NMBAQC

NE Atlantic Marine Biological Analytical Quality Control Scheme

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Macroalgae/Angiosperms Component Macroalgae and Seagrass % Cover Module Report – OMC RT08 2017

Emma Wells
Wells Marine Surveys
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Email: emma@wellsmarine.org

wells marine

MACROALGAE/ANGIOSPERMS COMPONENT REPORT FROM THE CONTRACTOR SCHEME OPERATION –2016-17

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 North East Atlantic 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 eighth year in which % cover estimations of macroalgae have been included as an element of the NMBAQC scheme and the sixth 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 – RT07). Test material was distributed to participating laboratories from which data forms were completed with macroalgae and seagrass % cover results and returned for analysis.

Fourteen laboratories were issued test material. Twelve laboratories completed the % cover macroalgae/seagrass component of the NMBAQC scheme with a total of 40 participants. Of those laboratories submitting results, all ten 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 could 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 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.

The NMBAQC scheme was originally set up for benthic invertebrates data submission to the NMMP (National Marine Monitoring Plan) to determine that data were fit for submission to the scheme. Macroalgal/angiosperms data are not submitted to any such scheme. However, they are used for classification, so it’s important they are correct.

1.1 Summary of Performance

This report presents the findings of the macroalgae/seagrass component for the eighth 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 – RT07). 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 using grid squares of varying quantities; open quadrat, 5 x 5 square grid, and 10 x 10 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. In general the correlation between image analysis results and mean % cover was closer than had been in previous years, which may have resulted from improved image analysis methods. As in previous years the preferred method was Test A (open quadrat) with Test B being the least favoured method. However, test C still remains the least accurate with regards to the number of 'Fails'.

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 can 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 2017. 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 RT07 with the two overlying grid systems. Opportunist algae consisted of species of *Ulva*, 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 result. 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

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 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.

2.4.3 Method C

Method C 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.

2.5 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.

Prior to analysis the quadrat photos were edited and modified within photoshop to ensure a substantial contrast between the seagrass and macroalgae enabling ImageJ to pick up the differences.

The photographs were first edited in photoshop to remove debris and any other spots that may influence the overall % cover such as green shells. Photoshop is then able to highlight only those areas of green seagrass or macroalgae with the background remaining pale and indistinguishable. The photographs are then opened within the ImageJ program which distinguishes contrasts in colour/tone and is therefore able to compare the macroalgae against the background substrate.

ImageJ is used to convert the black and white quadrat photo 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 definite 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 four will be recorded as MA2412c.

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 fourteen laboratories signed up for the % cover component of the macroalgae/seagrass element for RT08. Twelve laboratories returned data. Two laboratories failed to submit data within this round of the ring test. Of those laboratories that did submit data 29 completed method A, 12 completed method B and 24 completed method C for the macroalgae component. For the seagrass component 35 completed method A, 14 completed method B and 20 completed method C. Fifteen participants completed all three macroalgae and thirteen 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 RT08 Bulletin Report and the seagrass OMC RT08 Bulletin Report, which represent a summary of the results for RT08. 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 29 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 quadrats 5, 6 and 14 with ranges of 27%, 26% and 35% respectively. The remaining quadrats had ranges between 10 and 23; these ranges are slightly lower than for the same test last year. Z-scores calculated against the population mean resulted in nine laboratories failing between 1 and 6 quadrats. In total there was a 95% pass rate for test A when using Z-scores derived from the mean which is consistent with previous years results.

Although the number of 'Fails' produced when calculating Z-scores against image analysis participants showed an average % cover deviation from image analysis % cover ranging between 3.25% and 10.28%. The deviation from mean % cover was very similar ranging between 2.21 and 10.52. However, the pass rate was substantially lower for image analysis z-score at only 80% with 24 out of

29 participants failing at least one quadrat. These results were also consistent with those from RT07 with similar pass rates.

2.8.2.2 Test B Results (5 x 5 gridded quadrat)

Test B had the least number of participants with 12. As with test A there was a greater degree of correlation of % cover against population mean compared with the image analysis. A total of 75% of participants (9 out of the 12) consistently produced Z-scores of less than 2.0, which is regarded as a 'pass'. Two of the remaining labs 'failed' 1 quadrat each and the remaining lab 'failed' a total of 9 quadrats. The largest range of % covers per quadrat was a range of 23% (for quadrats 1 and 14) and 24% (for quadrats 4 and 5). The remaining quadrats had ranges between 6% and 22%. As seen in test A also, these ranges were also slightly lower than previous years. The lowest range of % cover estimates were for quadrat 7 which had a % cover range of 6.

Consistent with test A, test B also showed a higher degree of deviation from the image analysis results compared with the population mean, with 11 out of 12 participants failing at least one quadrat and an overall pass rate of only 71% compared with a pass rate of 94% using Z-score from the population mean although this result is consistent with last year (RT07). The greatest number of 'Fails' could be attributed to quadrat 7, with 10 'Fails' followed by quadrat 15 with 8 'Fails'. For 11 out of 12 participants the levels of deviations stayed under 5% when calculated against the mean. The range of deviation was broader with image analysis with more participants resulting in greater than 5% deviation.

2.8.2.3 Test C Results (9 x 9 crosshairs quadrat)

A total of 24 participants opted to complete Test C using the 100 square method with varying levels of deviation from the population mean. Although not the preferred method this year, Test C had a high number of participants, which was consistent with previous years. 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 22.4%, lower than for RT07, but higher than for tests A and B. Two quadrats had % cover ranges above 30% (34.3 for quadrat 14 and 32.3 for quadrat 4). Of the remaining quadrats 8 had ranges between 20% and 30% and 5 between 10% and 20%. The range of results submitted for test C was higher than for tests A and B. Six participants failed at least between 1 and 4 quadrats with an overall pass rate of 96%. There were also more 'Fails' using Z-scores from image analysis with 13 participants failing 1 quadrats and a further 7 'failing' between 2 and 9 quadrats and an overall pass rate of 87%. Quadrat 13 had the greatest number of 'Fails' with 16 out of the 24 participants scoring higher than +/- 2.0, the remaining quadrats had between 1 and 5 recorded 'fails' except quadrat 10 which has no 'fails'.

2.8.3 Seagrass Results from Participating Laboratories

2.8.3.1 Test A Results (open quadrat)

Test A consisted of 35 participants and as with the macroalgae this was the most popular method. The range of results submitted per quadrat also varied considerably but overall were much higher than for the macroalgae test. The largest range was for quadrats 9 and 13 with % and quadrat 12 with 55% and as in previous years the greatest range of results were recorded for those quadrats with the mid range of % cover. The lowest range of results were for quadrats 4 and 10 with 20% cover ranges. Z-scores

calculated against the population mean resulted in 9 participants failing between 1 and 5 quadrats. In total there was a 96% 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'. However, the overall pass rate was 91% with 15 participants 'passing' all quadrats. This is a considerably better result than for RT07. Those quadrats with the highest number of 'Fails' were quadrats 2 and 15 with 9 and 8 'fails' respectively. The average deviation of results were similar between image analysis (3.26 to 16.38) and mean % cover (2.04 to 14.67). These results were consistent with previous ring tests.

2.8.3.2 Test B Results (5 x 5 gridded quadrat)

Test B had the least number of participants with a total of 14 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 were considerably lower than for test A with all quadrats having % cover ranges in the order of between 10% and 40%. Quadrat 15 had the largest range of between 14% and 54%. Quadrat 4 has the smallest range of just 10% between 2% and 12%. Comparing % covers against the mean resulted in just 8 'Fails' distributed between 2 labs and an overall pass rate of 96%. In comparison, the total number of 'Fails' using image analysis was higher at 21 and was distributed among all 12 of the 14 participants. The overall pass rates using image analysis % cover was 90%. These results are considerably better than the previous year.

The overall deviation from the mean quadrat % cover and that calculated by image analysis was also very similar with a deviation from the mean ranging from 2.66% to 13.49% and deviation from image analysis ranging from 3.87% to 11.35. This was also an improvement from RT07.

2.8.3.3 Test C Results (9 x 9 crosshairs quadrat)

Test C had a total of 20 participants. The % cover ranges for test c were also consistent with those from Tests A and B with most quadrats having a % cover range between 10% and 40%. However, quadrat 15 had a % cover range between 16% and 67% proving to be consistently the most problematic quadrat within all test methods. Comparison of results against the mean resulted in 19 'Fails' with 5 participants 'failing' between 1 and 6 quadrats and an overall pass rate of 94%. Comparing results against the image analysis resulted in 36 'Fails' with pass rates of 88% with all 7 participants passing all quadrats.

Most 'Fails' against image analysis could be attributed to quadrat 2 which had a total of 10 participants failing. Although quadrat 15 had the largest range of % cover estimates this did not result in a high number of 'fails' due to the large standard deviation. Deviation from image analysis % cover was much higher than for the other two test methods with a range of between 3.53 and 22.77.

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 RT08 used the population mean and an image analysis method (ImageJ) 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 less subjectivity than skilled eye estimation. 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. During this eighth 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 that is consistent between quadrats.

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 other labs. 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 % covers 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 trend is more evident from the macroalgae quadrats. For seagrass the lower % cover ranges clearly show less 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.

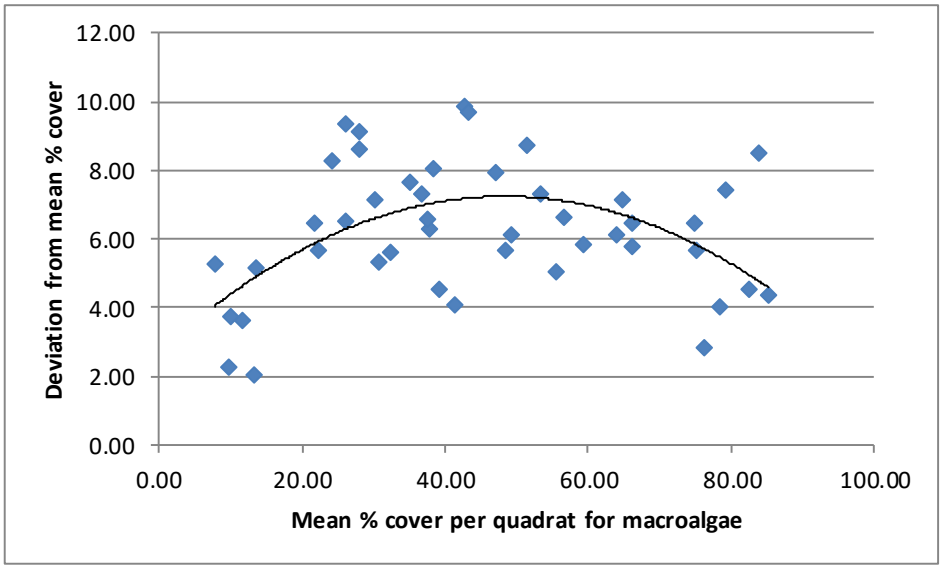

Figure 1: Scatter graph showing the mean level of deviation per quadrat from the mean % cover across all three test methods for macroalgae.

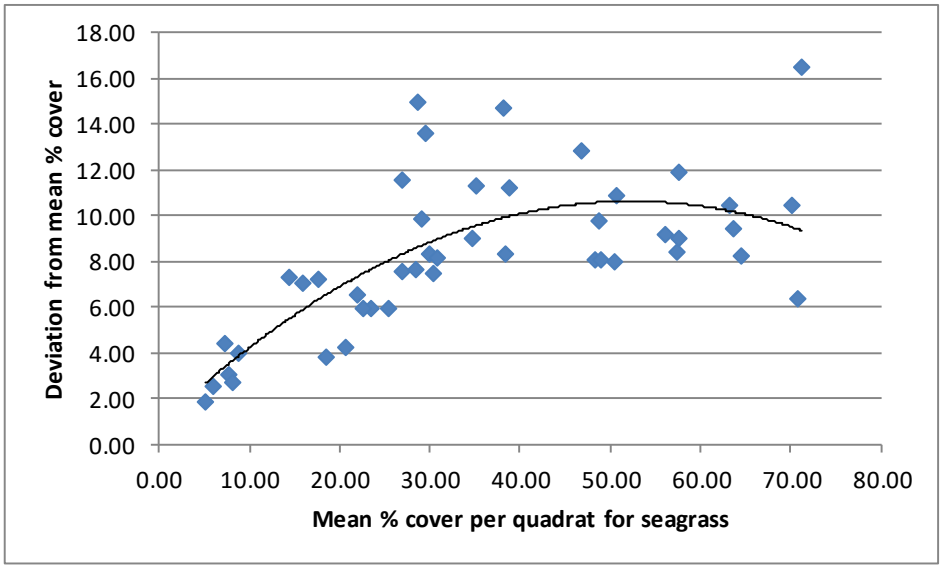

Figure 2: Scatter graph showing the mean level of deviation per quadrat from the mean % cover across all three test methods for seagrass.

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. IN previous years Test C (10 x 10 square grid) produced the least favourable results for both macroalgae and seagrass when comparing Z-scores from ImageJ but within the current ring test the pass rate has been better than that of Test A.

In general, the pass rate using Z-scores against image analysis showed a much higher number of 'Fails', in total this amounted to 187 and 106 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 50 'Fails' for the macroalgae and seagrass respectively. 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 (Figures 5 and 6).

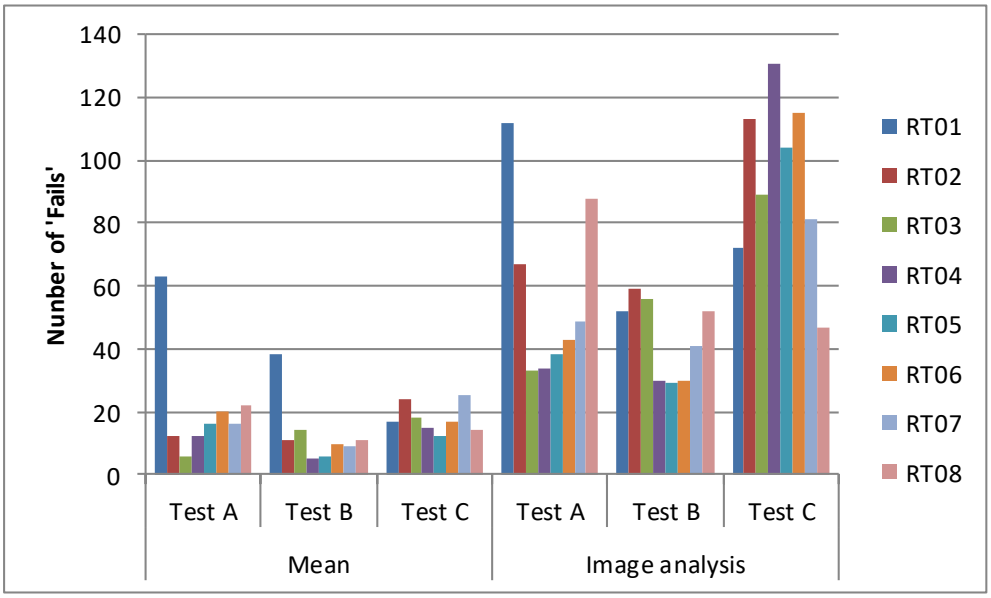

Figure 5: Number of 'Fails' recorded for each macroalgae test method from ring tests RT01 through RT07.

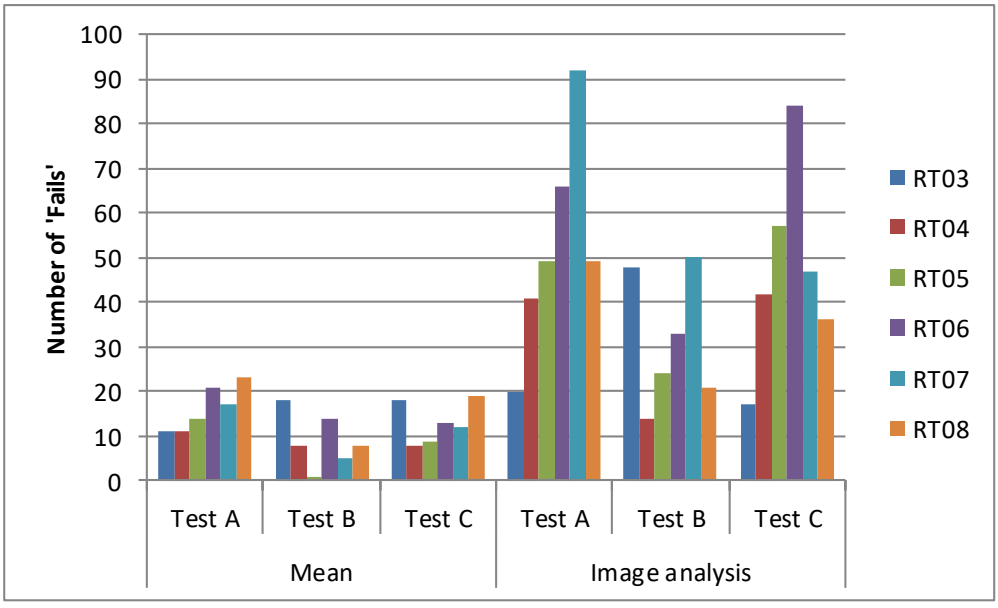

Figure 6: Number of 'Fails' recorded for each seagrass test method from ring tests RT03 through RT06.

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 a change in the number of 'fails associated with each method. Previously test C has results in a higher number of 'Fails' however within RT08 Test A produced the greatest overall number of 'Fails'. Consistent with previous years. Test B resulted in the highest pass rate. There is no definite explanation for this at present however the larger sample size for tests A and C will inevitably produce a broader spectrum of results possibly

contributing to a higher number of 'Fails'. This year the number of 'Fails' was also much higher for macroalgae than seagrass. As seagrass is a lot patchier than macroalgae and can be much harder to estimate % cover, the higher range of results would have contributed to an overall higher standard deviation and would lessen the risk of achieving a 'Fail', based on the Z-scores. These results along with those from previous years require further examination 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 RT08 Test method A was the most favoured method for macroalgae and seagrass albeit test B produced the most consistent results.

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 from which to compare participants results.

3. The image analysis method used during RT08 is considered more objective than skilled eye estimation and likely to produce a more accurate result; RT08 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.

4. During this eighth 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 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.

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.

6. It may be considered 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 and sun reflection may be a problem but all attempts will be made in the future to ensure clear photos are distributed with a broad range of % covers.

7. It was previously noted that when using the 9 x 9 cross hair method it was difficult to keep orientated when zooming in and out to check cross hair points, therefore it was recommended that a central grid in an alternative colour be place on both axis, thereby dividing the quadrat into four, to assist with the method. This was trialled within RT08 but one laboratory found the central orange cross hairs to be distracting. For the subsequent test, thinner orange lines will be trialled as a slight alternative.

8. Many labs use a slightly alternative method of a 10 x 10 grid and counting the presence within in each square. This is a point worth discussion should a workshop be held. 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. An alternative method has been suggested in which the quadrat is split into 4 equal cells. Although adding an additional test method at this stage may not be favoured by many labs alternative methods will always be considered for inclusion in subsequent years. At this stage it may be recommended that a review of all laboratories methodologies be undertaken to ensure the most appropriate methods are being included within the ring tests.

10. It has been suggested that the data collated from the current and previous OMC ring tests be used to produce a set of standard sheets to 'normalise' surveyors results. This proposal will be considered and put forward for investigation.

11. 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, but despite individual failures the overall pass rates are relatively high. In the future, 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.

12. It is requested that participants use the spreadsheets provided to submit results using the format provided. Each participants' 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). This ring test is now in its seventh year and although it has general approval we are still very happy to receive feedback particularly suggestions on how it may be improved.