The National Marine Biological Analytical Quality Control Scheme
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Macroalgae/Angiosperms Component Macroalgae and Seagrass % Cover Module Report – OMC RT07 2016

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MACROALGAE/ANGIOSPERMS COMPONENT REPORT FROM THE CONTRACTOR SCHEME OPERATION –2015-16

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 seventh year in which % cover estimations of macroalgae have been included as an element of the NMBAQC scheme and the five year for which seagrass has been assessed as a separate entity. This included a single exercise for macroalgae and one for seagrass both of which were split into three smaller exercises based on methodology. The format followed that of previous years (RT01 – RT06). 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 laboratories completed the % cover macroalgae/seagrass component of the NMBAQC scheme with a total of 27 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 in order 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) in order 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 seventh 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 – RT06). 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. A number of results deviated from the sample mean and from the % cover as calculated by image analysis. However deviation from the latter was more noticeable. There was a much higher range of results submitted for seagrass which appears to be more difficult to estimate % cover and may be attributed, in part, to its patchy nature. Although there was a slight preference for using method C (10 x 10 square grid) for the macroalgae the results using this method was less accurate as seen in previous years.

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 is able to 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 2016. 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 RT06 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. Each photograph was ground-truthed at the time of collection with additional drawings of areal coverage produced on a grid scale to ensure % cover could be determined accurately subsequent to field analysis and during image analysis.

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

2.4.1 Method A

Method A was an open quadrat, this allowed the analyst to estimate the percent cover in a $0.25m^2$ quadrat without visual obstruction or assistance from gridlines. A general estimation is conducted looking solely at the total area within the quadrat that is clearly covered by the opportunist macroalgae or seagrass.

2.4.2 Method B

Method B split the $0.25m^2$ quadrat into 25 squares with each square representing 4% of the total quadrat. The percent cover was estimated by counting the number of squares, to the nearest half square, that were covered by macroalgae/seagrass. Completely covered squares were counted as one each. Between 50% and 100% cover in individual squares was estimated to the nearest quarter and these portions were summed. For quadrats with sparse macroalgae cover (i.e. always < 50% cover per square) the participants accumulated the small portions of algal coverage (totalling to the nearest half square). The number of squares was divided by 25 and then multiplied by 100 to give a percentage.

2.4.3 Method C

Method C consisted of a 9 x 9 crosshair quadrat. This method splits the quadrat into 100 squares. The crosshair referred to the point at which the gridlines cross and within a 9 x 9 grid amounts to a total of 81 crosshairs. The method of cover estimation was achieved by recording the presence or absence of algae/seagrass under each of the crosshair points. Where present this was recorded as 1 and absence was recorded as 0. The number of cross hairs with algae/seagrass present was divided by 81, and then multiplied by 100 to give a percentage.

2.5 Quadrat image analysis

An image analysis programme called ImageJ was used to achieve a more precise measurement of % cover which could be compared with the traditional means of assessment. The photographs were opened within the ImageJ program which distinguishes contrasts in colour/tone and is therefore able to compare the colour of the macroalgae against the background substrate. Prior to analysis the images were modified within photoshop to ensure a substantial colour contrast and enable the

program to pick up the differences. ImageJ converts the colour image to a greyscale which is later changed into binary form to highlight the thresholds. The entire quadrat is calibrated against a known measurement scale from which the highlighted area can be spatially analysed. A percent cover is calculated using the area of macroalgae cover against the area of the quadrat as calibrated in ImageJ. These percentages were used as a comparison against the skilled eye estimations as submitted by the participants.

A 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 three will be recorded as MA2312c.

2.8 Results

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

2.8.1 General Comments

In total ten laboratories signed up for the % cover component of the macroalgae/seagrass element for RT07. All ten laboratories returned data. Of those laboratories that did submit data 19 completed method A, 15 completed method B and 26 completed method C for the macroalgae component. For the seagrass component 20 completed method A, 13 completed method B and 20 completed method

C. Fifteen participants completed all three macroalgae and thirteen completed all three seagrass methods. The results have been collated and represented in various formats to enable full comparisons between participants and against % cover as calculated by the image analysis.

Details of each participating laboratory performance were distributed in the macroalgae OMC RT07 Bulletin Report and the seagrass OMC RT07 Bulletin Report, which represent a summary of the results for RT07. 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 19 participants and was the second most popular of the three methods. The range of results per quadrat varied considerably with the largest range of results produced for quadrats 2, 12 and 14 with a range of 40%. Quadrats 5, 11, and 13 all displayed a range of 35% all of which lay between 30% and 75%. The smallest range was for quadrat 3 from 1% to 5%, the remaining quadrats had % cover ranges of between 15 and 28. Z-scores calculated against the population mean resulted in six laboratories failing between 1 and 5 quadrats. In total there was a 94% 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 ImageJ was much greater than seen when using the population mean. Participants showed an average % cover deviation from image analysis % cover ranging between 4.9% and 13.02%. The pass rate was equally much lower using Z-scores derived from image analysis estimates of % cover with 18 out of 19 participants failing at least one quadrat. The overall pass rate was lower at 82.8%. These results were also consistent with those from RT07 with similar pass rates.

2.8.2.2 Test B Results (5 x 5 gridded quadrat)

Test B had the least number of participants with 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 4 quadrats. The largest range of % covers per

quadrat was a range of 40% cover recorded in quadrat 15 and 12 differing considerably from the results seen in test A. The lowest range of % cover estimates was for quadrat 3 which resulted in the same range (4%) as for test A.

Consistent with test A, test B also showed a higher degree of deviation from the image analysis results compared with the population mean, with all 14 out of 15 participants failing at least one quadrat and an overall pass rate of only 82% compared with a pass rate of 96% using Z-score from the population mean although this results is better than seen in previous years. The greatest number of 'Fails' could be attributed to quadrat 7, with 9 'Fails' followed by quadrat 1 and quadrat 6, with 6 and 5 'Fails' respectively. Despite these results method B resulted in similar levels of deviation from % cover as calculated by ImageJ and 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 29%, higher than in RT06, with quadrat 12 producing the highest range of 59% with % cover ranges between 25% and 84% ((it is unclear at this stage if this was a typing error or misuse of the methodology). Ten participants failed at least one quadrat using Z-scores from the mean with 5 participants failing 1 quadrat and 3 participants failing between 5 and 8 quadrats and an overall pass rate of 94%. There were also more 'Fails' using Z-scores from image analysis with all participants failing between 1 and 6 quadrats and an overall pass rate of 79%. Quadrat 1 had the greatest number of 'Fails' with 23 out of the 26 participants scoring higher than +/- 2.0, followed by quadrats 10 and 4, with 15 and 14 'Fails' respectively.

2.8.3 Seagrass Results from Participating Laboratories

2.8.3.1 Test A Results (open quadrat)

Test A consisted of 20 participants and as with the macroalgae this was the second most popular method. The range of results submitted per quadrat also varied considerably as with the macroalgae test. The largest range was for quadrats 1, 4, 9 and 12 with between 40 and 45 percent ranges, these quadrats all had image analysis % cover results of between 48% and 69% cover providing evidence that this mid range of % cover is difficult to estimate. No quadrats had a particularly small range of results even for those with very little cover. Z-scores calculated against the population mean resulted in seven people failing between 1 and 4 quadrats. In total there was a 94% pass rate for test A when using Z-scores derived from the mean.

When comparing results against % cover as calculated using ImageJ, the number of 'Fails' per laboratory was greater with a total number of 92 'Fails' (84% pass rate) with all participants failing at least three quadrats. Those quadrats with the highest number of 'Fails' were quadrats 8, 10, 13 and 14 with between 15 and 17 'Fails' each , contributing to 72% of all 'Fails'.. The average deviation of results from image analysis % cover per lab ranged from 6.7 to 18.2, which was higher than the average deviation per participant when derived from the mean (2.4 to 13.3).

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 similar level of variation as described for test A with most quadrats having % cover ranges in the order of between 20% and 40% indicating a high level of discrepancy between participants. Quadrat 1 had the largest range of between 46% and 81%. Comparing against mean % covers resulted in a just 5 'Fails' distributed between 3 labs with the number of 'Fails' being distributed between several quadrats. There was an overall pass rate of 97%. In comparison, the total number of 'Fails' using image analysis was higher at 50 and was distributed among all 13 participants. The overall pass rates using image analysis % cover was 74%. These results are consistent with previous ring tests with similar numbers of 'Fails' and pass rates.

Consistent with method A the overall deviation from the mean quadrat % cover and that calculated by image analysis was considerably different with a deviation from the mean ranging from 2.49% to 11.97% and deviation from image analysis ranging from 8.18% to 16.07.

2.8.3.3 Test C Results (9 x 9 crosshairs quadrat)

Test C had a total of 20 participants. The % cover ranges were much higher for test C than for tests B and C with all bar one quadrat having a % cover range between 20% and 52% indicating a much high level of discrepancy between participants again with quadrat 11 having the largest range of between 23% and 75%. Comparison of results against the mean resulted in 12 'Fails' with one participant having 8 'Fails ' and the remaining 4 'Fails' being distributed among 3 other participants. Comparing results against the image analysis resulted in 47 'Fails' with pass rates of 84% with all participants failing at least one quadrat.

Most 'Fails' against image analysis could be attributed to quadrat 13 which had a total of 19 out 20 participants failing. Although the range for this quadrat was relatively small between 79% and 99% and a mean of 91% it was much higher than the % cover as calculated by image analysis which was 71.13% causing significant deviation from image analysis.

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 RT07 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 a lesser degree of subjectivity than skilled eye estimation. The ImageJ program is able to select areas of cover based on the colouration, identified by depth of colour. Each of the quadrat photographs is enhanced prior to analysis using Photoshop to ensure maximum contrast between algae and substrate by selecting the areas of algal coverage and in this instance converting to a black and white scale. Once the two distinct colours have been identified within the ImageJ program it is able to

calculate the total area covered thus reducing the degree of subjectivity experienced with skilled eye evaluations. During this 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 people regularly produced a higher deviation from the mean and ImageJ results than other labs. As with previous years this provides some evidence that different methods of % cover estimation provide varying levels of success for the different participants, making it difficult to conclude which method is the best in terms of producing the most accurate result. It seems this is highly dependent upon the participant.

The degree of deviation from the image analysis % cover value depended significantly upon the quadrat. Some quadrats were more problematic than others; this was consistent with the range of % cover and could be partly attributed to the more patchy coverage of opportunist algae in some quadrats which is much harder to estimate accurately. It is evident, as in previous years, that those quadrats with a mid percent cover range generally resulted in a higher level of deviation (Figures 1 and 2) with less consistency between estimates. Those quadrats with either a very high or low percent cover appeared much easier to estimate accurately total cover. This trend is more evident from the macroalgae quadrats. For seagrass the lower % cover ranges clearly show a lesser degree of deviation between the mean and the image analysis but this is not so apparent for the higher % cover ranges where there is a high degree of scatter. This is likely to be due to the nature of seagrass which is often thin and patchy with long strands making it difficult to estimate the % cover.

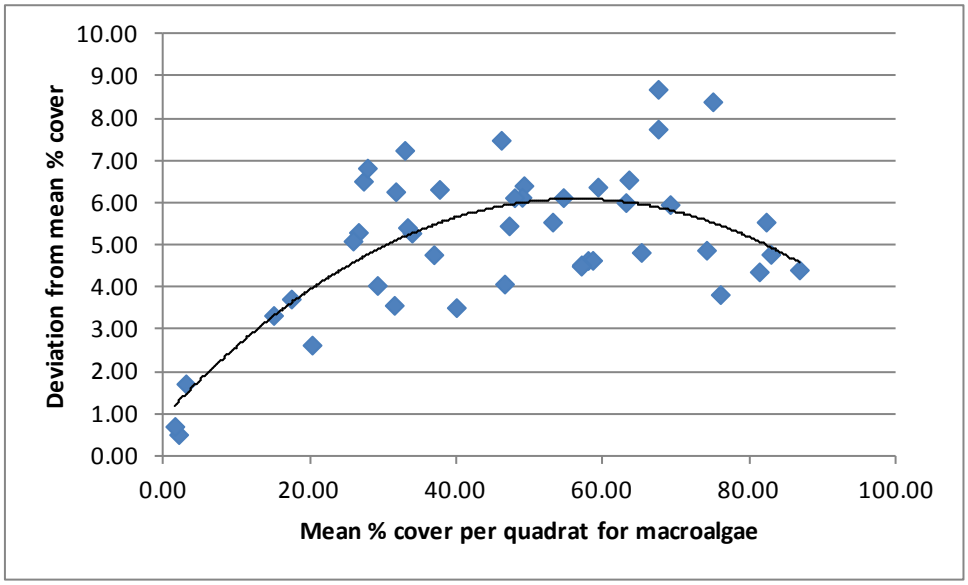

Figure 1: Scatter graph showing the mean level of deviation per quadrat from the mean % cover across all three test methods for macroalgae.

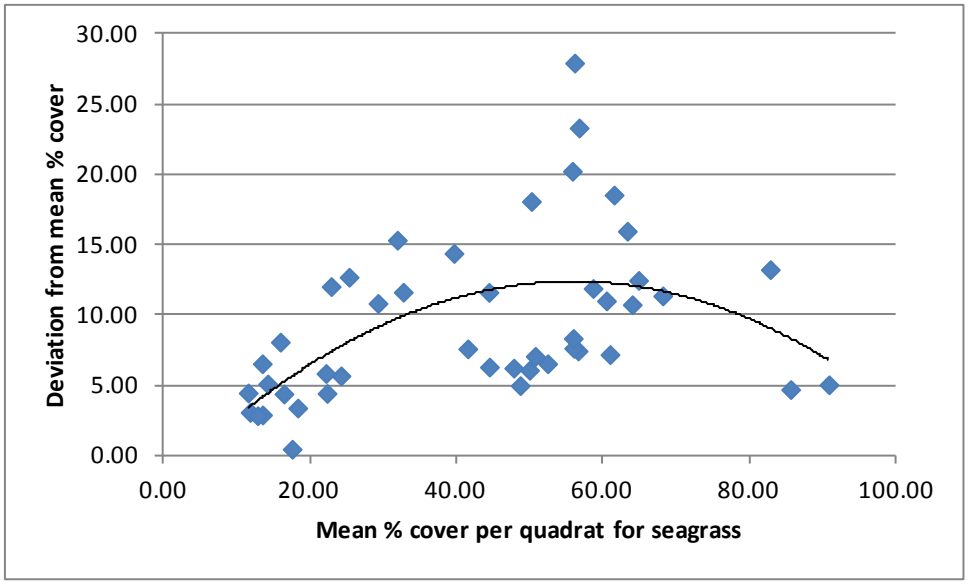

Figure 2: Scatter graph showing the mean level of deviation per quadrat from the mean % cover across all three test methods for seagrass.

There are also noticeable differences between the different methods of estimation used. Both the macroalgae and seagrass tests showed fewer 'Fails' in test B (5 x 5 square grid) when comparing against Z-scores from ImageJ and when comparing Z-scores from the population mean. Test C (10 x 10 square grid) continues to produce the least favourable results for both macroalgae and seagrass when comparing Z-scores from ImageJ but the pass rate is similar to Test A when comparing against the population mean.

In general the pass rate using Z-scores against image analysis showed a much higher number of 'Fails', in total this amounted to 171 and 189 within the macroalgae and seagrass tests respectively. This was significantly higher than when results were compared against the sample mean producing a total of 50 and 34 'Fails' for the macroalgae and seagrass respectively. This trend is also apparent across all years with image analysis z-scores consistently resulting in a higher number of 'Fails' compared with z-scores from the mean with the greatest number of 'Fails' consistently being recorded from test C (10 x 10 square grid) (Figures 5 and 6), particularly when compared against image analysis.

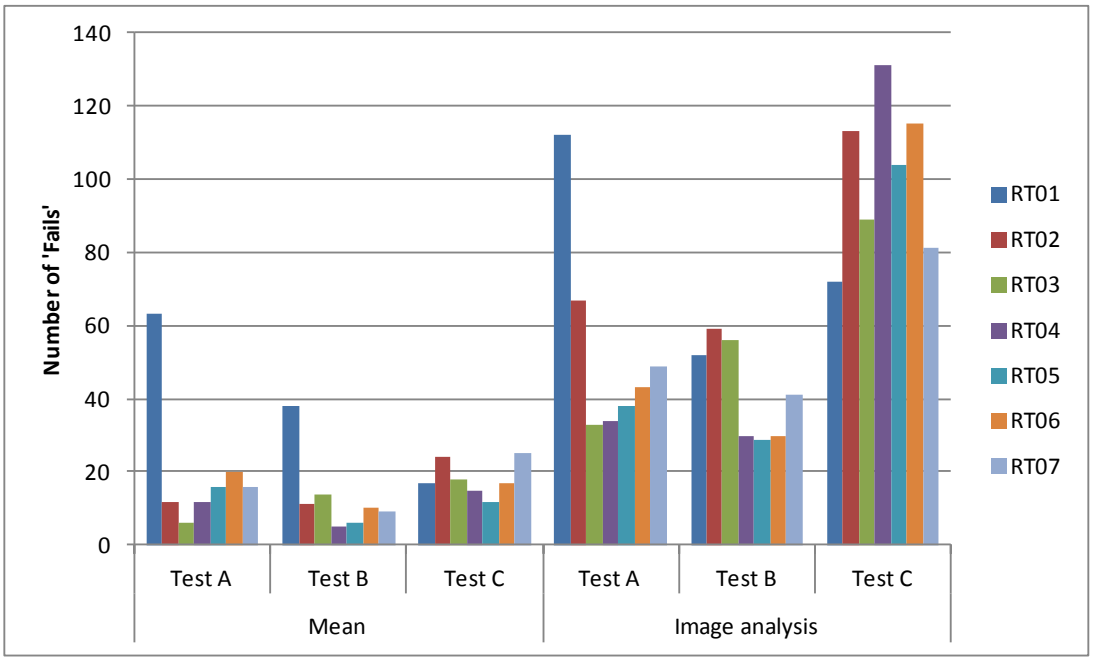

Figure 5: Number of 'Fails' recorded for each macroalgae test method from ring tests RT01 through RT07.

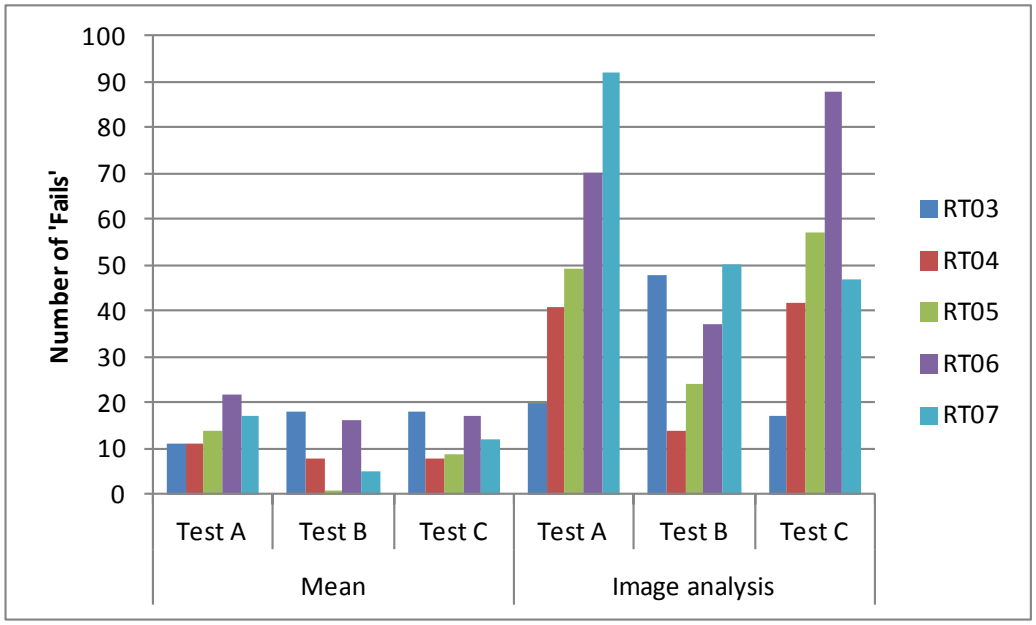

Figure 6: Number of 'Fails' recorded for each seagrass test method from ring tests RT03 through RT06.

The preferred test method is unclear although a greater number of participants completed tests A and C with both macroalgae and seagrass. This is also consistent with previous years and suggests this is the method most used by laboratories in the field. Most noticeable was the much higher number of 'Fails' associated with test C when comparing against the ImageJ analysis compared with all other tests. There is no definite explanation for this at present however the larger sample size will inevitably produce a broader spectrum of results further contributing to a higher number of 'Fails'. This year the number of 'Fails' was also much higher for macroalgae than seagrass. As seagrass is a lot patchier than macroalgae and can be much harder to estimate % cover, the higher range of results would have contributed to an overall higher standard deviation and would lessen the risk of achieving a 'Fail', based on the Z-scores. It is clear that these results along with those from previous years require further examination in order to improve the methodologies employed and the means in which the % cover is calculated both by field method and image analysis.

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 RT07 Tests A and C were the most favoured methods for macroalgae and seagrass.

2. There is still a high level of difference between z-scores calculated from the mean and z-scores calculated from image analysis results and given the varied levels of deviation between the two it is unclear which is the most accurate method from which to compare participants results.

3. The image analysis method used during RT07 is considered more objective than skilled eye estimation and likely to produce a more accurate result; RT07 also incorporated ground truthing to pick up subtleties of variations in cover within the defined affected area. However, this method is still under development and will continue to undergo improvements prior to the next round of tests. Despite this round incorporating a fully classified and ground truthed image analysis method with more accurate results it is recommended at this time that participants should use the Z-scores derived from comparisons with the mean if they are required for internal quality reports.

4. During this seventh cycle of the macroalgae % cover exercise all Laboratories completed the ring test within the allocated time period. All laboratories should continue to submit results within the requested deadlines as detailed at the beginning of the exercise. This is in both their own interests, and brings greater benefit to all participants in the scheme by increasing the dataset. In subsequent years reminders will continue to be distributed two weeks prior to the completion of the exercise to ensure the deadline is met, with a further reminder one week prior to the deadline. Any results submitted outside of this deadline will not be accepted and will not be included in the analysis.

5. Following consultation with current participants, it has been agreed that the tests are being distributed at the most appropriate time of year for the majority of labs, with a longer time scale within which to complete the exercises. Therefore tests will continue to be distributed early in

the New Year with a time limit of 6 weeks. It will remain the responsibility of the laboratory to ensure all results are submitted within the time provided.

6. It may be considered that during field sampling it may be possible to estimate % cover of opportunist algae with a higher degree of accuracy than when using photos. The nature of the photographs can produce difficulties when assessing the density of the algae and the presence of some shadows and the grids can hinder this further. This point has been highlighted by a couple of labs and in subsequent tests further efforts will be made to ensure this doesn't hinder the ability to accurately estimate the % cover. However, it is to be noted that many seagrass beds remain waterlogged regardless of tidal height and sun reflection may be a problem but all attempts will be made in the future to ensure clear photos are distributed with a broad range of % covers.

7. It has be noted that when using the 9 x 9 cross hair method it is difficult to keep orientated when zooming in and out to check cross hair points, therefore it has been 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.

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. 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.

10. 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.