NMBAQC

NE Atlantic Marine Biological Analytical Quality Control Scheme

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Macroalgae/Angiosperms % Cover Component Report – OMC RT09 2018

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MACROALGAE/ANGIOSPERMS COMPONENT REPORT FROM THE CONTRACTOR SCHEME OPERATION –2017-18

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 ninth year in which % cover estimations of macroalgae have been included as an element of the NMBAQC scheme and the seventh year for which seagrass has been assessed as a separate entity. This included one exercise for macroalgae and one for seagrass both of which were split into three additional exercises based on methodology. The format followed that of previous years (RT01 – RT08). Test material was distributed to participating laboratories from which data forms were completed with macroalgae and seagrass % cover results and returned for analysis.

Twelve laboratories were issued test material. All twelve laboratories completed the % cover macroalgae/seagrass component of the NMBAQC scheme with a total of 38 participants. Of those laboratories submitting results, all twelve 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 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 ninth 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 – RT08). 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, 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. Several results deviated from the sample mean and from the % cover as calculated by image analysis. Deviation from the latter was more noticeable and this has also been reported in previous years. There was a considerable lack of consistency between the three methods in terms of the degree of continuity between participants as well as how the data compared with the image analysis % cover. There was greater preference for methods A and C for both macroalgae and seagrass and as seen in previous years method B had far fewer participants. The number of total 'Fails' between test methods and comparison against mean or image analysis varied considerably with no apparent trend. The overall number of 'Fails' was the same for both the seagrass and macroalgae tests suggesting little difference in the approach or results between the two tests.

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 2018. 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 RT08 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

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.

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 against the background 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 MA2512c.

2.8 Results

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

2.8.1 General Comments

In total twelve laboratories signed up for the % cover component of the macroalgae/seagrass element for RT09. All twelve laboratories submitted data within this round of the ring test. Of those laboratories that submitted data 25 completed method A, 15 completed method B and 26 completed method C for the macroalgae component. For the seagrass component 26 completed method A, 14 completed method B and 23 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 RT09 Bulletin Report and the seagrass OMC RT09 Bulletin Report, which represent a summary of the results for RT09. 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 25 participants and was the second most popular of the three methods, albeit marginally. The range of results per quadrat varied considerably with the largest range of results produced for quadrat 5 with a range of 40%. Quadrats 1, 6, 8 and 9 displayed a range of between 30% and 40%, these quadrats were mid-range % cover quadrats of between 40% and 60%. The smallest range was for quadrat 5 (16%) and the remaining quadrats had % cover ranges of between 16 and 28. Z-scores calculated against the population mean resulted in seven laboratories failing between 1 and 3 quadrats. In total there was a 96% pass rate for test A when using Z-scores derived from the mean which is consistent with previous years results.

The deviation from % cover as calculated using Image J was much greater than seen when using the population mean. Participants showed an average % cover deviation from image analysis % cover ranging between 3.10% and 14.12%. The pass rate was equally much lower using Z-scores derived from image analysis estimates of % cover with 9 out of 25 participants failing between 1 and 4

quadrats and a remaining 2 participants failing both 9 and 11 quadrats. The overall pass rate was lower at 90%. However, these results are improved from RT08 in which most participants failed at least one quadrat.

2.8.2.2 Test B Results (5 x 5 gridded quadrat)

Test B had the least number of participants with 15. As with test A there was a greater degree of correlation of % cover against population mean compared with the image analysis. A total of 73% of participants (11 out of the 15) consistently produced Z-scores of less than 2.0, which is regarded as a 'pass'. The remaining 4 labs failed between 1 and 6 quadrats. The largest range of % covers per quadrat was a range of 32% cover recorded in quadrat 14 and the lowest range of % cover estimates was for quadrat 2 (6%), differing considerably from the results seen in test A. Overall the % ranges were lower than seen in test A with most laying between 16% and 23% cover range.

Consistent with test A, test B also showed more deviation from the image analysis results compared with the population mean, with 12 out of 15 participants failing at least one quadrat and an overall pass rate of only 83% compared with a pass rate of 96% using Z-score from the population; this is also consistent with previous years. The greatest number of 'Fails' could be attributed to quadrat 3, with 7 'Fails' followed by quadrat 7 with 4 'Fails'. Method B also resulted in slightly elevated levels of deviation from % cover as calculated by ImageJ than by mean % cover.

2.8.2.3 Test C Results (9 x 9 crosshairs quadrat)

A total of 26 participants opted to complete Test C using the 100 square method with varying levels of deviation from the population mean. As seen in previous years this was the most 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%, slightly lower than in RT08, with quadrat 5 producing the highest range of 42% with a very broad % cover range of between 25% and 67% and with a total of 11 quadrats with % cover ranges over 20%. Nine participants failed between 1 and 3 quadrats using Z-scores against the mean an overall pass rate of 96%, which is a slight improvement on the previous year. There were also more 'Fails' using Z-scores from image analysis with 18 participants failing between 1 and 5 quadrats and an overall pass rate of 91% which is an increase of 12% compared with RT08 (79% pass rate). Quadrats 7 and 9 had the greatest number of 'Fails' (6) this is also far fewer than seen in previous years.

2.8.3 Seagrass Results from Participating Laboratories

2.8.3.1 Test A Results (open quadrat)

Test A consisted of 26 participants and this was the most popular method. The range of results submitted per quadrat also varied considerably as with the macroalgae test. The largest range was for quadrat 10 with a % cover range of 45 and results varying between 25% and 70 %. A further 5 quadrats had % cover ranges over 30% and only 2 quadrats had % cover ranges less than 20%. The average range across all participants and quadrats was 27%. Z-scores calculated against the population mean resulted in eight people failing between 1 and 3 quadrats. In total there was a 97%

pass rate for test A when using Z-scores derived from the mean which is a marginal increase on RT08 (94%).

When comparing results against % cover as calculated using ImageJ, the number of 'Fails' per laboratory was greater with a total number of 51 'Fails' (87% pass rate) which is far fewer than in RT08 with 92 'Fails' showing considerable improvement. 21 out of the 26 participants failed at least one quadrat. Quadrat 9 had the highest number of 'Fails' against image analysis with a total of 14 followed by quadrats 11 and 12 both with seven 'Fails'. The average deviation of results from image analysis % cover per lab ranged from 3.3 to 12.7, which was higher than the average deviation per participant when derived from the mean (2.7 to 9.8) but both of which are lower than the previous year.

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 showed a lower level of variation than described for test A with most quadrats having % cover ranges in the order of between 15% and 30% indicating a lower level of discrepancy between participants, also compared with previous years. Comparing results against mean % cover resulted in a 13 'Fails' distributed across 5 labs with an overall pass rate of 94%. In comparison, the total number of 'Fails' using image analysis was higher at 31 and was distributed among all 12 out of the 14 participants. The overall pass rates using image analysis % cover was 85%, an increase on the previous year (74%).

The deviation from image analysis (4.2 - 10.5) and mean % cover (2.3 - 9), the difference between the two means of calculation is far less than previously calculated. Although the number of 'Fails' against the mean could be attributed to all but 2 of the quadrats this was not the case for 'Fails' against image analysis whereby most 'Fails' could be attributed to 3 quadrats, quadrat 12 (8), quadrat 11 (4) and Quadrat 9 (4).

2.8.3.3 Test C Results (9 x 9 crosshairs quadrat)

Test C had a total of 23 participants. The % cover ranges were much higher for test C than for tests A and B with all except two quadrats having a % cover range between 20% and 50% and a further one quadrat with a range of 90%, indicating a much high level of discrepancy between participants than seen with the other two methods. The total % cover range over all participants and quadrats was 37%. Comparison of results against the mean resulted in 18 'Fails' with one participant having 10 'Fails' and the remaining 8 'Fails' being distributed among seven other participants and a total pass rate of 95%. Comparing results against the image analysis resulted in 31 'Fails' with pass rates of 91% again with 10 'Fails' attributed to a single participant.

Most 'Fails' against image analysis could be attributed to quadrat 6 which had a total of 10 'Fails' although the range for this quadrat was relatively small between 75% and 87%. Deviation from mean % cover and image analysis was also considerably higher than for the other two tests.

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 RT09 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 can 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 seventh 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 participants 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 provides 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 possibly be attributed to the more patchy coverage of opportunist algae in some quadrats which is much harder to estimate accurately. However, unlike in previous years it was not so evident that those quadrats with a mid percent cover range generally resulted in a higher level of deviation (Figures 1 and 2) with a broad range of deviations from mean across all quadrats. In previous years it has been apparent that those quadrats with either a very high or low percent cover have appeared much easier to accurately estimate total cover. This trend is still slightly more evident

from the macroalgae quadrats, albeit not as conclusive as previously seen. For seagrass there is a high degree of scatter across all % cover ranges. 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.

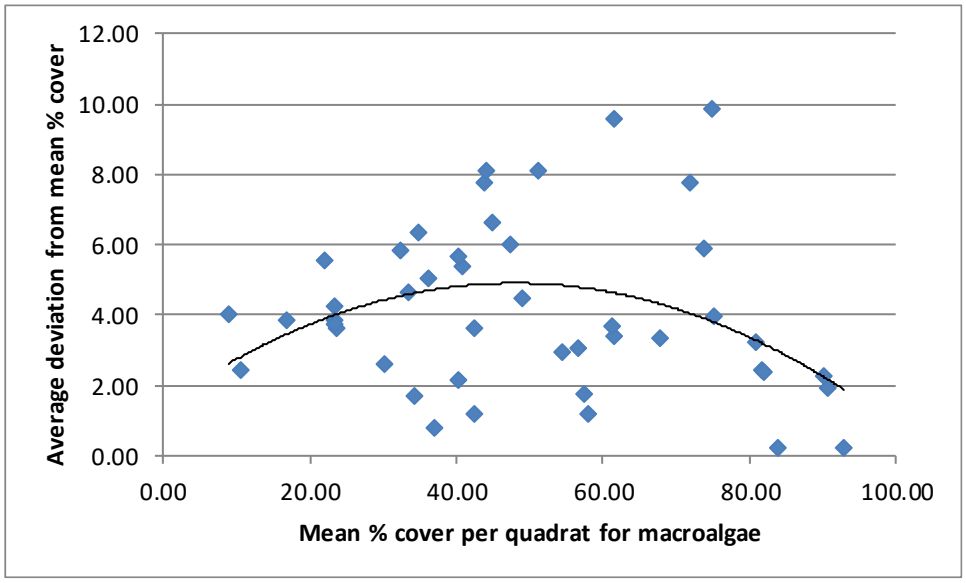

Figure 1: Scatter graph showing the mean level of deviation per quadrat from the mean % cover across all three test methods for macroalgae.

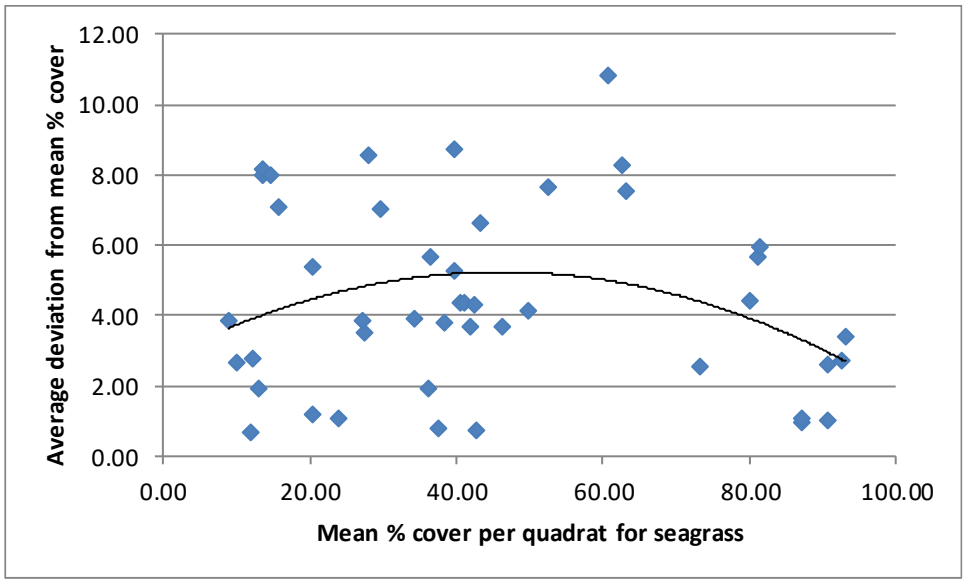

Figure 2: Scatter graph showing the mean level of deviation per quadrat from the mean % cover across all three test methods for seagrass.

There are also noticeable differences between the different methods of estimation used although this is also not consistent with last years results. The macroalgae component resulted in a greater number of 'Fails' for test C compared with the seagrass component with a significantly greater number of

'Fails' for test A. Test C (10 x 10 square grid) had the highest pass rate against image analysis for both macroalgae and seagrass with the lowest level of deviation between mean and image analysis. Tests A and B consistently produced results that were lower than the image analysis estimates of % cover. In contrast Test C produced mean results both higher and lower than that of the image analysis suggesting a greater degree of continuity between Test C and the image analysis results.

In general, the pass rate using Z-scores against image analysis showed a much higher number of 'Fails', in total this amounted to 114 and 113 within the macroalgae and seagrass tests respectively. This was significantly higher than when results were compared against the sample mean producing a total of 40 and 41 'Fails' for the macroalgae and seagrass respectively. The total number of 'Fails' however, is significantly lower than in previous years and is more consistent between seagrass and macroalgae components than has been previous recorded. The overall trend is apparent across all years with image analysis z-scores consistently resulting in a higher number of 'Fails' compared with z-scores from the mean however the greatest number of 'Fails' can no longer be confidently attributed to one species test method.

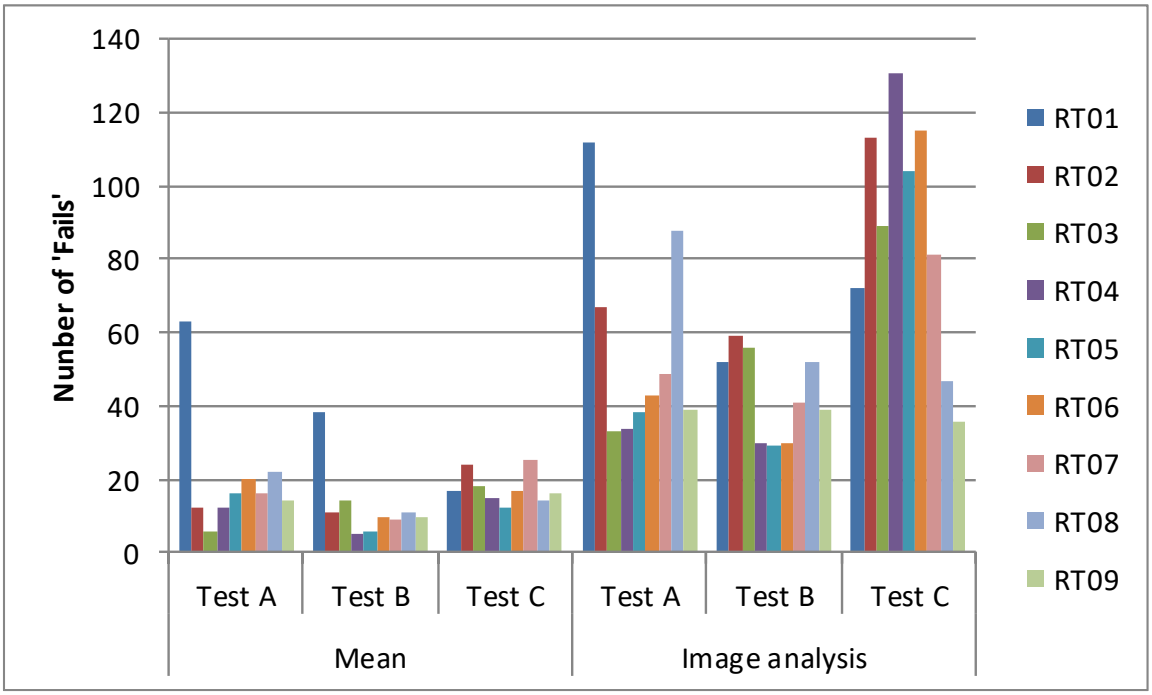

Figure 5: Number of 'Fails' recorded for each macroalgae test method from ring tests RT01 through RT09.

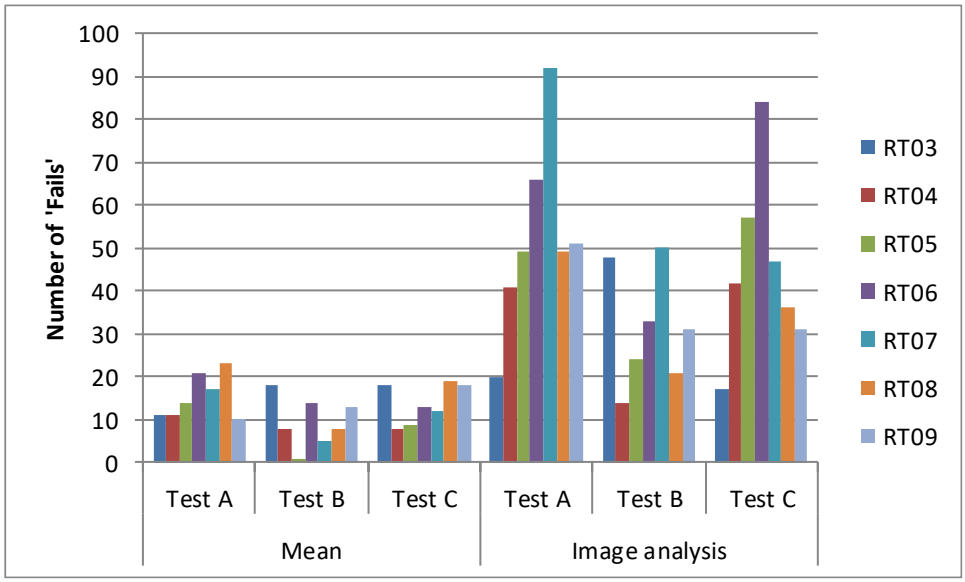

Figure 6: Number of 'Fails' recorded for each seagrass test method from ring tests RT03 through RT09.

The preferred test methods remained unchanged with a greater number of participants completing 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 fewer number of 'Fails' associated with test C when comparing against the ImageJ analysis compared with previous indicating more agreement between analysis methods and participants results. This year the number of 'Fails' was comparable between macroalgae and seagrass unlike in previous years. As seagrass is a lot patchier than macroalgae and can be much harder to estimate % cover and often results in a broader range of % cover estimates. It may be that the higher range of results would contribute to an overall higher standard deviation and would lessen the risk of achieving a 'Fail', based on the Z-scores or possibly that the level of consistency between participants is improving. 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.

The results from both components and across all test methods still produced a high degree of variation with the % cover ranges still beyond what could be considered acceptable levels. It is often the practice that where two field workers are estimating % cover they should remaining within 5% of one another. This is evidently not the situation with these results and causes some concern with regards to the application of the data particularly with reference to the use of the WFD macroalgae algae % cover metric. The constant broad range of results are creating high values of standard deviation and often not highlighting the severity of the deviation from mean and image analysis and producing far fewer 'Fails' than would be expected. The broad range of results across participants and laboratories is a problem that needs to be addressed either by adapting a less subjective means of estimating % cover of macroalgae or seagrass, or by stimulating a field meeting with which to synchronise such methodologies to remain the high degree of error currently being recorded within the NMBAQC macroalgae and seagrass % cover tests. The current test methods do not provide a current solution and where one method may work best with macroalgae this may not be the case for seagrass, so this remains an area requiring significant further investigation.

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 RT09 Test methods A and B 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 from which to compare participants results.

3. The image analysis method used during RT09 is considered more objective than skilled eye estimation and likely to produce a more accurate result. However, this method is still under development and will continue to undergo improvements prior to the next round of tests. 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 ninth 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 most 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 more 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 noted during RT08 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 was suggested that a central grid in an alternative colour be place on both axis, thereby dividing the quadrat into four, to assist with the method. However, feedback suggests the additional colour added to assist with counting cross hairs is also distracting, this will need to be considered in subsequent tests.

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. Further suggestions have been made to consider a 2 x 2 squared quadrat as partially achieved by the additional coloured cross hairs in Method C. Adding an additional method at this stage is likely to be unfavourably received due to the amount of time already required to use the current three methods however, should a field workshop be organised for the future this is a method worth incorporating for comparison against other methods.

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 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.

11. 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.