't hinder the ability to accurately estimate the % cover. However, it is to be noted that many seagrass beds remain waterlogged regardless of tidal height. It is equally accepted that sometimes it is difficult to accurately count algal cover when obscured under cross hairs, this would not be an issue in the field, but cannot be prevented within the test, therefore it remains important to include the open quadrat test method for a full view of the quadrat. There was no comment this year over the range of % covers included in the test so it is assumed that these were acceptable.

6. This year there was good approval on the current methods of estimation used and the descriptions provided, therefore no further methods will be considered at this time for future tests. However, some labs highlighted the problems associated with counting presence of algae under cross hairs and that this method can often over estimate the % cover. This has also been illustrated in the results in which test B consistently has higher % covers than the other two tests. This is something that is worth considering if this is your laboratory's preferred method.

   The methods that are currently included within the ring test were those considered to be most frequently used. It is agreed that where laboratories use alternative methods such as subtidal quadrat % cover estimations these methods may not accurately represent their commonly used procedures. However, by completing all three methods for both seagrass and macroalgae it is still possible to compare results with other laboratories in order gauge the level of accuracy.

7. As many laboratories take quadrat photos whilst estimating % cover for in house quality control, it has been suggested that a reverse ring test could be included in the % cover component. This would enable laboratories to submit their own quadrat photos for analysis. This still remains to be discussed for inclusion in future ring tests.

8. **Due to the presence of some anomalies within the results submitted it is recommended that all laboratories review their data prior to submission.** Such anomalies can skew the results and fail to recognise any small deviations from the mean; they can also cause the mean to be exceptionally high or low also affecting the outcome of other laboratories. Such data may be rejected as outliers. Care should also be taken to ensure the results are in the correct format and page within the spreadsheets provided.

9. Comment has been made on the order of the three tests and that currently it is inconsistent between documents. This has already been amended for subsequent years.

If anyone has further thoughts on this, or disagrees with any of the interpretation, please pass forward your comments to Dr Emma Wells (emma@wellsmarine.org) or Dr Clare Scanlan (clare.scanlan@sepa.org.uk). This ring test is now in its fourth year and although it has general approval we are still very happy to receive feedback particularly suggestions on how it may be improved.