**The NE Atlantic Marine Biological Analytical Quality Control Scheme**

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# Macroalgae/Angiosperms Percentage Cover

# Component Report

# Ring Test OMC RT13 2022


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**22nd April 2022**

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## Contents

## List of Figures

# 1. Introduction

## 1.1 Background

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 aims to improve consistency between analysts and increase confidence in ecological quality status.

The NE 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:

- Estimation of percentage cover
- Comparison of methodologies

This is the thirteenth year in which percentage cover estimations of macroalgae have been included as an element of the NMBAQC scheme and the eleventh year for which seagrass has been assessed as a separate entity. This included one exercise for macroalgae and one for seagrass, each of which were subdivided into three separate tests based on different methodologies. The format followed that of previous years (RT03 - RT12). Test material was distributed to participating laboratories along with standardised data forms, which were completed with macroalgae and seagrass percentage cover estimates and returned for analysis.

Graphical representations of the performance of each participating laboratory were distributed in the macroalgae and seagrass OMC RT13 Bulletin Report. This bulletin included the z-score based ‘pass’ and ‘fail’ flags assigned to each result to highlight deviation from sample means and image analysis values. The current report describes the results in more detail and should be read in conjunction with the OMC RT13 Bulletin

## 1.2 Participating Laboratories

Eleven laboratories were issued test material. Nine laboratories completed the macroalgae/seagrass component percentage cover of the NMBAQC scheme with a total of 18 individual participants. Of those laboratories submitting results, all nine were government organisations. Due to the nature of the exercise, there was no limit on the number of participants per laboratory.

Judging percentage cover by eye is subjective and the preferred method of estimation varies between laboratories. Participants were given the option to complete the percentage cover test that best represented the methodology used within their laboratory. However, participants were also encouraged to complete all three variations of both the macroalgae and seagrass exercises so that the results obtained using different methodologies could be compared.

The NMBAQC scheme was originally set up for benthic invertebrate 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 is important that they are correct. At present this scheme does not specify a definite qualifying performance level, and NMBAQC ring tests may be treated as training exercises. However, in previous years certain indicative targets have been applied to the assessment of the results based on calculated z-scores to allow 'pass' or 'fail' flags to be assigned, which provides competent monitoring authorities with an option for internal monitoring of performance. For consistency with previous years, these same criteria have been maintained for the current year. The ring tests offer a means to assess personal and laboratory performance and to identify training requirements or potential areas for improvement in existing field and laboratory procedures.

# 2. Summary of the Percentage Cover Exercises

## 2.1 Introduction

There was one exercise for the assessment of percentage cover of opportunistic macroalgae and one for seagrass. Each test included three methodology options. The exercise is described in full below to include details of distribution and logistics, procedures for estimation of percentage 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 accurately estimate various levels of opportunistic macroalgae and seagrass percentage cover. The exercise can determine the level of inter-laboratory variation and the degree of deviation from percentage 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 2022. Participating laboratories were required to estimate the percentage cover of the opportunistic macroalgae and seagrass using one or more of the methodologies provided. The photographs were taken to be consistent with those provided for RT12, with two of the sets modified with overlaid grid systems. Opportunistic macroalgae consisted of species of *Ulva*, and seagrass was identified as *Zostera noltii*.

## 2.3 Logistics

The test material was distributed via an online file sharing link sent to each laboratory. The files contained the six tests, a description of methods and standardised forms for data submission. Participants were given six weeks to complete the test and return their results. There were no restrictions on the number of participants per laboratory.

Email has been the primary means of communication for all participating laboratories.

## 2.4 Confidentiality

To preserve the confidentiality of participating laboratories, each participant was randomly allocated a four-digit laboratory code to allow them to identify their results. The two-letter prefix ‘MA’ refers to the scheme component and this is followed by two digits representing the current NMBAQC scheme year, and the final two digits representing the laboratory. However, it appears the macroalgae component is out of synchrony with the rest of the NMBAQC scheme components, since the current scheme year is twenty-eight, but the ‘28’ prefix has already been used for macroalgae RT12. For the sake of continuity with the previous macroalgae ring tests, a ‘29’ prefix has been used this year for RT13, but this will be corrected in the 2022-23 scheme year to ensure standardisation across all scheme components. For those laboratories that provided multiple submissions, the laboratory code is followed by a letter suffix to distinguish each participant of that laboratory. For example, participant c from laboratory twelve in scheme year twenty-eight would be recorded as MA 2912c.

## 2.5 Preparation of the Samples

To assess the accuracy of opportunistic macroalgae and seagrass percentage cover determination, photographs were taken of quadrats placed to include varying amounts of macroalgae or seagrass cover. In total of 15 representative photographs of macroalgae and 15 of seagrass were taken by APEM Ltd for the purpose of this exercise.

Each set of 15 photographs was modified with two different overlaid grids to produce the total of three tests for each component to facilitate different methods of percentage cover estimation.

### *2.5.1 Method A*

Method A used an open quadrat, which allowed the participant to estimate the percent cover in the 0.25m² quadrat without visual obstruction or assistance from gridlines. A general estimation was conducted looking solely at the total area within the quadrat that is clearly covered by the opportunistic macroalgae or seagrass.

### *2.5.2 Method B*

Method B used an overlaid grid to divide the divide the 0.25m² quadrat into 25 squares, with each square representing 4% of the total quadrat area. The percentage 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 total number of covered squares was then divided by 25 and multiplied by 100 to provide the total percentage cover.

### *2.5.3 Method C*

Method C used an overlaid 9 x 9 crosshair grid to divide the 0.25m² quadrat into 100 squares. The ‘crosshair’ refers to each point at which the gridlines cross and with a 9 x 9 grid there are a total of 81 crosshairs. The estimation of percentage cover was calculated by recording the presence or absence of macroalgae/seagrass under each of the crosshair points. Where seagrass or macroalgae were present a crosshair was given a score of 1 and where absent a score of 0. The total number of crosshairs with macroalgae/seagrass present was then divided by 81 and multiplied by 100 to provide a total percentage for the quadrat.

## 2.6 Quadrat Image Analysis

An image analysis programme called ImageJ was used to calculate a more objective measurement of percentage cover that could be compared with the traditional means of assessment following the methodology described by Xiong *et al*. (2019). Previous ring tests have sought a full, impartial image analysis comparison as part of the QC exercise. Initially this was attempted using GIS software, but this did not provide a fully independent analysis of percentage cover. ImageJ image analysis software was chosen to be less subjective by providing a more accurate analysis based on colour/tone contrast. Image analysis has been carried out to demonstrate how the comparisons would work but may still require further modification and discussion as to its applicability and accuracy, therefore cannot be taken as a definite measure of percentage cover.

Prior to analysis each quadrat photo was edited using Photoshop, cropping each image to the exact 0.25m² inside area of the quadrat and increasing the green colour saturation to ensure a substantial contrast between the seagrass and macroalgae against the background. The photograph was then processed using the ImageJ program. Firstly, the image measurements were calibrated according to the quadrat dimensions. Then each image was separated into two portions of green and non-green areas by adjusting the hue, saturation and brightness colour threshold settings to match the areas of macroalgae or seagrass. The resulting green area selection was used to calculate the area of coverage in cm² and this was converted to a percentage by dividing the result by 2500 (i.e. the total quadrat area) and multiplying by 100. The resulting percentages were used as a comparison against the skilled eye estimations as submitted by the participants.

## 2.7 Analysis and Data Submissions

A results workbook was distributed to each participating laboratory along with the exercise instructions to standardise the format in which the results were submitted. These results will be retained and stored appropriately. 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 allow a comparison of methodologies and levels of accuracy achieved within each.

For each test the participant had to estimate the percentage cover of opportunistic macroalgae or seagrass species only, excluding any additional species that might be present within the quadrat and that were not considered to belong to either of these types of species. The assessment included a broad range of variation in percentage cover to represent the full range that could be experienced in the field.

### 2.8 Z-Scores

Z-scores were calculated to determine how many standard deviations each participant's percentage cover value was separated from the mean percentage cover value using the following formula:

$$Z = \frac{x - \mu}{\sigma}$$

Where:

$\boldsymbol{x}$ is the raw percentage cover value to be standardised;

$\boldsymbol{\mu}$ is the mean of the participants' percentage cover values;

$\boldsymbol{\sigma}$ is the standard deviation of the participants' percentage cover values.

Z-scores were calculated separately using the mean of the participants' percentage cover scores and then using the percentage cover score derived from the ImageJ analysis. For consistency with previous ring tests, a z-score value of greater than +/- 2.00 was considered to be outside an acceptable limit of deviation from the mean and this cut-off point was used determine a 'fail' or 'pass' flag on the submitted data.

## 3. Results

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

### 3.1 Participant Data Received

Of the laboratories that submitted data, there were five participants that completed method A, six that completed method B and twelve that completed method C for the macroalgae exercise. For the seagrass exercise six completed method A, eight completed method B and nine completed method C. Only two participants completed all three macroalgae and seagrass tests. The results have been collated and represented in various formats to enable full comparisons between participants and against the percentage cover calculated using image analysis.

## 3.2 Macroalgae Results

### *3.2.1 Macroalgae Test A (Open Quadrat)*

Test A was completed by the fewest participants, with only five choosing this method. The ranges of percentage cover estimates per quadrat was generally lower than methods B and C, varying from 1% to 17% per quadrat. Nine quadrats had ranges of less than 10% cover between participants and the remaining six quadrats had percentage cover ranges of between 10 and 20%. These were much lower ranges than were recorded for the previous year, most likely influenced by the smaller sample size in the current year. None of the percentage cover values were deemed ‘fails’ when using the z-scores calculated from the mean percentage cover value.

In comparison, when using z-scores calculated from the ImageJ analysis percentage cover value there were a total of 17 ‘fails’. Quadrats 1, 7, 14 and 15 had one ‘fail’ each, quadrats 6 and 8 both had four ‘fails’ and all five participant results were deemed ‘fails’ against the ImageJ results for quadrat 9. All of the ‘fails’ against ImageJ analysis were due to higher estimations than the ImageJ calculated values. For quadrats 6, 8 and 9 there was mud present on the surface of some of the algal fronds, which appears to have led to an underestimate of percentage cover in the ImageJ analysis, with all five participants recording higher estimates than the ImageJ analysis.

Z-scores calculated against the population mean resulted in a 100% pass rate for test A, which is higher than previous years, whilst the pass rate against ImageJ results was 77.33%, a decrease from previous years, most likely due to the lower sample size in the current year.

Deviation from the mean varied between participants, ranging from 2.04% and 3.29% taken as an average across all quadrats. Deviation from ImageJ was slightly higher ranging between 3.74 and 5.91. Both these results were an improvement on the previous year, but as with the results above, this is likely due to the lower number of participants in the current year. The average deviation of the mean participant percentage cover from ImageJ calculated values was 3.06%, indicating that there was an overall tendency towards overestimation.

### *3.2.2 Macroalgae Test B (5 x 5 Gridded Quadrat)*

Test B had one more participant than Test A, with a total of six choosing this method. The ranges of percentage cover estimates were slightly higher than Test A, varying from 1% to 21%. Again, there were nine quadrats with ranges of less than 10% cover between participants. Four quadrats had ranges between 10% and 20% cover and the remaining two quadrats each had a percentage cover ranges of 21%. These were lower ranges than previous years, but as with Test A this is likely to be due to the lower number of participants in the current year.

Only one of the nine participants ‘failed’ a quadrat when compared against the mean, giving a 98.89% pass rate for this test component. In comparison the total number of ‘fails’ when compared with image analysis was 33, with a pass rate of only 63.33%. All six participants ‘failed’ at least four quadrats with 9 ‘fails’ attributed to one participant. Unlike Test A, these ‘fails’ were an almost equal mixture of underestimation (17 ‘fails’) and overestimation (16 ‘fails’) of percentage cover compared to the ImageJ calculated values. Quadrats 6 and 12 resulted in ‘fails’ for all six participants when compared against the ImageJ analysis. For quadrat 6 all of these ‘fails’ were overestimates, whilst all of the ‘fails’ for quadrat 12 were underestimates.

As with Test A, the average deviation per participant compared to the mean was relatively low, ranging from 1.51 to 4.12. Average deviation from ImageJ analysis was slightly higher, ranging from 2.99 to 6.93. The average deviation of the mean participant percentage cover from ImageJ calculated values was 0.84%, indicating a mixture of overestimation and underestimation of percentage cover, with a slight tendency towards overestimation.

### *3.2.3 Macroalgae Test C (9 x 9 Crosshairs Quadrat)*

As with previous years, Test C had the highest number of respondents, with a total of 12 participants opting to use the 100 square method of percentage cover estimation. The range of results was also correspondingly higher than the other two test methods, with only five quadrats having ranges of less than 10% cover and eight quadrats varying by 10-20% cover between participants. The largest ranges were recorded for quadrats 9 and 11 with ranges of 27.3% and 29.1% cover, respectively.

Using z-scores based on participants’ mean percentages, five participants ‘Failed’ one quadrat and one participant ‘failed’ four quadrats to give a total of 9 ‘fails’. The overall pass rate was 95% which is consistent with previous years. All nine of the ‘fails’ were for different quadrats. Comparisons against image analysis resulted in 49 ‘fails’ and a 72.8% pass rate, which is lower than the previous year. All but two of these ‘fails’ were overestimates compared to the ImageJ calculated results. All of the participants ‘failed’ quadrat 15 compared to ImageJ, as method C yielded higher percentage cover values for this quadrat compared to Test A and Test B.

The average deviation from the mean across all quadrats (1.26 - 5.65) was only slightly higher than Methods A and B. Average deviation from the ImageJ results ranged from 3.56 to 8.66, which is also only slightly higher than methods A and B and lower than in previous years. The average deviation of the mean participant percentage cover from ImageJ calculated values was 4.29%, indicating more of a tendency towards overestimation of percentage cover than was seen with Test A.

## 3.3 Seagrass Results

### *3.3.1 Seagrass Test A (Open Quadrat)*

As with the macroalgae, seagrass Test A had the lowest number of respondents, with six participants opting for this method. The results submitted showed more variation than the macroalgal test, with ranges between 1% and 42% per quadrat. The largest range of 42% was recorded for quadrat 6 with estimates varying between 28% and 70%. A further 4 quadrats had percentage cover ranges of 30% and only two quadrats had ranges below 10%. The average range across all participants and quadrats was 22%, which is lower than the RT12 results, although there were more than twice as many participants (15) for this test RT12.

Z-scores calculated using the population mean only resulted in one ‘fail’, for quadrat 4. This appears to be due to the very low standard deviation, as the actual result differed from the mean by less than 1%. In total there was a 98.89% pass rate for test A when using z-scores derived from the mean, which is consistent with last year. When comparing results against percentage cover calculated using ImageJ the number of 'fails' was 21, a pass rate of 76.67%. Five of the participants had between two and three 'fails' and one participant 'failed' seven quadrats, with consistent overestimation of percentage cover compared to the ImageJ results.

The average deviation of results from the mean and image analysis percentage cover per laboratory ranged from 2.67 to 13.64 and 4.27 to 17.60 respectively. These deviations are higher than those recorded for macroalgae Test A but are consistent with seagrass test results from previous years. The average deviation of the mean participant percentage cover from ImageJ calculated values was 3.96%, indicating a tendency towards overestimation of percentage cover.

### *3.3.2 Seagrass Test B (5 x 5 Gridded Quadrat)*

Test B had two more respondents than Test A, giving a total of 8 participants opting to complete the 5 x 5 square grid quadrat method, in contrast to previous years where this has consistently been the least popular method. The range of results for this test was narrower than for Test A, varying between 0 and 34% with a mean range of 16.9%. Quadrat 2 had the highest range of results, with estimations varying between 34% and 68% cover. Four of the quadrats had ranges of less than 10% cover between participants, five had ranges between 10 and 20% cover and a further five ranged from 20-30% cover between participants.

Comparing z-scores against mean percentage cover resulted in three 'fails' between two participants, with an overall pass rate of 97.5%. In comparison, the total number of 'fails' when comparing against ImageJ results was much higher at 28 and was distributed amongst all eight participants. The overall pass rates using ImageJ analysis percentage cover was 76.67%, which is consistent with the results of Test A, but lower than previous years.

The deviation from mean percentage cover (2.58 - 9.06) was slightly lower than the previous year. Deviation from ImageJ analysis (4.63 - 12.05) was slightly higher than deviation from the mean but was consistent with the previous year's results. The average deviation of mean participant results from ImageJ analysis values was lower than for test method A, at 2.41 but still indicates a general trend of overestimation in comparison to the ImageJ analysis percentage cover values.

### *3.3.3 Seagrass Test C (9 x 9 Crosshairs Quadrat)*

Test C was the most popular method, having one more participant than Test B. The percentage cover ranges were much higher for test C than for either Test A or B, varying from 0% to 45%. Only one quadrat had a range of less than 10% cover between participants, four had ranges between 10 and 20% cover and a further four ranged from 20-30% cover between participants. Six quadrats had ranges of 30% or above, with the highest range for quadrat 2, which had results varying between 27% and 72% cover.

Comparison of z-scores calculated from the mean resulted in two 'fails' distributed between two participants, giving a total pass rate of 98.5%. Comparing results against the ImageJ calculated values gave 29 'fails' with a pass rate of 78.52%. One participant 'failed' ten quadrats, followed by another that 'failed' six quadrats. The remaining participants 'failed' between one and three quadrats each. All of the 'fails' were overestimates compared to the ImageJ analysis calculated values.

Deviation from mean percentage cover varied between 3.51 and 13.9, which was comparable to Test B and a decrease from the previous year. The deviation from the ImageJ analysis values (4.54 – 21.40) is higher than the deviation from the mean but is also a decrease from the previous year. The average level of deviation between percentage cover estimates and image analysis across all quadrats and participants was 7.46 suggesting a high level of overestimation of percentage cover compared to ImageJ results.

# 4. Discussion

The percentage cover of opportunistic macroalgae or seagrass in 0.25 m² quadrats is usually estimated in the field based on a skilled eye observation using either an open quadrat or gridded quadrat with an aim of variance of less than 5% between surveyors. It is highly unlikely that this method of percentage cover estimation is 100% accurate due to the subjectivity of individuals, although over time people can become highly skilled. It is difficult to establish an unambiguous 100% reliable method for determining percentage cover of opportunistic macroalgae or seagrass. Based on the methodology established in previous ring tests, OMC RT13 used the population mean and an image analysis software program (ImageJ) to calculate a more objective percentage cover for comparison with individual participants' results. The use of image analysis software is considered to provide less subjectivity than skilled eye estimations.

The exact methodology used to prepare and analyse images in ImageJ in previous ring tests was not specifically defined, and the program offers multiple possible techniques for the calculation of percentage cover. These include manual definition of areas of macroalgae/seaweed, defining areas based on colour thresholds or converting the image to binary (i.e. black and white) based on a defined contrast threshold. The resulting calculation of percentage cover therefore still has scope for variability depending on the settings used and in particular the way the selection threshold is defined. The methodology described by Xiong *et al*. (2019) for determination of vegetation cover was used for the current year. This involved defining the areas of macroalgae or seagrass cover by adjusting hue, saturation and brightness threshold settings to match the observed areas of macroalgae or seagrass.

Z-scores were used to establish a level of acceptance for results submitted by participants following the same methodology used in previous ring tests. Separate z-scores were calculated using both the mean percentage cover per quadrat recorded by participants and the percentage cover as calculated using ImageJ analysis. The results could then be compared between participants and between methodologies of cover estimation for both macroalgae and seagrass. As in previous years the number of 'fails' was much higher when comparing results against ImageJ analysis values rather than against the population mean. This is unsurprising given that the mean is calculated directly from the participant data whereas the ImageJ value is derived from independent analysis. This was apparent across all three tests for both macroalgae and seagrass. This indicates either a lack of accuracy in percentage cover estimations or inaccurate percentage 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. This does not negate the value of using ImageJ analysis for comparison as this still the most objective method determined so far and using the mean is naturally going to allow more estimates to sit within the z-score +/- 2.00 range.

The degree of deviation from the image analysis percentage cover value also varied considerably between quadrats, with some proving more problematic than others. Three of the macroalgae quadrats had mud overlying some of the macroalgae, which whilst not unrealistic of conditions likely to be encountered in the field, creates difficulty in using automated colour recognition software and as such resulted in underestimates for the ImageJ values for these quadrats.

The range of results was generally lower this year than in previous years, but still much greater than the recommended +/- 5% between surveyors, with some quadrats having estimated ranges up to 45%. The lower range in current year may be at least partly due to the lower number of submissions compared to previous years. The average range per test ranged between 8.13 and 13.09 for macroalgae and between 16.90 and 25.77 for seagrass. One of the limitations of using z-scores is that high result ranges give high standard deviation values, which reduces the chance of achieving a ‘fail’ based on the resulting +/- 2.00 cut-off value. These results, along with those from previous years require further examination to improve the methodologies employed and the means in which the percentage cover is calculated both by field methods and ImageJ analysis.

In previous years it was observed that quadrats with either a very high or low percentage cover have been easier to accurately estimate total cover, whereas quadrats with a percentage cover in the middle range (30 – 70%) generally result in a higher level of deviation with a much broader range of results. This trend continues in the current year and could be attributed to the patchier coverage of opportunistic macroalgae and seagrass in some quadrats which is much harder to estimate accurately. There is also a broader range of percentage cover estimations and deviations for both the mean and ImageJ analysis for seagrass than for macroalgae. Seagrass displays a much patchier nature of growth; its thin long strands often make it difficult to estimate percentage cover leading to a broader range of results and high levels of deviation.

Figure 1 and Figure 2 (overleaf) show this range of percentage cover results for macroalgae and seagrass, respectively. These scatter graphs indicate that in general Test B for both macroalgae and seagrass had smaller ranges of results across participants, whilst Test C had the greatest ranges. Overall, the broad range of percentage cover estimates submitted by participants is concerning in terms of consistency between laboratories as well as within laboratories.

There are noticeable differences both between seagrass and macroalgae results and between the different methods of estimation used and the resulting number of ‘fails’ (Figure 3). The macroalgae results for comparisons against the mean and comparisons against ImageJ both had the highest number of fails for Test C and lowest for Test A. The seagrass results also had lowest numbers of ‘fails’ for Test A but had less difference between Tests B and C than was evident in the macroalgae results. For comparisons against the mean, seagrass had slightly more ‘fails’ for Method B than C, whilst for comparisons against the ImageJ results the number of ‘fails’ was slightly higher for Test C.

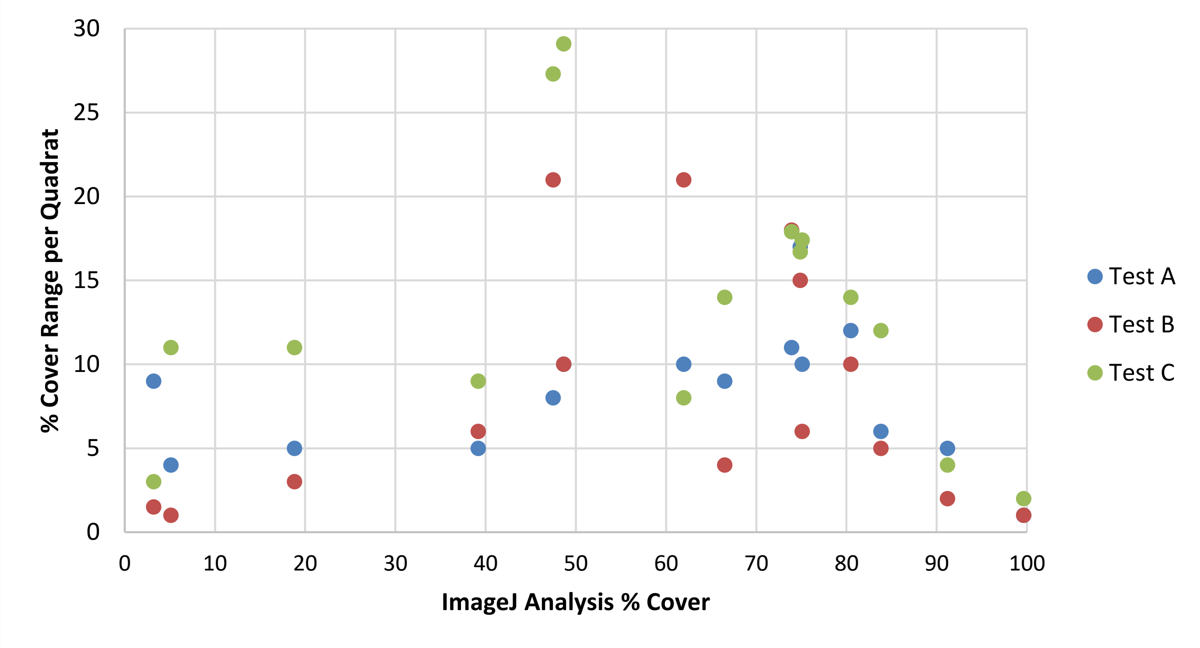


**Figure 1** **Scatter graph showing the range of percentage cover results per quadrat across all three opportunistic macroalgae test methods**

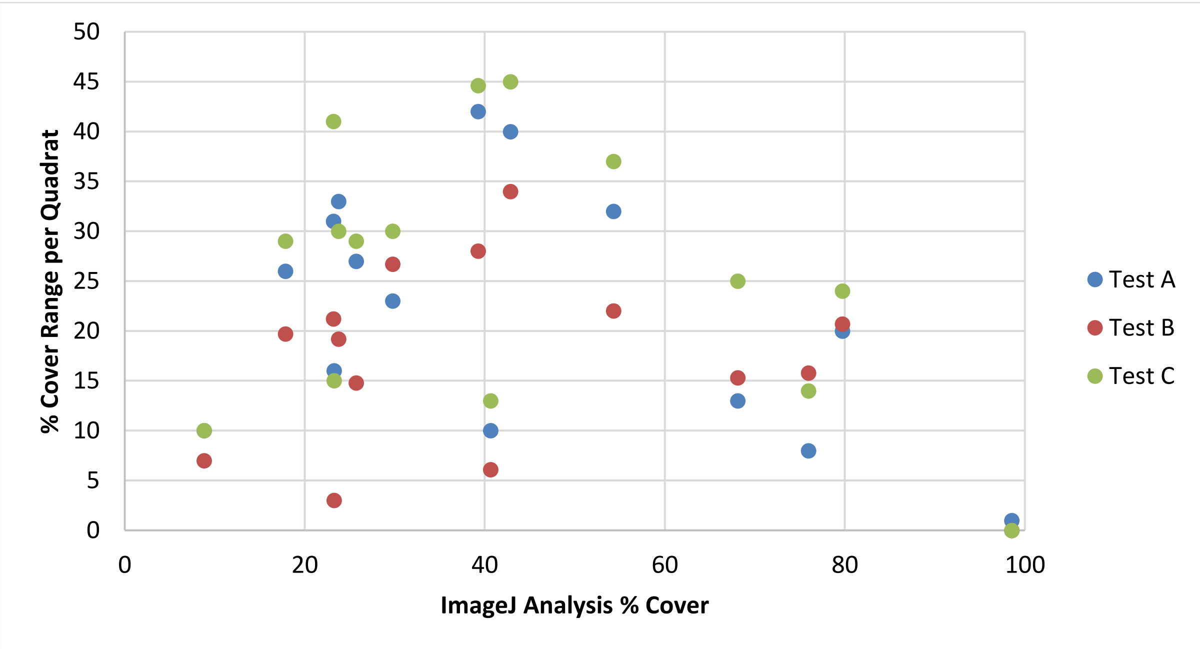


**Figure 2** **Scatter graph showing the range of percentage cover results per quadrat across all three seagrass test methods**

There do not appear to be consistent patterns of variation in results between years, although direct comparisons are hampered by the variations in numbers of participants each year. Figure 4 shows the results from the OMC RT12 for comparison. The number of 'fails' against the mean were higher than the current ring test, whilst the number of 'fails' against ImageJ were lower. There were also differences in the results for the different methods, with a higher number of 'fails' against the mean for Test A than Test B for both seagrass and macroalgae. The comparisons against ImageJ were the inverse of the current year for macroalgae, with the highest number of 'fails' for Test A and the lowest for Test C.

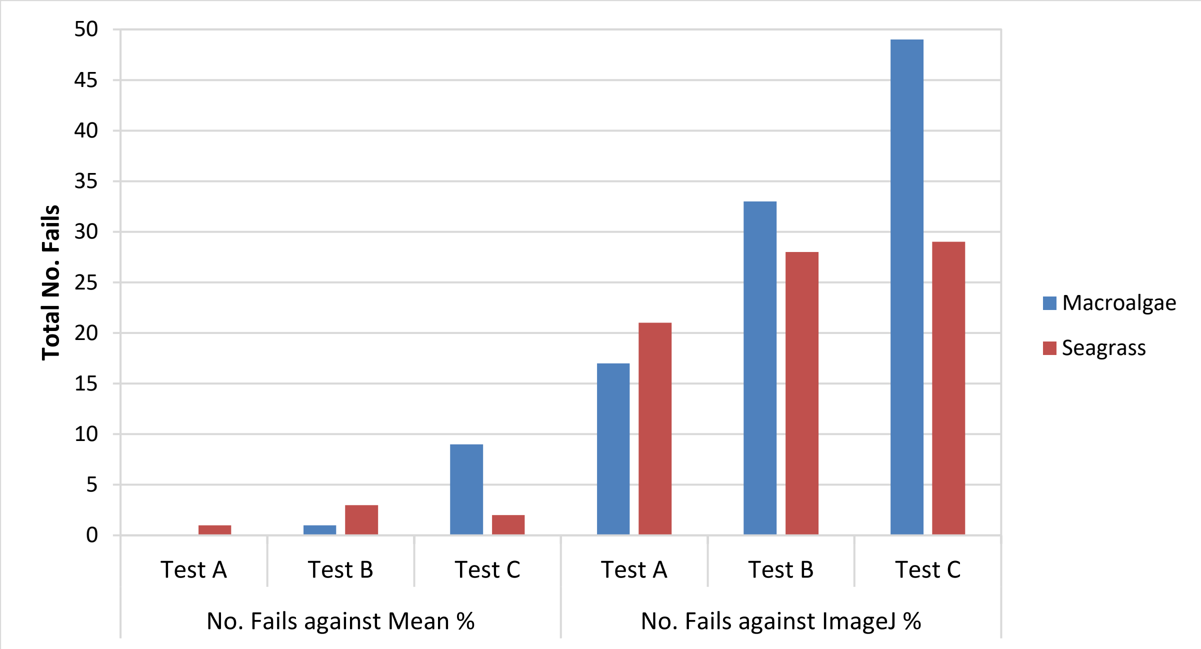


**Figure 3** **Number of fails recorded in each test for macroalgae and seagrass quadrats for OMC RT13.**

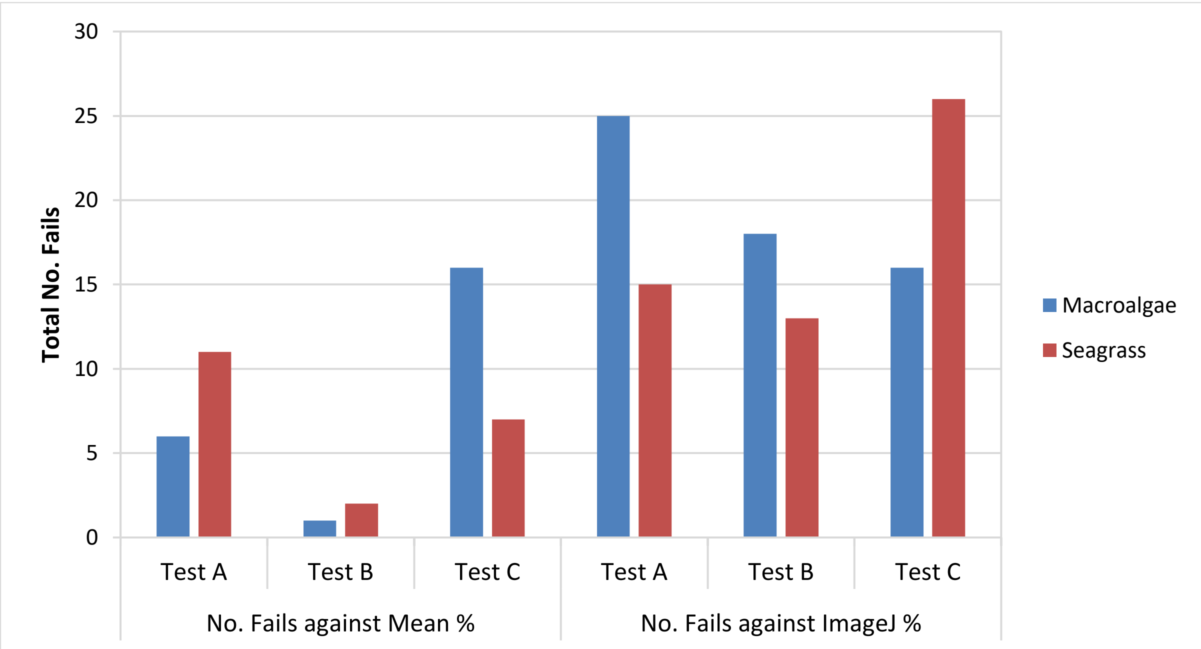


**Figure 4** **Number of fails recorded in each test for macroalgae and seagrass quadrats for OMC RT12.**

Test C still appears to be the preferred methodology, which is consistent with previous ring tests. However, the current year also saw a slight preference for Test B over Test A, in contrast to previous years. This may be related to the reduced number of participants in the current year and the preferred methods of their respective laboratories. Some participants also cited time restraints preventing them repeating the tests using alternative methods.

In theory test method C should provide the least 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 large disparity in results, often much higher than for the other test methods that may suggest the method is not being used consistently between participants. One participant commented that for some images it was unclear if seagrass or macroalgae is present under a particular crosshair and in the field the quadrat could be lifted to confirm this whereas with only an image it can remain ambiguous.

Not all participants completed all three different test methods, which makes it difficult to differentiate variance between methodologies from variance between individual participants. This makes it harder to conclude which of the test methods is the most accurate and consistent as it is highly dependent upon the participant and the sample size. The broad range of results across participants and laboratories is a problem that needs to be addressed either by adopting a less subjective means of estimating percentage cover of macroalgae or seagrass, or by holding a field meeting with which to synchronise such methodologies and reduce the high degree of variation currently being recorded within the NMBAQC macroalgae and seagrass percentage cover tests. The current test methods do not provide an obvious 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.

## 5. Conclusions and Recommendations

1. There is still a high degree of variation in results both between tests and between participants and this may prompt the need for a specific workshop whereby methods can be discussed, and possibly percentage cover estimations compared in the field. It is not possible from the current ring test to conclude which percentage cover estimation method provides the most accurate results; however, based on the dominant proportion of the data returns, during OMC RT13 Test method C was the most favoured method for both macroalgae and seagrass.

2. There are still large differences 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 RT13 aims to provide a more objective result than skilled eye estimation. However, the precise methodology used to prepare the images and calculate the percentage cover in ImageJ is still being investigated and will continue to undergo further refinement prior to the next round of tests. It is recommended at this time that participants should continue to use the z-scores

derived from comparisons with the mean if they are required for internal quality reports.

4. During this thirteenth cycle of the macroalgae percentage cover exercise nine of the eleven laboratories were able to complete the ring test within the allocated timescale. It is appreciated that conflicts with work and the ongoing delays caused by the Covid-19 pandemic may have prevented laboratories from meeting the deadline. 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 timescale. In subsequent years reminders will continue to be distributed one week prior to the completion of the exercise to ensure the deadline is met. 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. It was commented that during field sampling it may be possible to estimate percentage cover of opportunistic macroalgae with less ambiguity than when using photographs. The nature of the photographs can produce difficulties when assessing the density of the macroalgae due to differences in lighting/weather and the presence of some shadows/reflections or sediment over the macroalgae can hinder this further. However, it is to be noted that in their natural conditions many seagrass beds remain waterlogged regardless of tidal height and sun reflection may be unavoidable. In future ring tests attempts will be made to ensure reflections and sediment over algal fronds in the photographs are minimised as far as is possible.

6. This year all participants who submitted results 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 to 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.

7. This year the test material was distributed via an emailed link to a file sharing website. None of the participants reported any difficulties in accessing the test material in this way. This method both reduces unnecessary postage and ensures arrival of the test materials on the designated start date.

8. The feedback forms indicated that there is currently insufficient time or resources to complete all three test methods. Having results for the same participants across all three test methods would allow for a more direct comparison of the methods used and the results obtained. It is still recommended that all participants try to complete all three methods where possible.

9. There was a suggestion that some quadrats could contain a mix of seagrass and other macroalgae (i.e. not opportunistic macroalgae) as well as other substrata such as stones to represent a range of natural habitats in which these species might be found. At present the results are still highly variable there is no evidence to suggest that a combined quadrat would be of benefit and may further confuse the results. This would also create difficulties distinguishing seagrass from macroalgae in the ImageJ analysis. However, it is acknowledged that in the field there can be a mixture of seagrass and macroalgae in the same location.

If anyone has further thoughts on this, or disagrees with any of the interpretation, please forward your comments to nmbaqc@apemltd.co.uk. This ring test is now in its thirteenth year and although it has general approval, we are still very happy to receive feedback particularly suggestions on how it may be improved.

# 6. References

Xiong, Y., West, C.P., Brown, C.P. & Green, P.E. (2019). Digital image analysis of old world bluestem cover to estimate canopy development. *Agronomy Journal* 111(3), 1247-1253.