NMBAQC

NE Atlantic Marine Biological Analytical Quality Control Scheme

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Macroalgae/Angiosperms % Cover Component Report – OMC RT11 2020

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MACROALGAE/ANGIOSPERMS COMPONENT REPORT FROM THE CONTRACTOR SCHEME OPERATION –2019-20

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 eleventh year in which % cover estimations of macroalgae have been included as an element of the NMBAQC scheme and the nineth 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 (RT03 – RT10). Test material was distributed to participating laboratories from which data forms were completed with macroalgae and seagrass % cover results and returned for analysis.

Ten laboratories were issued test material. All ten laboratories completed the % cover macroalgae/seagrass component of the NMBAQC scheme with a total of 24 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 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 methodologies.

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 eleventh 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 RT03 – RT10). 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 'Fails' between test methods and comparison against mean or image analysis varied considerably with no apparent trend. The overall number of 'Fails' was higher for macroalgae than seagrass particularly when compared against ImageJ. The seagrass tests resulted in a much broader range of results thereby increasing the standard deviation, so it is likely that the Z-scores were unable to pick up slight deviations from mean or ImageJ analysis % cover, therefore resulting in fewer '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 misuse 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 2020. 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 RT10 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 objective 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-seven will be recorded as MA2712c.

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 ImageJ calculated % cover estimations for both macroalgae and seagrass.

2.8.1 General Comments

In total ten laboratories signed up for the % cover component of the macroalgae/seagrass element for RT10. All ten laboratories submitted data within this round of the ring test. Of those laboratories that submitted data 20 completed method A, 14 completed method B and 20 completed method C for the macroalgae component. For the seagrass component 19 completed method A, 13 completed method B and 19 completed method C. Thirteen participants completed all three macroalgae and twelve 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 RT11 Bulletin Report and the seagrass OMC RT11 Bulletin Report, which represent a summary of the results for RT11. 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 is 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 20 participants and was the joint most popular of the three methods. The range of results per quadrat varied considerably with the largest range of results produced for quadrat 10 with a range of 40%. Quadrats 9, 12 and 14 had % cover estimate ranges of between 31% and 34%, and a further 9 quadrats had % cover ranges of between 20 and 30%. The lowest range was for quadrat 8 with a 10% cover range. Despite quadrat 10 resulting in a broad range of results there was only one ‘Fail’ when using z-score against the mean, probably due to the higher standard deviation of 10.8, and overall there were no more than 2 ‘Fails’ per quadrat and most quadrats on recorded one ‘Fail’. In comparison the total number of ‘Fails’ when using Z-scores derived from ImageJ analysis % cover was higher with a total of 23 which is double the number of 'Fails' from analysis against the mean. However, this is far lower than previous years with most quadrats recording between 1 and 2 'Fails' and only one quadrat having three 'Fails'. Z-scores calculated against the population mean resulted in a 96% pass rate for test A, which is consistent with previous years results and a 92% pass rate for analysis against ImageJ.

Deviation from the mean varied between participants, ranging from 2.16% and 9.32% taken as an average across all quadrats, this was a slight improvement on the previous year. Deviation from ImageJ was slightly higher ranging between 2.86 and 12.8 which is also consist with last years results.

Seven participants 'Failed' at least 1 quadrat when comparing against the mean, using ImageJ 50% of participants 'Failed' at least 1 quadrat. However, 12 out of 23 'Fails' could be attributed to just two participants when using ImageJ. These two participants often underestimated the % cover by 15 – 20%. Test A recorded an average deviation from Image analysis of -3.5% with all bar two quadrats being underestimated

2.8.2.2 Test B Results (5 x 5 gridded quadrat)

Test B had the least number of participants with 14. As with test A there was a greater degree of correlation of % cover against population mean compared with the image analysis. Overall, there was a far smaller range of results recorded for test B. The highest range was for quadrat 4 (27%). Most quadrats had ranges between 10% and 20% with two quadrats displaying ranges of below 10%. This is a much lower range than has been previously recorded. The lowest range of % cover estimates were for quadrat 5 (4%) and quadrat 8 (7%), consistent with results from Test A.

Only one of the fourteen participants 'Failed' at two quadrats when compared against the mean with a 99% pass rate for this test component. In comparison the total number of 'Fails' when compared with image analysis was 49 with a pass rate of only 77%. All except two participants 'Failed' at least 1 quadrat with 7 'Failing' between 4 and 9 quadrats.

Average deviation, per participant, from the mean was relatively low between 2.0 and 7.0. Average deviation from image analysis was consistently higher ranging between 3.1 and 10.9. Overall deviation from image analysis was -4.98, indicating that % cover estimates from participants were consistently lower than those as calculated by image analysis. This is also consistent with test A results.

2.8.2.3 Test C Results (9 x 9 crosshairs quadrat)

A total of 20 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 also 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 range of results was consistent with the other two test methods. Three quadrats had % cover ranges between 30% and 40%, a further 8 had % cover ranges of between 20 and 30%, the remaining had less than 20% ranges with quadrat 8 recording the lowest range of 8%. As per the previous two test methods quadrats 5 and 8 had the lowest % cover ranges.

Six participants 'Failed' between 1 and 4 quadrats, with a total of 11 'Fails' for comparisons against the mean. The overall pass rate was 96% which is consistent with the previous year. The 'Fails' were distributed across all quadrats with no more than 1 'Fail' per quadrat. Comparisons against image analysis resulted in 16 'Fails' and a 94% pass rate, which is far lower than the previous two test methods. Most quadrats resulted in 1 'Fail' with quadrat 11 and quadrat 6 resulting in two 'Fails' and 8 participants 'Failing' between 1 and 4 quadrats.

The degree of deviation from mean (2.34 – 8.72) and image analysis (2.93 – 8.87) was comparable and were lower than the other two test methods. Overall deviation was very low at only -0.14 showing a good degree of agreement between test method c and image analysis.

2.8.3 Seagrass Results from Participating Laboratories

2.8.3.1 Test A Results (open quadrat)

Test A consisted of 19 participants and this was the joint most popular method. The range of results submitted per quadrat varied considerably as with the macroalgae test. The largest range was for quadrat 6 with a large % cover range of 45% and results varying between 20% and 65 %. A further 4 quadrats had % cover ranges over 40% with only 2quadrats with % cover ranges less than 20%. The average range across all participants and quadrats was 32%, which is consistent with last years results.

Z-scores calculated against the population mean resulted in three people 'Failing' between 2 and 4 quadrats and 9 'Fails' overall. In total there was a 96.8% pass rate for test A when using Z-scores derived from the mean which is consistent with last year. When comparing results against % cover as calculated using ImageJ, the number of 'Fails' per laboratory was marginally greater with a total number of 13 'Fails' (95.4% pass rate) which is much more consistent comparison against the mean. However, this may be because of high values for standard deviation derived from the large range of results thereby resulting in fewer 'Fails'. Eight out of the 19 participants 'Failed' between 1 and 3 quadrats and these were distributed across all quadrats with quadrats 1 and 15 having 3 'Fails'. However, these two quadrats did not have the broadest range of results.

The average deviation of results from mean and image analysis % cover per lab ranged from 2.79 to 15.26 and 3.02 to 14.73 respectively. These deviations, which although consistent between analysis methods, are higher compared with the macroalgae % cover estimates. The overall deviation of mean from image analysis was only -0.52% indicating both over and underestimations of % cover.

2.8.3.2 Test B Results (5 x 5 gridded quadrat)

Test B had the least number of participants with a total of 13 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 9 quadrats having % cover ranges in the order of between 10% and 30% indicating a lower level of discrepancy between participants, although 6 quadrats still had % cover ranges between 30% and 35%. Quadrat 7 had a range of only 7%.

Comparing results against mean % cover resulted in only 5 'Fails' distributed across 4 participants, with an overall pass rate of 97%. In comparison, the total number of 'Fails' using image analysis was higher at 27 and was distributed among 7 out of the 13 participants. The overall pass rates using image analysis % cover was 86%. Both sets of results were consistent with last years results.

The deviation from mean % cover (2.81 – 9.22) and image analysis (3.45 – 13.18) was slightly lower than the previous year and can be attributed to the slightly lower range of results across some quadrats. The overall deviation of mean from image analysis was higher than for test method A, at -4.81 indicating a greater number of quadrats with estimations lower than the image analysis % cover calculations.

2.8.3.3 Test C Results (9 x 9 crosshairs quadrat)

Test C had a total of 19 participants. The % cover ranges were much higher for test C than for both tests A and B with all except four quadrats having a % cover range between 30% and 51% indicating a much higher level of discrepancy between participants. The total % cover range over all participants and quadrats was 33% with quadrat 12 recording the highest range of 51%

Comparison of results against the mean resulted in 8 'Fails' distributed between 4 participants and a total pass rate of 97%. Comparing results against the image analysis resulted in 19 'Fails' with pass rates of 93% with participants resulting in between 2 and 7 'Fails'. Given the broad range of results it would be anticipated that there would be a much higher number of 'Fails'. 'Fails' against image analysis and mean were spread broadly between all quadrats.

Deviation from mean % cover (2.50 – 15.79) and image analysis (3.17 – 19.91) was consistent with that of the previous year but still higher than test B. The average level of deviation between % cover estimates and image analysis across all quadrats and participants was 4.13 indicating more overestimations.

2.9 Discussion

The % cover of opportunist algae or 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 RT11 used the population mean and an image analysis method (ImageJ) to calculate a more objective % 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 is thought to provide less subjectivity than skilled eye estimations. 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 eleventh 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 up to 51% indicating a high degree of participant error and inconsistency. 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 patchier coverage of opportunist algae and seagrass in some quadrats which is much harder to estimate accurately. However, as in some previous years it was evident that those quadrats with a mid percent cover range generally resulted in a higher level of deviation with a much broader range of results. 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 evident throughout both macroalgae and seagrass quadrat. There is also a broader range of % cover estimations and deviations for both the mean and ImageJ analysis for seagrass than macroalgae. Seagrass displays a much patchier nature of growth; its thin long strands often make it difficult to estimate % cover leading to a broader range of results and high levels of deviation.

As per previous years there is a very broad range of results provided for each quadrat, far greater than the recommended +/- 5%. The range of results also varies according to the % cover of the quadrat with those in the mid-range (30 – 70%) having less consistency in % cover estimates than those quadrats whose % cover is at the higher or lower end of the range. This is seen across all test methods and for seagrass and macroalgae alike.

Figures 1 and 2 show this range of % cover results. The scatter graphs also indicate that Test method B for both macroalgae and seagrass has the smallest range of results across participants. However, there are also far fewer participants for this test method so it is unclear whether, given similar number of participants, this would result in a broader range of results. Overall, the broad range of % cover estimates submitted by participants is concerning in terms of consistency between laboratories as well as within laboratories.

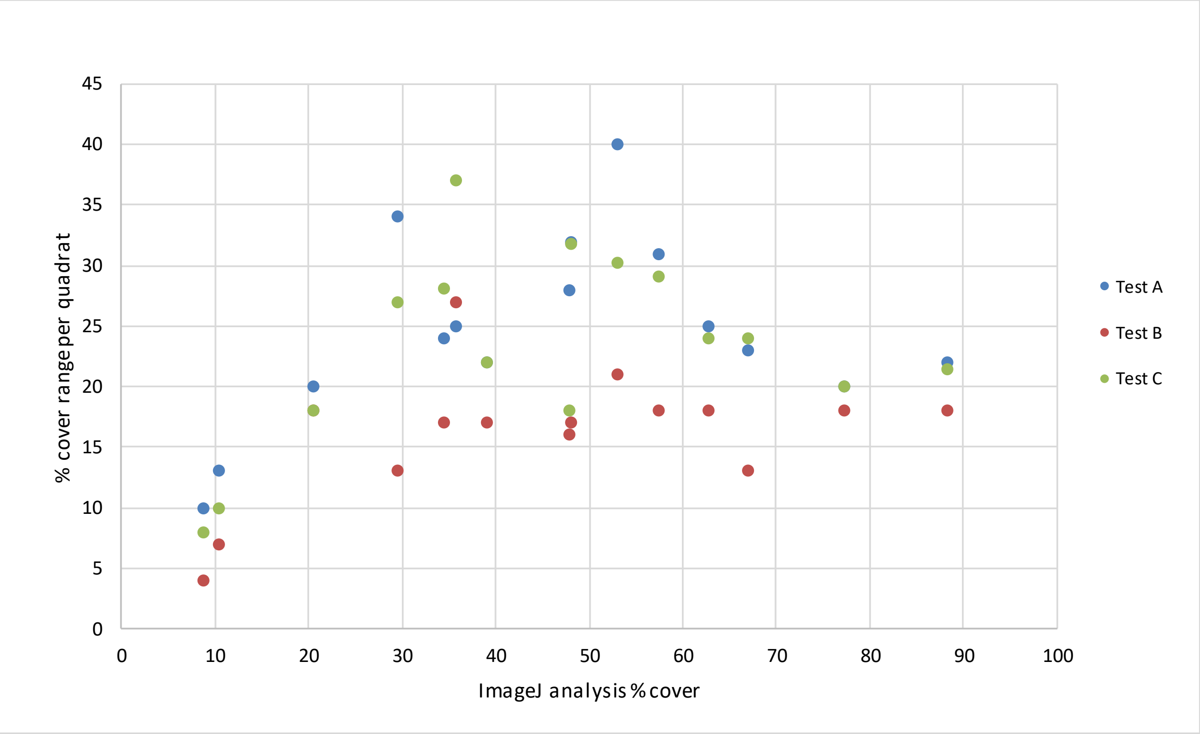

Figure 1: Scatter graph showing the range of % cover results per quadrat across all three test methods for opportunist macroalgae.

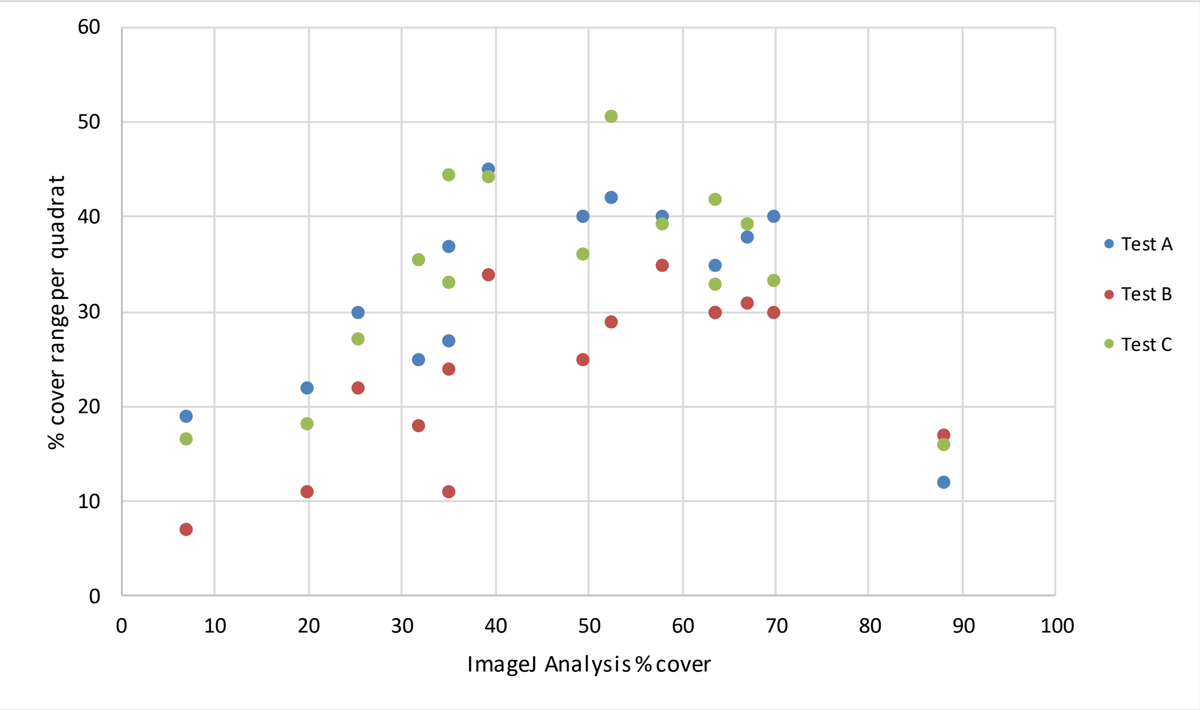

Figure 2: Scatter graph showing the range of % cover results per quadrat across all three test methods for seagrass.

There are also noticeable differences between the different methods of estimation used and the resulting number of 'Fails' (Figure 3). The macroalgae component resulted in the greatest number of 'Fails' when comparing against both mean and ImageJ analysis with 24 and 88 'Fails' respectively. Unlike last year, test B had the most 'Fails' recorded for both methods of analysis. In comparison the number of 'Fails' for seagrass were much lower but test B also resulted in the greatest number of 'Fails'. As in previous years the number of 'Fails' was much higher for comparison against ImageJ than against mean. This has come to be expected but does not negate the value of using ImageJ analysis with which to compare results as this is ultimately the least subjective method and using the mean is naturally going to allow more estimates to sit within the Z-score +/- 2.00 range. Therefore, the two methods cannot be directly compared.

Figure 3 also shows that the results for macroalgae are producing far more 'Fails' than for the seagrass. Given that the seagrass could be considered more difficult to estimate, and the broad range of results submitted by all participants, it may be considered that the higher pass rate is due to the high standard deviation produced from the high range of results and is not due to more consistent estimates of % cover.

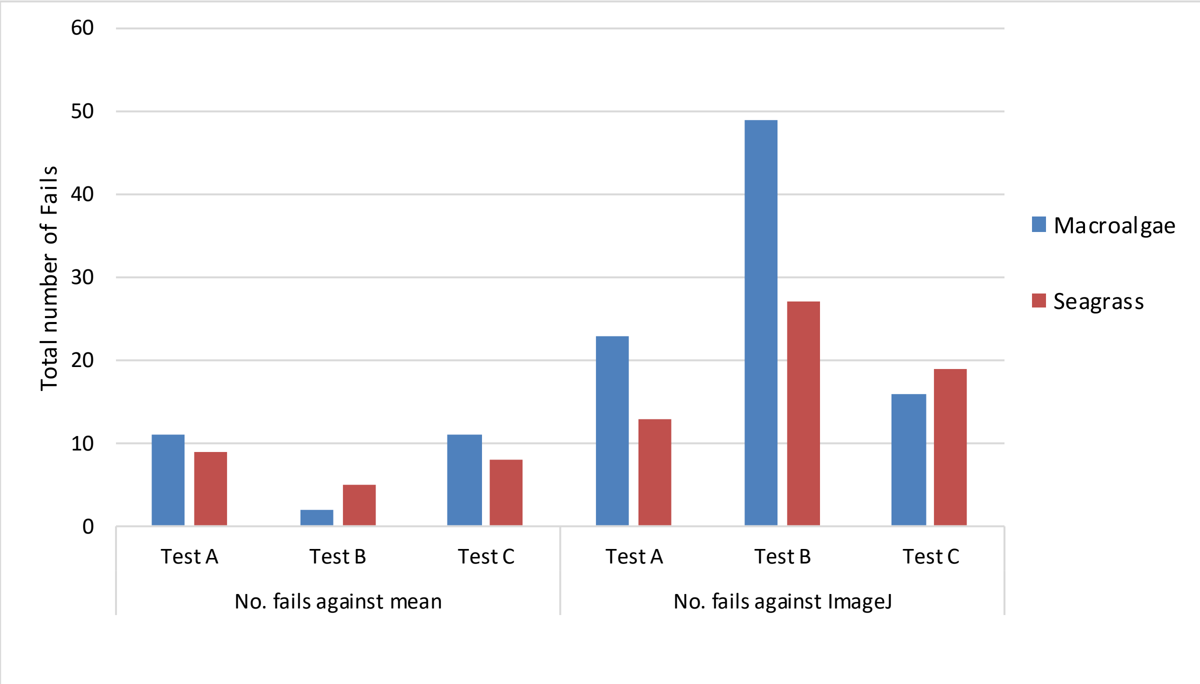

Figure 3: Number of Fails recorded across all tests for macroalgae and seagrass quadrats for OMC RT11.

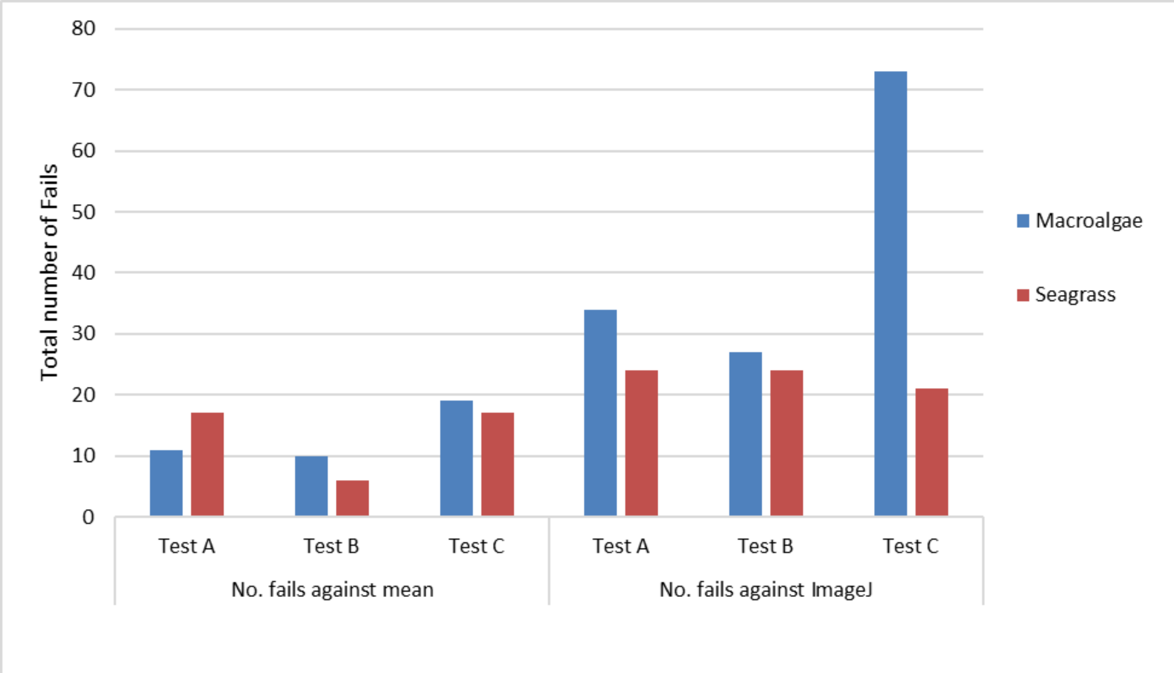

Figure 4: Number of Fails recorded across all tests for macroalgae and seagrass quadrats for OMC RT10.

Figure 4 shows the results from the OMC RT10. The number of 'Fails' against the mean are very comparable to the current ring test. However, the number of 'Fails' against the mean show that last year the greatest number of 'Fails' was recorded for test C and not Test B as seen this year. Albeit the overall number of 'Fails' has been reduced since last year which is a positive trend and may suggest greater agreement between laboratories and participants.

Tests A and B produced results that were, on average, lower than the image analysis estimates of % cover for both macroalgae and seagrass. In contrast Test C produced mean results that were higher than that of the image analysis. Clearly there is no continuity between test methods none of which agree regularly with the % cover results from the ImageJ analysis, some of which appear to be overestimating % cover while other methods underestimate. If different laboratories continue to use different methods of % cover estimation, then it is like that there will be no continuity in the quality of the results.

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. However, with test A regularly resulting in lower estimates of % cover than use of method C there need to be some form of calibration between the two methods to produce consistent results.

It may be considered that test method C would provide a less subjective method of estimation, as counting the number of cross hairs under which macroalgae or seagrass lay should be a relatively straight forward method. However, there is still a huge disparity in results, often much higher than for the other test methods that may suggest the method is not being used consistently between participants. It may be that for some quadrats it is unclear if seagrass or macroalgae is present under a cross hair which affects the % cover estimate. This method should clearly be used with some caution.

This year the number of 'Fails' was far less for seagrass than for macroalgae. 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 estimate. The higher range of results is contributing to an overall higher standard deviation which lessens 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.

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 remain 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. If it is to be taken that two field personnel should agree a % cover within +/- 5%, and therefore implicating a standard deviation of 5.0, then there would be a much higher number of fails. The average standard deviation per method ranges between 4.8 and 6.5 for macroalgae and 7.14 and 9.6 for seagrass, with very few quadrats with resulting in a standard deviation lower than 5. The macroalgae component picks up a greater number of 'Fails' because the range of results isn't as broad as those for the seagrass, so it is able to highlight outliers more easily. Many quadrat standard deviations for the seagrass component were above 10.0 which is an indication of the huge range of results and lack of consistency between field workers.

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 and remove 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 OMC RT11 Test methods 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 from which to compare participants results.

However, the high standard deviation across all test methods is having a significant impact on the overall results.

3. The image analysis method used during RT11 is considered more objective than skilled eye estimation and likely to produce a more objective 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 eleventh cycle of the macroalgae % cover exercise one laboratory was unable to complete the ring test within the allocated time period. It is appreciated that conflicts with work may prevent laboratories from meeting the deadline and where necessary allowances can be made. However, it is important that all laboratories continue to attempt 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 and ensuring preliminary bulletins and reports are circulated within the set time period. 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. It is also requested that those laboratories unable to meet the deadline should give prior notice of two weeks.

5. This year there were no problems with the distribution of test material over the Christmas period and all laboratories received their test material on time. 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. Some labs still use slightly alternative methods of estimating % cover. It may be useful to review which methods are the most appropriate for the ring test and which methods are most commonly being employed. 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.

8. This year all participants successfully filled out the spreadsheets provided and removed any prior calculations, particularly with regards to Test method C. This has made the analysis process much easier and reduced the risk of error during subsequent calculations. It is requested that participants continue and exclude all 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.

9. It is evident that not all computers or laptops have means of playing a CD or DVD. This is highly problematic due to the test material using this medium as its means of distribution. Other methods of distribution, that are accessible to all laboratories will be invested prior to the next round of ring tests, to ensure the material can be accessed at the start date. We are looking into the use of file transfers for subsequent test material.

10. Of the feedback provided two laboratories expressed some concern with the use of the crosshair method. It was more open to disparities between participants and more prone to mistakes with general overestimation. This is also evident in the results returned from all the participants. This is a method that needs further assessment as to its viability as a means of measuring % cover.

11. There were some concerns that those quadrats in which seagrass was present within the macroalgae test quadrats or where Zostera had already died back, that these may affect the ImageJ analysis. It can be assured that these factors are removed from the image prior to analysis so as not to overestimate the % cover.

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.