The National Marine Biological
Analytical Quality Control Scheme
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Macroalgae/Angiosperms Component
Macroalgae and Seagrass % Cover Module
Report – OMC RT06 2015

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MACROALGAE/ANGIOSPERMS COMPONENT REPORT FROM THE CONTRACTOR SCHEME OPERATION –2014-15

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. The Healthy, Biologically Diverse Seas Evidence Group (HBDSEG), part of UK Marine Monitoring and Assessment Strategy, sets the key areas for UK agencies in which this external quality control is particularly needed. For 2015/2016 the components are: Benthic invertebrates, Fish, Particle Size Analysis, Macroalgae/Seagrass, Phytoplankton, Epibiota and Zooplankton. 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, and this report focuses on two of these:

- The estimation of % cover (macroalgae and seagrass)
- The comparison of methodologies

This is the sixth year in which % cover estimations of macroalgae have been included as an element of the NMBAQC scheme and the fourth 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 – RT05). 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. All laboratories completed the % cover macroalgae/seagrass component of the NMBAQC scheme with a total of 38 participants. Of those laboratories submitting results, all were government organisations. 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.

Data for macroalgal blooming and seagrass are currently used in relation to WFD classification and assessments for other Directives/purposes. In the UK at present they are not reported through a national database such as Merman; consequently they do not have definite national qualifying performance levels. They may be treated as training exercises. However, certain indicative targets have been applied to the assessments of the results based on Z-scores allowing “Pass/Fail” flags to be assigned as appropriate. 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.

2 Summary of Performance

This report presents the findings of the macroalgae/seagrass component for the sixth 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 two biological quality elements are treated in the same report, as the methods of assessment are the same, so are covered by the same ring test module.

The analytical procedures of the exercise remained consistent with previous rounds of the scheme (OMC RT01 – RT05). 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 replicated to produce the three separate options incorporating the different quadrat 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, 5 x 5 square grid and 9 x 9 cross hairs. 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 slightly wider range of results submitted for seagrass which appears to be more difficult to estimate % cover for, and may be attributed, in part, to its patchy nature. Test B (5 x 5 square grid) had the least number of results submitted with most laboratories indicating a preference towards tests A and C. Method B also resulted in fewer 'Fails' against both the mean and the image analysis % cover for both seagrass and macroalgae tests. Method C had the greatest number of 'Fails' which was highest for % cover estimates when compared against image analysis % cover results for both macroalgae and seagrass. Although there was a slight preference for using method C (9 x 9 cross hairs) for both macroalgae and seagrass the results using this method were less accurate than seen in previous years. It was noticed that methods A and B for both macroalgae and seagrass resulted in both over and underestimates of % cover when compared against image analysis % cover, however Method C resulted in consistent over-estimation of % cover.

3 Summary of Exercise

3.1 Introduction

The macroalgae and seagrass % cover 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.

3.2 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, or the mean of participants' estimates. 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 early January 2015. 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 RT05 with the two overlying grid systems. Opportunist algae consisted of species of *Ulva* (previous known as *Enteromorpha*) and seagrass was identified as *Zostera noltii*.

3.3 Logistics

The test material was distributed on CD to each laboratory. Each disc contained the six tests, description of methods and data submission forms, plus a feedback form. Participants were given six weeks to complete the test and return the results. 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.

3.4 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 (Macroalge/Angiosperms); 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 two will be recorded as MA2212c.

3.5 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.

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

3.5.1 Method A (open quadrat)

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.

3.5.2 Method B (5 x 5 square grid)

Method B 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.

3.5.3 Method C (9 x 9 cross hairs)

Method C consisted of a 10 x 10 grid square quadrat. This method splits the quadrat into 100 squares. The crosshair referred to the point at which the gridlines cross and within a 10 x 10 grid amounts to a total of 81 crosshairs (9 horizontal and 9 vertical lines). 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.

3.6 Quadrat image analysis

The image analysis programme, ImageJ, was used to achieve a more objective 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 fully objective image analysis comparison was sought as part of the QC exercise as it should be less subjective than visual estimation by 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, and therefore cannot be taken currently as a definitive measure of % cover.

3.7 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. The robustness of statistical analysis of results is dependent to some extent on the number of results returned, so it was in the interests of all participants to return a full set of results.

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. 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.

3.8 Analysis

The macroalgae and seagrass % cover exercise provides ‘Pass’ and ‘Fail’ flags to each data set to highlight deviation from sample mean and image 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.

4 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.

4.1 General Comments

In total thirteen laboratories signed up for the % cover component of the macroalgae/seagrass element for RT06 with all laboratories returning data. Of those laboratories that did submit data 31 completed method A, 16 completed method B and 27 completed method C for the macroalgae component. For the seagrass component 30 completed method A, 16 completed method B and 22 completed method C. Fifteen participants completed all three macroalgae and fifteen 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 RT06 Bulletin Report and the seagrass OMC RT06 Bulletin Report, which represent a summary of the results for RT06.

4.2 Macroalgae Results

4.2.1 Test A Results (open quadrat)

Test A consisted of 31 participants and was the 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 50% from 35% to 85%. Quadrats 7, 10 and 14 all displayed a range of 40%. The smallest range was for quadrat 4 and ranged from 1% to 7%, the remaining quadrats had % cover ranges of between 12% and 35%. Z-scores calculated against the population mean resulted in 8 participants failing just one quadrat, one participant failed 2 quadrats and one participant failing 10 quadrats. In total there was a 96% 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 with a total of 43 failed quadrats and an overall pass rate of 91%. Twenty three participants failed between 1 and 9 quadrats. Participants showed an average % cover deviation from mean and image analysis % cover ranging between 2.95% and 15.9% and 4.08% and 14% respectively. These results are consistent with those from RT05 with similar pass rates. The average deviation in %

cover estimates against image analysis % cover was 0.88 suggesting a relatively good agreement between the two methods.

4.2.2 Test B Results (5 x 5 gridded quadrat)

Test B had the smallest number of participants with only 16. The largest range of % cover per quadrat was a range of 32% cover recorded in quadrat 3. The lowest range of % cover estimates was for quadrat 4 with a range of only 3%. As with test A there was a greater degree of correlation of % cover against population mean compared with the image analysis. Seven participants produced Z-scores of less than 2.0, which is regarded as a 'pass' and failing between 1 and 3 quadrats and an overall pass rate of 95%.

Consistent with test A, test B also showed a higher degree of deviation from the image analysis results compared with the population mean, with 12 of the 16 participants failing at least one quadrat and an overall pass rate of 87.5%. The levels of deviation for individual participants were similar between comparisons against the mean and against image analysis % cover. The average deviation of mean from image analysis % cover was 2.11 with a greater number of quadrats resulting in estimated over that of the image analysis results.

4.2.3 Test C Results (9 x 9 cross hairs)

A total of 27 participants opted to complete Test C using the 100 square grid method. This method also had varying levels of deviation from the population mean. 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 total number of 'Fails' was considerably lower for comparisons against mean than against image analysis results with a total of 17 and 115 and pass rates of 96% and 72% respectively. Only three participants out of the 27 received no 'Fails' using z-scores against image analysis. The % cover ranges were more consistent between quadrats with most having a range of between 20% and 30%. The smallest range was also for quadrat 4 with estimates between 1.2% and 5%. The mean % cover for each quadrat was consistently greater than the image analysis with an average deviation per quadrat of 8.25, this is considerably higher than for tests A and B and suggests this test results in generally higher levels of % cover estimates.

4.3 Seagrass Results

4.3.1 Test A Results (open quadrat)

Test A consisted of 30 participants and as with the macroalgae this was the most popular method. The range of results submitted per quadrat varied considerably as with the macroalgae test. The largest variation was for quadrats 1, 2, 3, 8, 10 and 14 all with between 40 and 45 percent ranges, these quadrats all had image analysis % cover results of between 30% and 50% cover suggesting this to be a difficult level of cover to estimate. No quadrats had a particularly small range of results even for those with very little cover. Z-scores calculated against the population mean resulted in nine people failing between 1 and 5 quadrats. In total there was a 95% 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 70 'Fails' (85% pass rate) with all except four labs failing at least one quadrat. The average deviation of results from image analysis % cover per lab ranged from 5.41 to 14.2, which was also similar to the average deviation per participant when derived from the mean (2.57 to 13.92). Quadrat 12 appeared to be the most problematic, with 13 participants 'Failing' against image analysis % cover. Overall across all quadrats the deviation between the mean quadrat % cover and that calculated by image analysis was -0.16with some quadrats having a mean above and some below that of the image analysis % cover.

4.3.2 Test B Results (5 x 5 gridded quadrat)

Test B had the smallest number of participants with a total of 16 participants opting to complete the 5 x 5 square grid 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 40% indicating a high level of discrepancy between participants. Quadrat 8 had the largest range of between 15% and 72%. Comparing against mean % covers resulted in a total of 14 'Fails' distributed between 5 labs with 10 'Fails' being attributed to a single participant and an overall with a pass rate of 94%. In comparison, the total number of 'Fails' using image analysis was higher at 33 and was distributed among 14 of the 16 participants. The overall pass rates using image analysis % cover was 86%. These results are consistent with RT04 and RT05 with similar numbers of 'Fails' and pass rates.

Consistent with method A the overall deviation between the mean quadrat % cover and that calculated by image analysis across all quadrats was quite small at -0.60 with some quadrats having a mean above and some below that of the image analysis % cover.

4.3.3 Test C Results (9 x 9 cross hairs)

Test C had a total of 22 participants. Similar to method B most quadrats had % cover ranges in the order of between 20% and 40% indicating a high level of discrepancy between participants again with quadrat 8 having the largest range of between 16% and 74%. As with method C in the macroalgae test this one also had large difference in the pass rate between z-scores from mean and z-scores from image analysis. Comparison of results against the mean resulted in 13 'Fails' where as comparing results against the image analysis resulted in 84 'Fails' with pass rates of 96% and 75% respectively.

Only 6 participants failed between 1 and 7 quadrats using Z-scores from the mean compared with all participants 'Failing' at least one quadrat when using z-scores from image analysis. The most number of 'Fails' per participant was also relatively high with a total of 10.

5 Discussion

The % cover of opportunist algae/seagrass 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 RT06 used the population mean and an image analysis method (ImageJ) to calculate a more objective % cover for comparison with individual participants' records. The ImageJ program is able to select areas of cover based on the colouration, identified 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.

Z-scores were used to establish a level of acceptance for results submitted by participants. These Z-scores used either the mean % cover per quadrat or 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. Reasons for the discrepancy are being considered.

The overall range of results submitted is still highly variable with some quadrats having estimated ranges in excess of 50% indicating a high degree of participant error. For some participants this was more noticeable than others. 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 others. 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 participant.

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, and particularly seagrass, 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 total cover accurately. This trend is more evident from the macroalgae quadrats. For seagrass the lower % cover ranges clearly show a lesser degree of deviation between the mean and the image analysis but this is not so apparent for the higher % cover ranges where there is a high degree of scatter. This is likely to be due to the nature of seagrass which is often thin and patchy with long strands making it difficult to estimate the % cover.

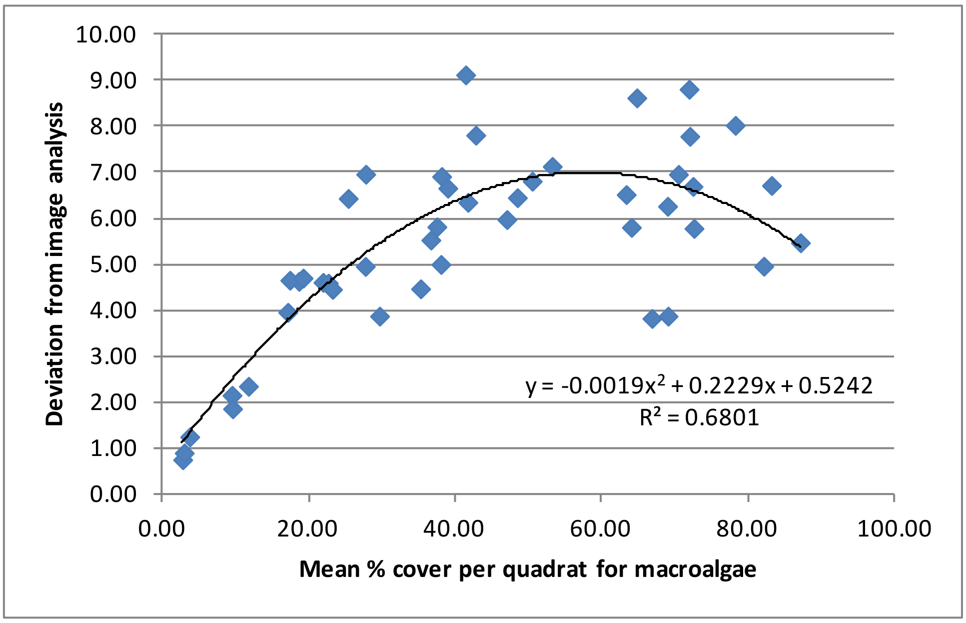

Figure 1: Deviation of mean macroalgae % cover per quadrat from image analysis across all three test methods with polynomial trend line (deviation is calculated as an average of all participants deviation from the image analysis).

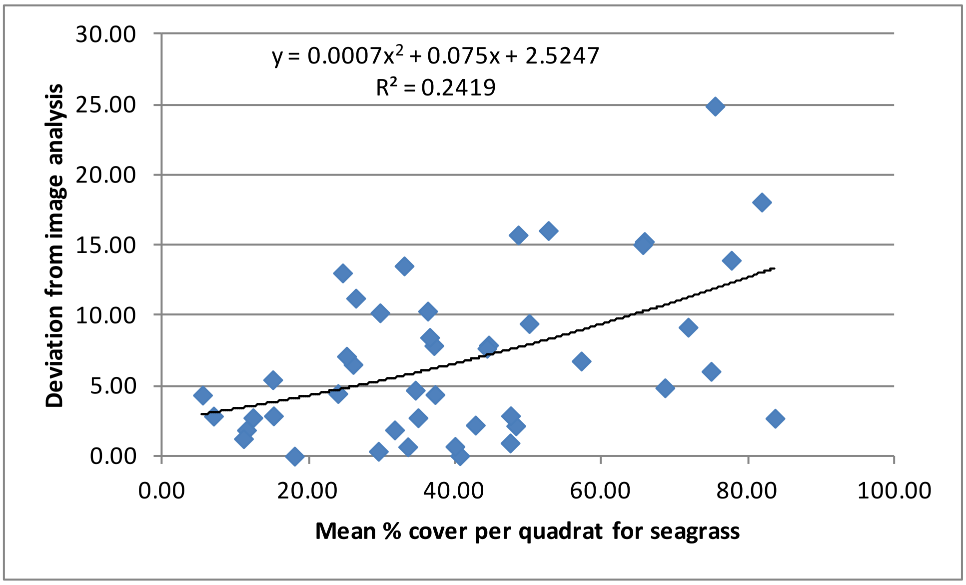

Figure 2: Deviation of mean seagrass % cover per quadrat from image analysis across all three test methods with polynomial trend line (deviation is calculated as an average of all participants deviation from the image analysis).

Figures 3 and 4 below include data for all tests and all years; RT01 – RT06 for macroalgae and RT03 – RT06 for seagrass, whereby the level of deviation was calculated from the mean against the image analysis and not as an average of participants degree of deviation as in Figures 1 and 2 above. A similar trend can be seen albeit with a much higher degree of scatter. It is evident that the level of deviation is strongest on those quadrats with a mid range of % cover which is seen by the large degree of scatter. Those quadrats with little cover show less deviation between the mean and image analysis. It is also evident that much of this scatter is attributed to test method C (100 square grid or 81 crosshairs quadrat) and this is apparent for both the seagrass and macroalgae.

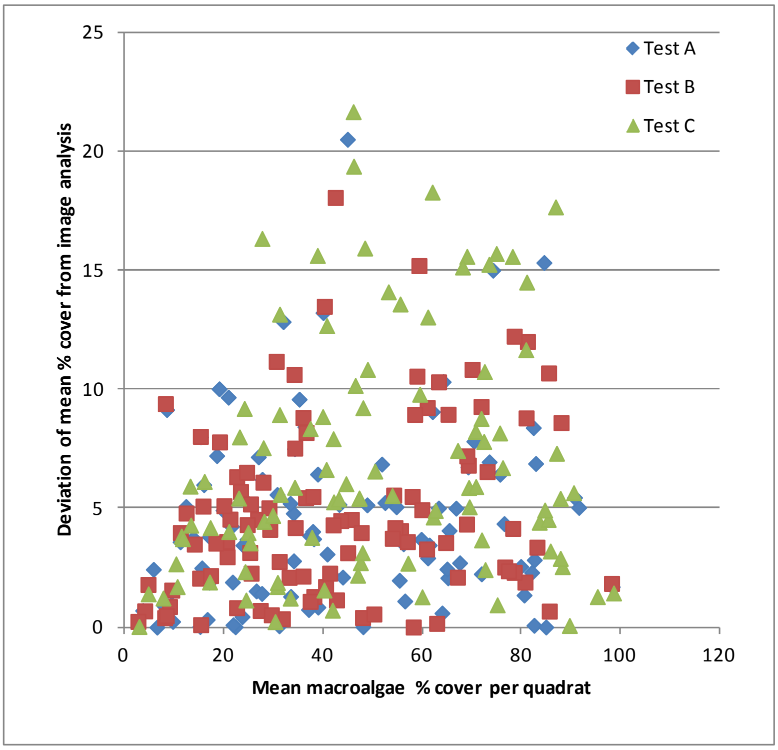

Figure 3: Deviation of mean macroalgae % cover from image analysis all three test methods and all years of data.

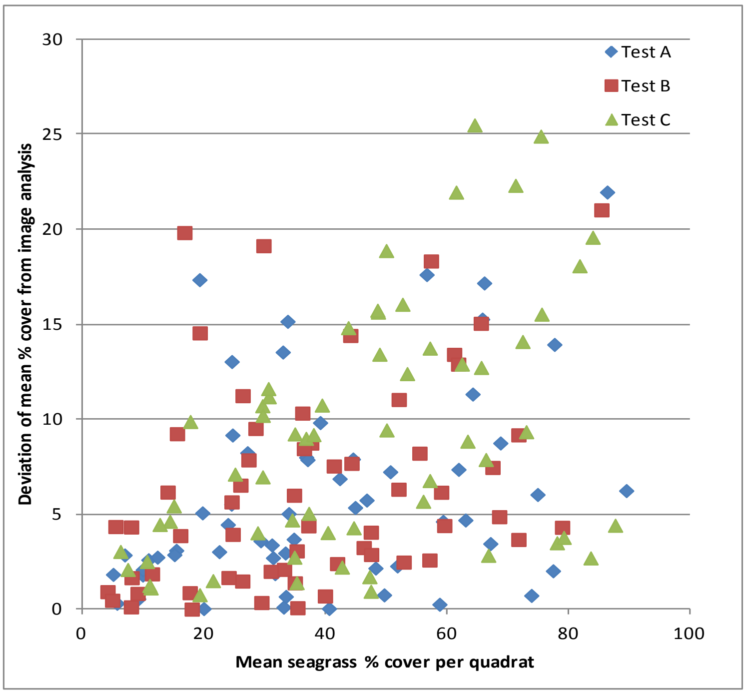

Figure 4: Deviation of mean seagrass % cover from image analysis all three test methods and all years of data.

There are also noticeable differences between the different methods of estimation used. Both the macroalgae and seagrass tests showed fewer 'Fails' in test B (5 x 5 square grid) when comparing against Z-scores from ImageJ and when comparing Z-scores from the population mean. However these also had the fewest participants making the data slightly less statistically robust. Test C (9 x 9 cross hairs) continues to produce the least favourable results for both macroalgae and seagrass when comparing Z-scores from ImageJ but the pass rate is similar to Test A when comparing against the population mean.

In general the pass rate using Z-scores against image analysis showed a much higher number of 'Fails', in total this amounted to 188 and 183 within the macroalgae and seagrass tests respectively. This was significantly higher than when results were compared against the sample mean producing a total of 47 and 48 'Fails' for the macroalgae and seagrass respectively. This number of 'Fails' is also higher than for previous years suggesting a difficult test in terms of % cover ranges. This trend is also apparent across all years with image analysis z-scores consistently resulting in a higher number of 'Fails' compared with z-scores from the mean with the greatest number of 'Fails' consistently being recorded from test C (9 x 9 cross hairs) (Figures 5 and 6)

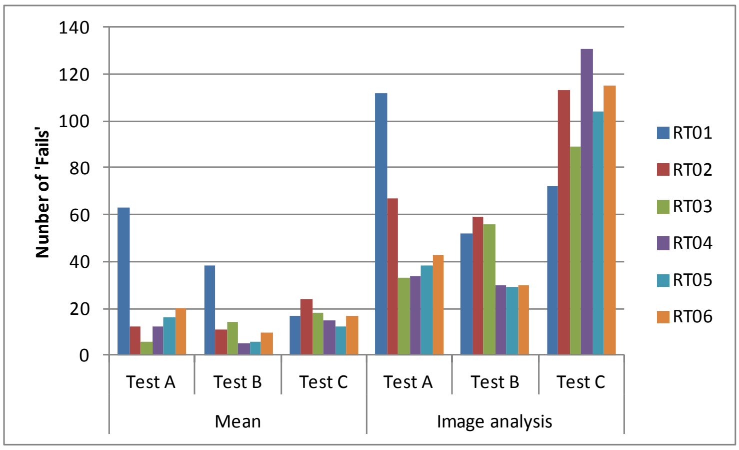

Figure 5: Number of 'Fails' recorded for each macroalgae test method from ring tests RT01 through RT06.

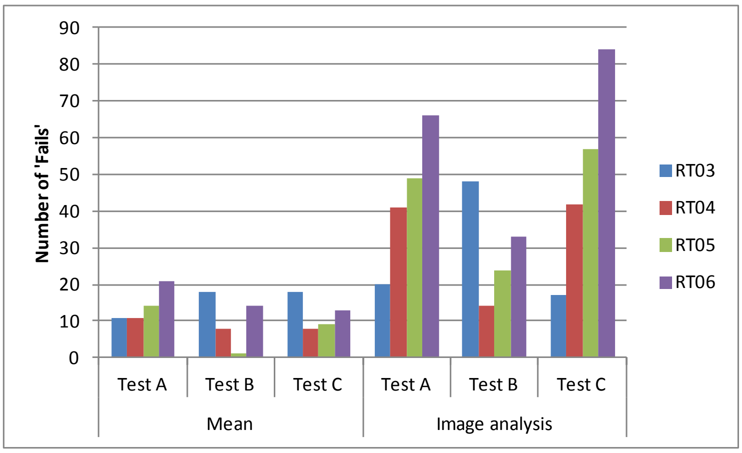

Figure 6: Number of 'Fails' recorded for each seagrass test method from ring tests RT03 through RT06.

Comparing the mean % covers across all quadrats for each of the tests (Table 1) shows that test C (9 x 9 cross hairs) results in a higher % cover than for the other two tests for both macroalgae and seagrass. 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 (Figures 7 and 8).

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	39.93	0.88	41.17	2.11	47.31	8.25
Seagrass	37.92	-0.16	37.48	-0.60	45.99	7.91

Figure 7: Deviation of mean macroalgae % cover from image analysis for all test methods and scheme years.

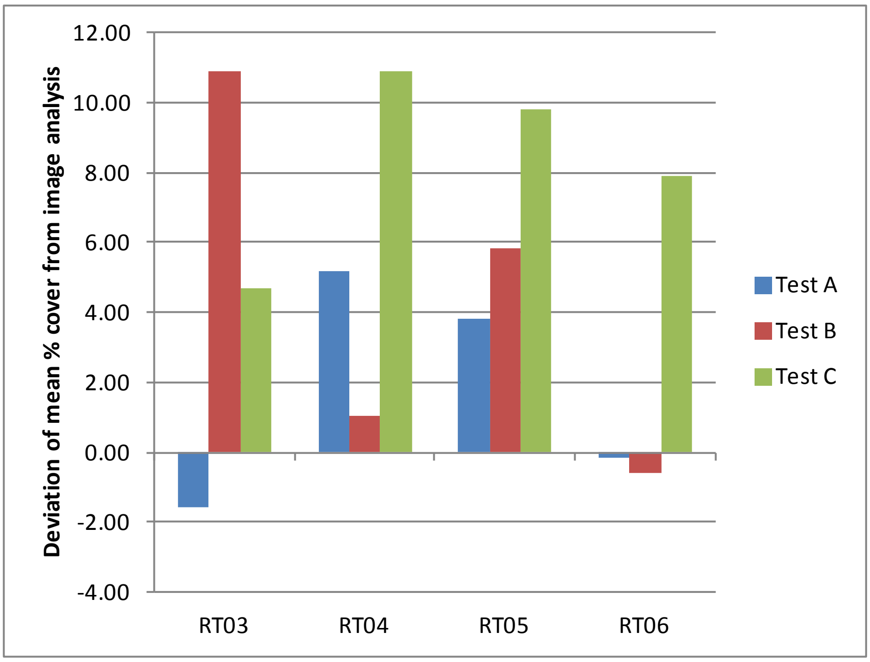

Figure 8: Deviation of mean seagrass % cover from image analysis for all test methods and scheme years.

Figures 7 and 8 above indicate the level of variability in deviation between the mean % cover and image analysis % cover. Test methods A and B show the image analysis neither over nor underestimates the % cover compared with the mean speculating that it is dependent upon the test material and general levels of % cover. However test method C provides clear evidence that the mean % cover estimated by participants is consistently higher than that of the image analysis suggesting that this method overestimates the % cover.

The preferred test method is unclear although a greater number of participants completed tests A and C 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 C 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.

6 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 Tests A and C were the most favoured methods for macroalgae and seagrass.

2. There is still a high level of difference between z-scores calculated from the mean and z-scores calculated from image analysis results and given the varied levels of deviation between the two it is unclear which is the most accurate method. Tests A and B neither over nor underestimate % cover when compared with image analysis instead it varies between quadrats. In contrast test C method results are consistently above those of the image analysis. It could be speculated that because distribution is random and often quite stringy it can regularly sit under the cross hairs even when there isn't much algae present resulting in over-estimated % cover. This will to be investigated using all data at some stage in the future.

3. Image analysis should be more objective than skilled eye estimation and likely to produce a more accurate result; RT06 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 image analysis method with more accurate and objective 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.

4. During this sixth cycle of the macroalgae % cover exercise all laboratories completed the ring test within the allocated time period. 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 allowing results to be distributed on time. 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.

5. 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.

   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. However attempts will be made for subsequent ring tests to make the grids opaque to increase the level of visibility under the cross hairs. Participants can also zoom into photos to help check cross-hairs.

6. There was no comment this year over the range of % covers included in the test so it is assumed that these were acceptable.

7. This year there was good approval on the current methods of estimation used and the method descriptions provided. However there has been a request for the inclusion of sublittoral photos of both macroalgae and seagrass, this is something that will be discussed for possible future ring tests.

8. Some labs highlighted the problems associated with counting presence of algae under cross hairs and that not only can this method often over estimate the % cover but that it is considered fairly inaccurate when compared with a visual estimate of cover and would be impractical and time consuming in a field situation. 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.

9. 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.

10. 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 for 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.

11. Participants must use the spreadsheets provided to submit results; other formats will not be accepted. Each participant's results should be submitted on a separate sheet and exclude calculations. Where calculations or formulas are included there is greater chance of error when transferring data to a single spreadsheet and during subsequent data analysis.

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) and copy to Dr Clare Scanlan (clare.scanlan@sepa.org.uk). This ring test is now in its sixth year and although it has general approval we are still very happy to receive feedback particularly suggestions on how it may be improved. Participants are encouraged to use the feedback form provided with the exercises.