The National Marine Biological Analytical Quality Control Scheme
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Ring Test Bulletin – OMC RT02

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RING TEST DETAILS

Ring Test - OMC 02

Type /Contents - % cover estimations of opportunist macroalgae

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Number of Participating Laboratories - 14

Total Number of Participants - 29

Number of Results Received Per Test - 17 (Test A), 27 (Test B), 14 (Test C)

*multiple data entries per lab submitted

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1 Introduction

There has been a quality control over the submission of biological data for a number of years. This has now been extended through all biological elements including macroalgae. Quality control ensures the consistency of data reported for environmental management purposes and has been primarily driven, within the sphere of marine plants, by international analytical standards due to the Water Framework Directive. The QC scheme aims to facilitate improvements in biological assessment whilst maintaining the standard of marine biological data. The scheme is able to ensure consistency between laboratories and field staff with improved confidence in ecological quality status.

The National Marine Biological Analytical Quality Control (NMBAQC) Scheme addresses two main areas relating to macroalgae and angiosperm data collection:

- The estimation of % cover
- The comparison of methodologies

This is the second year in which % cover estimations of macroalgae have been included as an element of the NMBAQC scheme and included a single exercise which was split into three smaller modules based on methodology. Test material was distributed to participating laboratories from which data forms were completed and returned.

Fourteen laboratories completed the macroalgae and angiosperm component of the NMBAQC scheme including a total of 29 participants. Thirteen of the participating laboratories were government organisations and only one was a private consultancy.

Laboratories were able to complete all three % cover methodologies or whichever procedure was most appropriate for their laboratory, however, they were encouraged to complete all three variations of the exercise.

Currently this scheme does not provide a means of qualifying performance levels. It offers 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. Certain targets have been applied to the assessment of the results based on Z-scores allowing “Pass” or “Fail” flags to be assigned accordingly; however, these have no weighting and merely act to identify those results which were considered significantly different based on comparisons between laboratories. These flags have no current bearing on the acceptability of data from such participating laboratories.

1.1 Summary of Performance.

This report presents the findings of the macroalgae component for the second year of operation within the National Marine Biological Analytical Quality Control (NMBAQC) Scheme. This component consisted of a single exercise which was subsequently split into three alternative means of assessment which could be considered as separate modules from which laboratories could complete one or more module.

The results for each of the methods within the one exercise are presented and discussed with comments provided on the overall participant performance and methods used.

2 Summary of Macroalgae Component

2.1 Introduction

There was one exercise for the assessment of % cover of macroalgae which took the form of three separate method 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 Logistics

The test material was distributed on CD to each laboratory. Each disc contained the three tests, description of methods and data submission forms. Participants were given a month to complete the test and return the results. 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.

2.3 Methods

The percent cover estimation test consisted of a set of 20 photographs. These quadrat photos 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 accurately determined subsequent to field analysis.

The set of 20 photographs were adapted to produce three tests that utilised different methods of % cover estimation.

1. **Test A** was an open quadrat, this method 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 opportunist macroalgae.

2. **Test B** 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 lines 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 under each of the crosshair points. Where alga was present under the crosshair this was recorded as 1 and absence was recorded as 0. The number of cross hairs with algae present was divided by 81, and then multiplied by 100 to give a percentage.

3. **Test C** method 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. 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. Where only a

small portion (i.e. <50%) of the square was covered these small portions were added until they equated to at least a half square. 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 Analysis and Data Submissions

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 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 algal species including *Ulva* sp., *Chaetomorpha* sp., *Porphyra* sp., *Ectocarpus* sp. and *Pilayella* sp and excluded any additional species that were present within the quadrat but were not considered opportunist algae. 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.

2.5 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 eighteen will be recorded as MA1812c.

2.6 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 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. The program puts the image into its binary form. The entire quadrat, and the area highlighted as macroalgal opportunist cover, were spatially analysed and a subsequent percent cover was calculated from the areas. 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 contrast. Image analysis has been conducted to demonstrate how the comparisons would work, but may require further modification and discussion as to its applicability.

2.7 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 GIS calculated % cover estimations. A summary of these results can be found at the end of the report.

2.7.1 Test A (Open Quadrat)

Table 1. Results for % cover estimations received from participating laboratories for test A.

Lab Code		MA1812a	MA1831	MA1806	MA1805a	MA1802a	MA1802b	MA1805b	MA1812b	MA1805c	MA1803a	MA1803b	MA1802d	MA1807a	MA1807b	MA1807c	MA1807d	MA1807e
Image analysis results																		
Test A	Algal coverage %																	
Quadrat 1	74.55	70	60	60	55	65	62	65	65	60	70	60	60	75	65	70	65	65
Quadrat 2	58.20	60	55	45	60	64	65	60	60	60	75	60	60	70	70	60	60	55
Quadrat 3	63.10	70	60	75	65	65	70	70	65	50	70	70	65	80	68	60	55	50
Quadrat 4	34.16	35	25	26	20	27	36	20	25	20	40	40	25	30	25	25	20	20
Quadrat 5	82.61	80	85	85	80	77	76	85	80	90	90	85	80	90	85	80	80	75
Quadrat 6	44.84	45	30	20	25	37	37	30	30	25	40	30	35	35	35	35	30	25
Quadrat 7	7.74	5	5	8	10	12	12	5	10	10	10	10	10	5	10	10	10	5
Quadrat 8	79.17	90	90	80	75	75	72	90	75	80	85	80	75	87	70	80	80	85
Quadrat 9	30.61	30	15	12	25	25	24	10	25	15	25	20	25	15	35	15	20	20
Quadrat 10	33.98	35	25	18	35	35	34	15	20	30	40	35	30	25	30	20	25	20
Quadrat 11	38.62	40	25	25	30	40	38	20	25	35	45	45	40	30	40	25	40	25
Quadrat 12	69.21	85	90	90	65	77	79	95	80	85	90	92	75	94	90	90	75	85
Quadrat 13	31.11	30	25	15	25	37	38	30	25	40	40	40	40	30	40	20	30	25
Quadrat 14	20.95	15	15	10	12.5	17	17	10	20	30	20	25	20	20	20	15	15	10
Quadrat 15	9.98	6	5	5	5	12	12	5	7.5	20	15	8	10	15	15	10	10	5
Quadrat 16	28.97	25	15	20	25	22	28	10	20	40	30	27	25	27	35	15	20	15
Quadrat 17	59.23	75	70	75	65	60	70	85	65	75	75	75	70	92	85	75	80	70
Quadrat 18	38.15	40	27	30	40	40	39	30	30	50	45	47	45	40	55	35	40	30
Quadrat 19	25.83	20	20	15	20	20	25	15	15	25	30	23	25	29	30	15	25	15
Quadrat 20	17.68	10	5	5	5	10	13	5	10	10	10	10	10	10	12	5	10	5

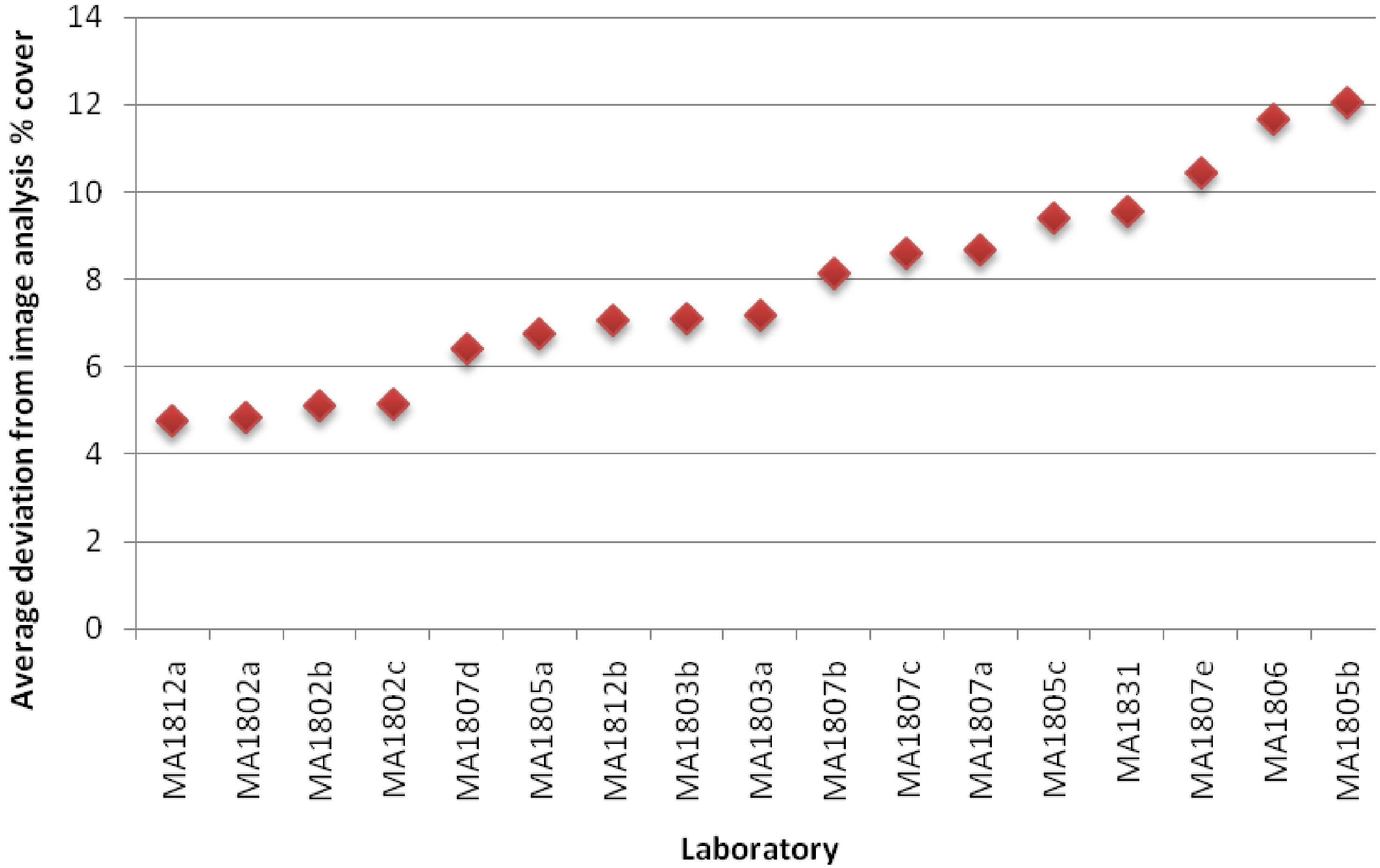


Figure 1. Difference in % cover between submitted results and image analysis results, displayed as the average difference across all quadrats in test A for each participant.

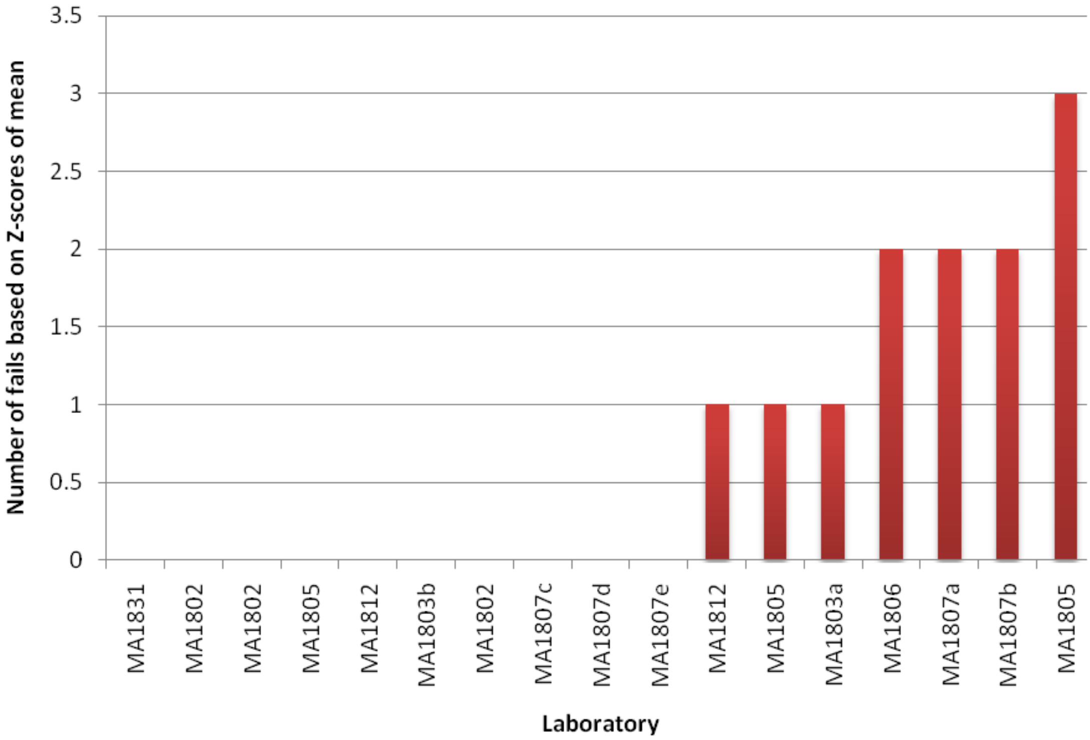


Figure 2. Number of quadrat fails per participant based on z-scores derived from mean % cover estimates for test A.

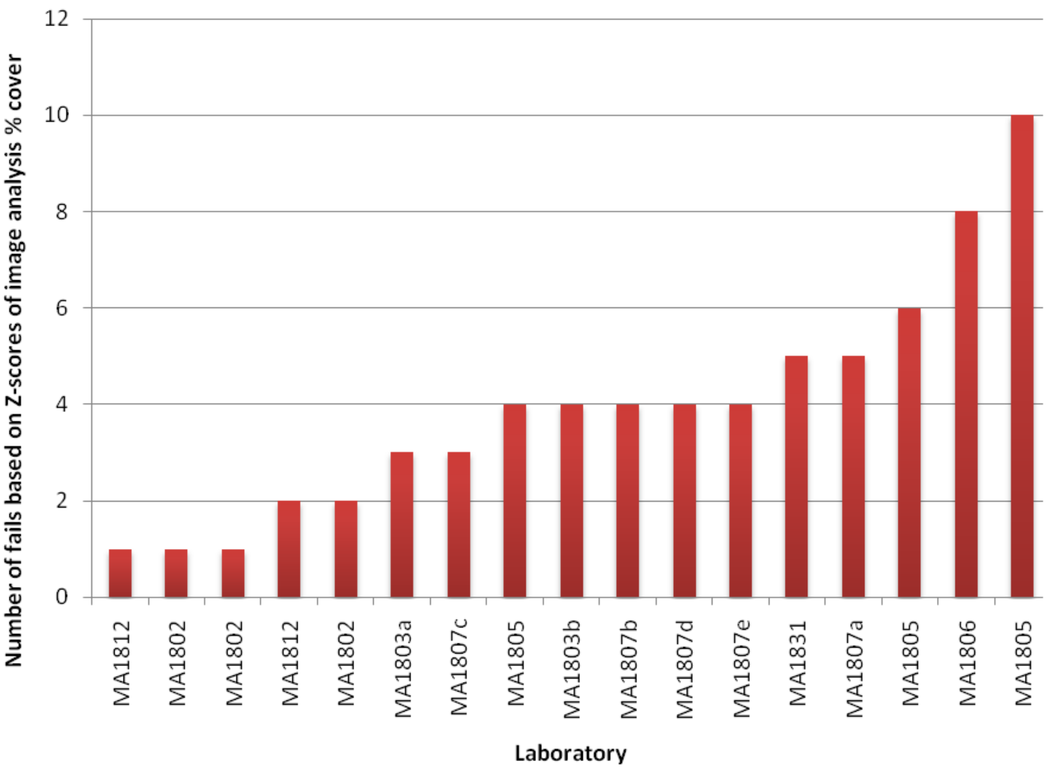


Figure 3. Number of quadrat fails per participant based on z-scores derived from % cover as calculated using image analysis for test A.

2.7.2 Test B (9 x 9 Gridded Quadrat)

Table 2. Results for % cover estimations received from participating laboratories for test B.

Lab Code Test B	Image analysis results Algal coverage %	MA1812a	MA1830a	MA1830b	MA1830c	MA1817a	MA1817b	MA1831	MA1806	MA1805a	MA1802a	MA1802b	MA1805b	MA1812b	MA1811	MA1818	MA1805c	MA1802c	MA1802d	MA1801	MA1809	MA1803a	MA1803b	MA1803c	MA1807a	MA1807b	MA1807d	MA1807e
Quadrat 1	74.55	65	75	74	63	79.0	80.2	64	78	60	60	64.2	67	65.4	69	72	70	66	63	67	65.4	79.0	75.3	68	54	73	80	80
Quadrat 2	58.20	74	77	76	65	82.7	82.7	75	81	55	64	71.6	74	72.8	74	78	79	70	65	69	72.8	81.5	84.0	84	52	76	70	77
Quadrat 3	63.10	75	69	69	67	76.5	84.0	69	80	53	66	72.8	73	64.2	70	70	79	69	65	70	67.9	82.7	80.2	89	54	74	75	76
Quadrat 4	34.16	40	37	43	33	56.8	59.3	35	63	31	26	45.7	28	40.7	46	46	50	36	33	33	38.3	54.3	56.8	56	29	48	25	47
Quadrat 5	82.61	89	85	84	83	90.1	92.6	83	90	76	76	81.5	88	85.2	85	89	93	83	79	84	86.4	90.1	93.8	90	70	89	90	91
Quadrat 6	44.84	48	44	48	39	58.0	60.5	48	60	31	35	39.5	49	48.1	44	52	53	43	41	47	50.6	53.1	56.8	56	33	52	40	54
Quadrat 7	7.74	14	11	10	6	11.1	11.1	7	15	6	9	11.1	10	8.6	10	11	15	7	6	15	12.3	13.6	14.8	12	8	9	8	9
Quadrat 8	79.17	84	81	88	79	92.6	91.4	79	89	73	74	80.2	85	77.8	85	89	90	78	75	80	81.5	86.4	88.9	89	72	93	90	85
Quadrat 9	30.61	31	32	36	25	34.6	32.1	25	43	20	20	28.4	22	27.2	35	33	41	20	22	28	30.9	35.8	35.8	49	21	36	25	31
Quadrat 10	33.98	38	37	37	38	40.7	39.5	37	37	35	37	38.3	37	32.1	40	37	40	37	35	38	38.3	39.5	40.7	41	36	40	33	41
Quadrat 11	38.62	43	39	49	43	56.8	56.8	38	59	32	30	44.4	41	43.2	46	51	48	39	37	44	48.1	51.9	53.1	64	30	52	30	36
Quadrat 12	69.21	93	84	93	83	92.6	93.8	91	96	72	82	86.4	81	84.0	88	93	93	85	75	79	84.0	88.9	92.6	95	76	93	85	86
Quadrat 13	31.11	43	38	40	36	49.4	44.4	37	53	30	32	38.3	37	35.8	41	43	49	37	30	40	33.3	44.4	45.7	59	33	41	30	38
Quadrat 14	20.95	25	22	28	22	23.5	28.4	22	33	11	17	22.2	21	23.5	30	28	32	21	24	26	28.4	28.4	35.8	38	18	27	15	22
Quadrat 15	9.98	16	13	17	16	14.8	17.3	13	26	7	13	16	16	11.1	15	16	24	12	11	17	21.0	22.2	18.5	22	15	19	10	16
Quadrat 16	28.97	32	29	30	28	34.6	30.9	27	51	24	17	28.4	25	27.2	33	37	38	26	25	30	34.6	33.3	34.6	51	25	32	20	30
Quadrat 17	59.23	79	72	72	67	77.8	81.5	69	85	67	59	76.5	68	82.7	79	78	85	67	61	69	77.8	82.7	85.2	85	67	79	75	76
Quadrat 18	38.15	51	50	47	47	59.3	61.7	45	60	38	35	46.9	49	48.1	49	54	53	38	37	47	53.1	56.8	55.6	65	40	51	36	48
Quadrat 19	25.83	33	32	35	27	32.1	34.6	31	40	23	27	29.6	27	32.1	28	33	36	30	24	32	30.9	34.6	40.7	42	25	37	22	30
Quadrat 20	17.68	12	15	17	11	16.0	19.8	7	20	7	8	14.8	9	12.3	15	21	17	7	10	14	17.3	16.0	17.3	27	7	8	7	9

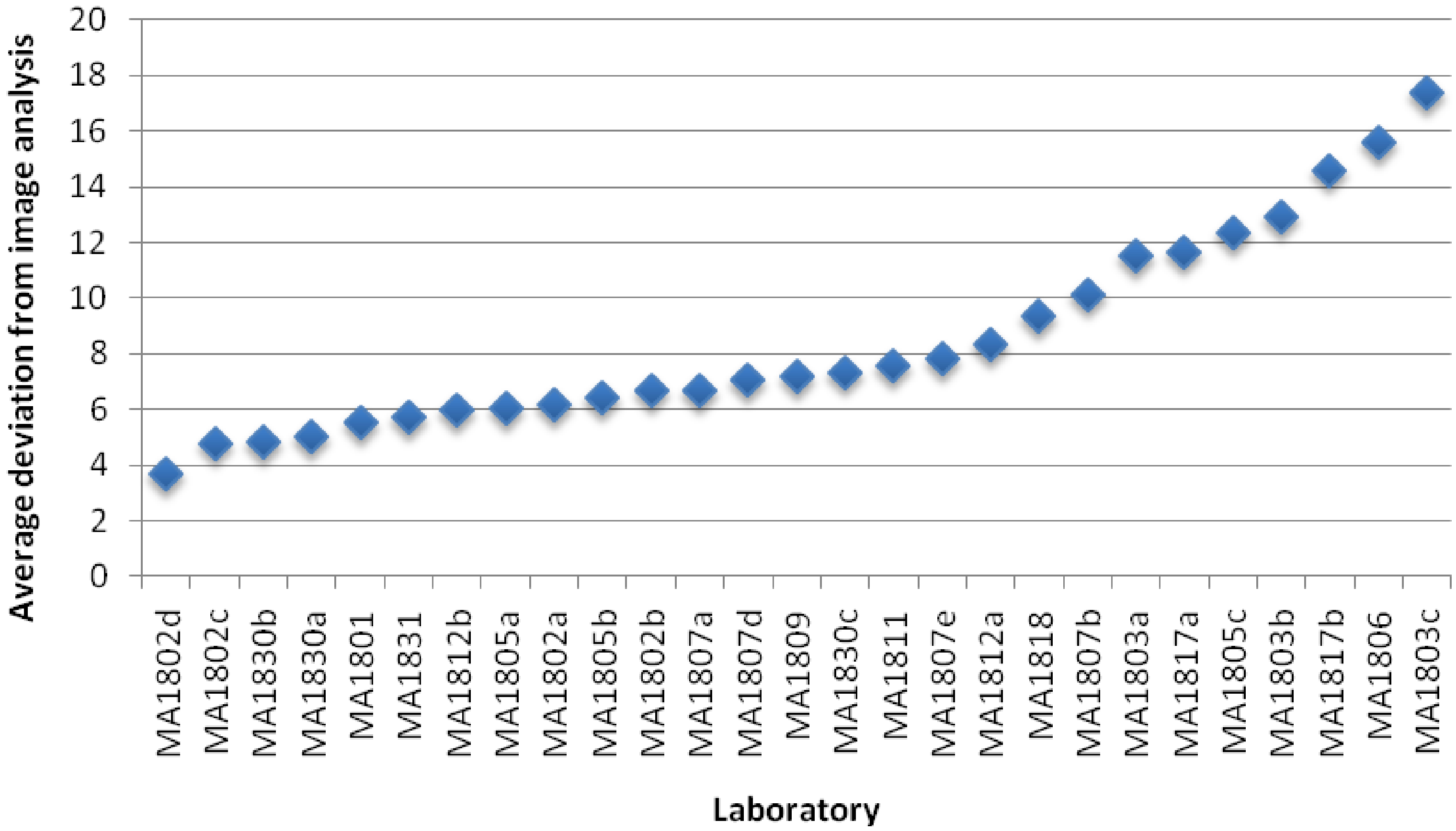


Figure 4. Difference in % cover between submitted results and image analysis results, displayed as the average difference across all quadrats in test B for each participant.

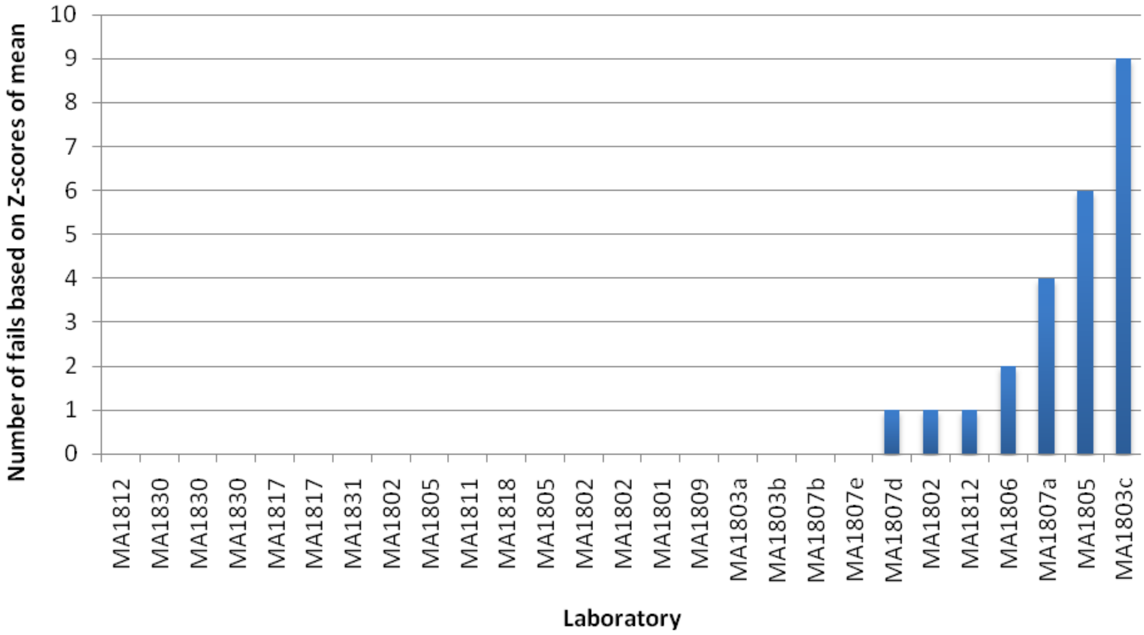


Figure 5. Number of quadrat fails per participant based on z-scores derived from mean % cover estimates for test B.

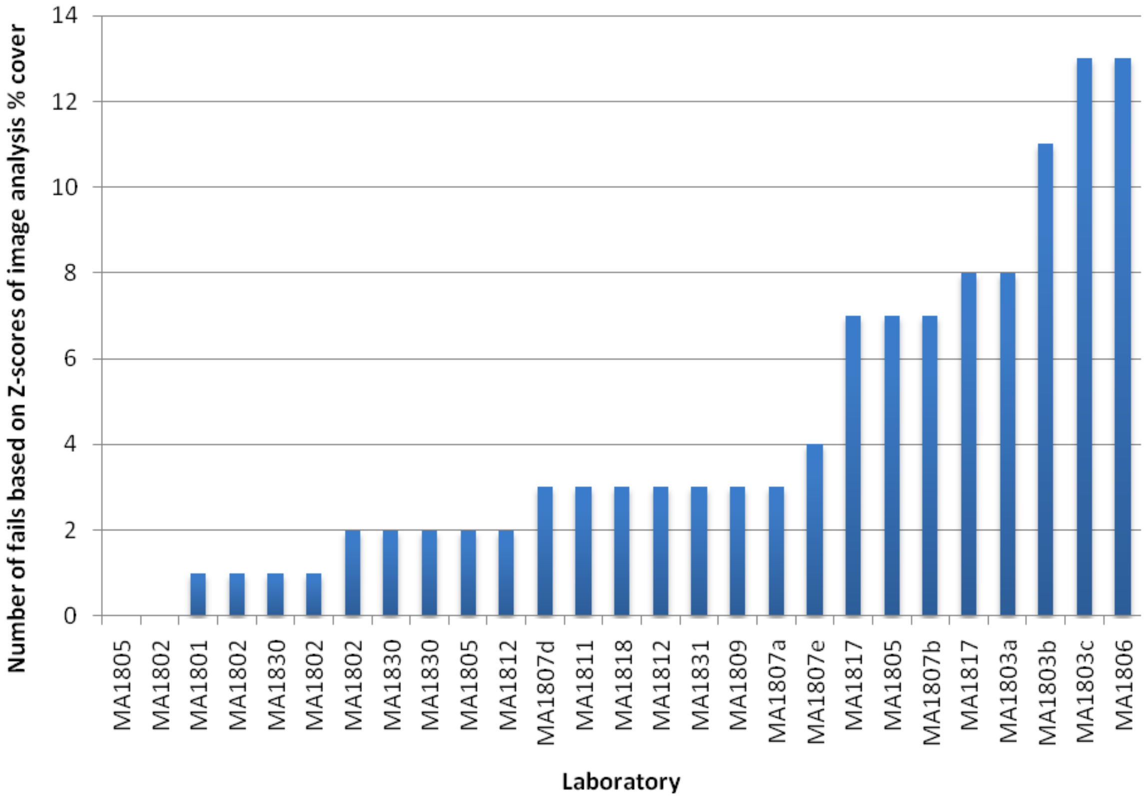


Figure 6. Number of quadrat fails per participant based on z-scores derived from % cover as calculated using image analysis for test B.

2.7.3 Test C (4 x 4 Gridded Quadrat)

Table 3. Results for % cover estimations received from participating laboratories for test C.

Lab Codes		MA1812a	MA1831	MA1810	MA1805a	MA1805b	MA1812b	MA1803a	MA1803b	MA1805c	MA1807a	MA1807b	MA1807c	MA1807d	MA1807e
Test C	Image analysis results Algal coverage %														
Quadrat 1	74.55	64	52	80	52	68	44	66	48	52	52	68	55	70	60
Quadrat 2	58.20	58	48	72	50	62	48	66	56	52	52	74	45	80	52
Quadrat 3	63.10	69	46	72	58	71	52	69	58	60	68	70	65	75	48
Quadrat 4	34.16	41	22	28	26	32	29	33	32	28	28	35	20	30	24
Quadrat 5	82.61	88	76	88	76	85	68	81	82	84	72	85	85	80	80
Quadrat 6	44.84	45	28	36	28	36	30	38	40	32	28	42	30	40	26
Quadrat 7	7.74	8	8	8	6	10	4	8	8	12	8	15	5	8	6
Quadrat 8	79.17	81	60	84	70	82	66	82	72	80	64	85	80	85	82
Quadrat 9	30.61	29	18	28	22	27	22	29	30	24	24	35	20	24	24
Quadrat 10	33.98	35	30	32	34	30	26	35	32	34	36	35	30	24	24
Quadrat 11	38.62	41	26	32	30	33	32	38	36	42	32	46	30	36	28
Quadrat 12	69.21	79	84	88	72	81	68	87	80	84	76	90	80	80	88
Quadrat 13	31.11	42	30	28	32	31	28	41	40	38	32	40	25	32	26
Quadrat 14	20.95	22	14	12	12	13	12	20	20	20	12	20	15	16	14
Quadrat 15	9.98	10	8	8	6	6	4	14	10	12	8	20	7.5	8	6
Quadrat 16	28.97	26	24	24	20	18	30	26	24	24	20	30	15	16	20
Quadrat 17	59.23	70	60	76	66	64	60	73	68	76	72	77	75	72	72
Quadrat 18	38.15	37	30	36	34	32	36	43	38	34	32	55	35	28	34
Quadrat 19	25.83	23	18	22	20	20	16	27	24	20	20	35	15	20	18
Quadrat 20	17.68	11	10	6	6	6	6	14	10	10	4	15	4	8	6

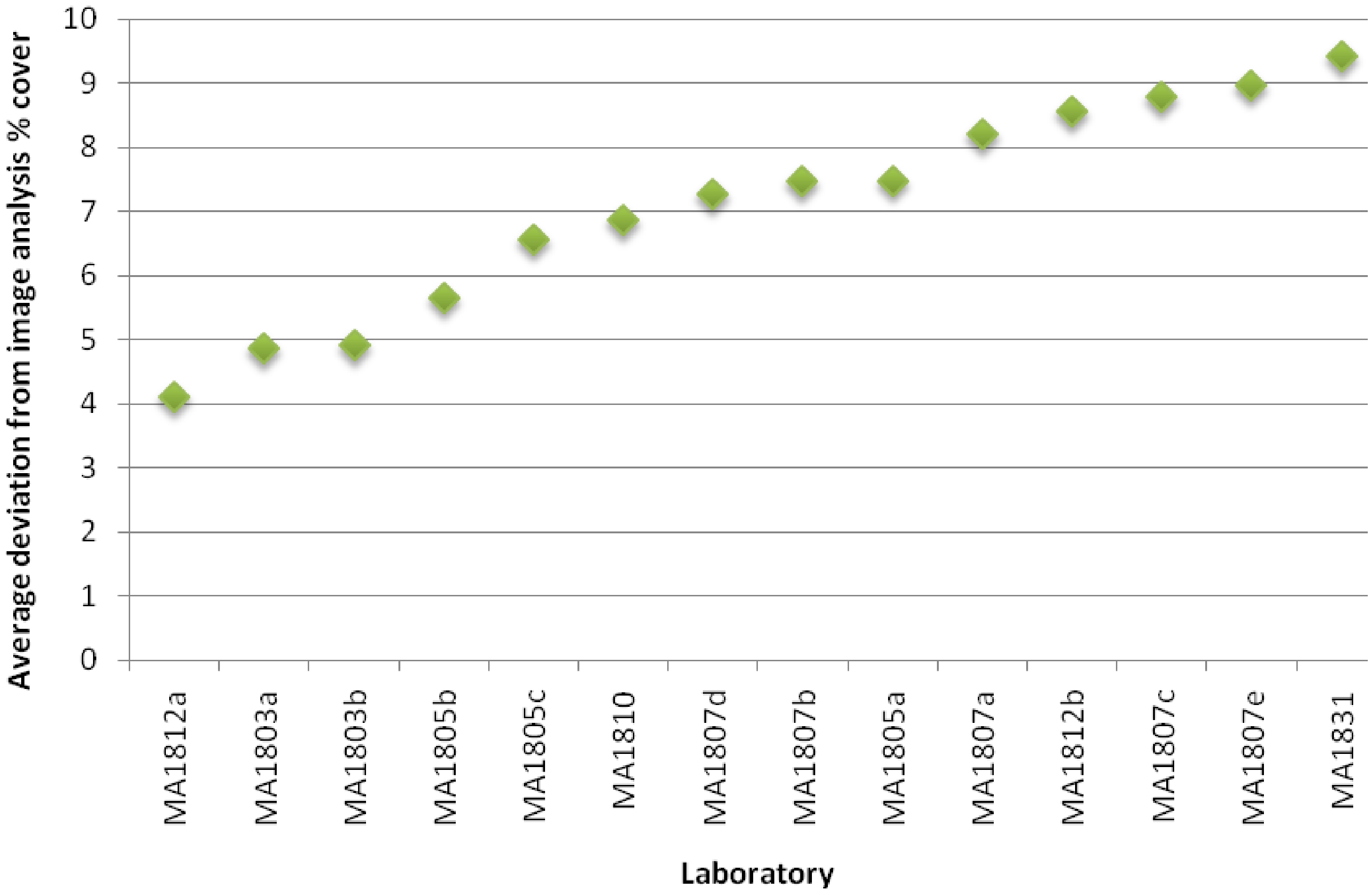


Figure 7. Difference in % cover between submitted results and image analysis results, displayed as the average difference across all quadrats in test C for each participant.

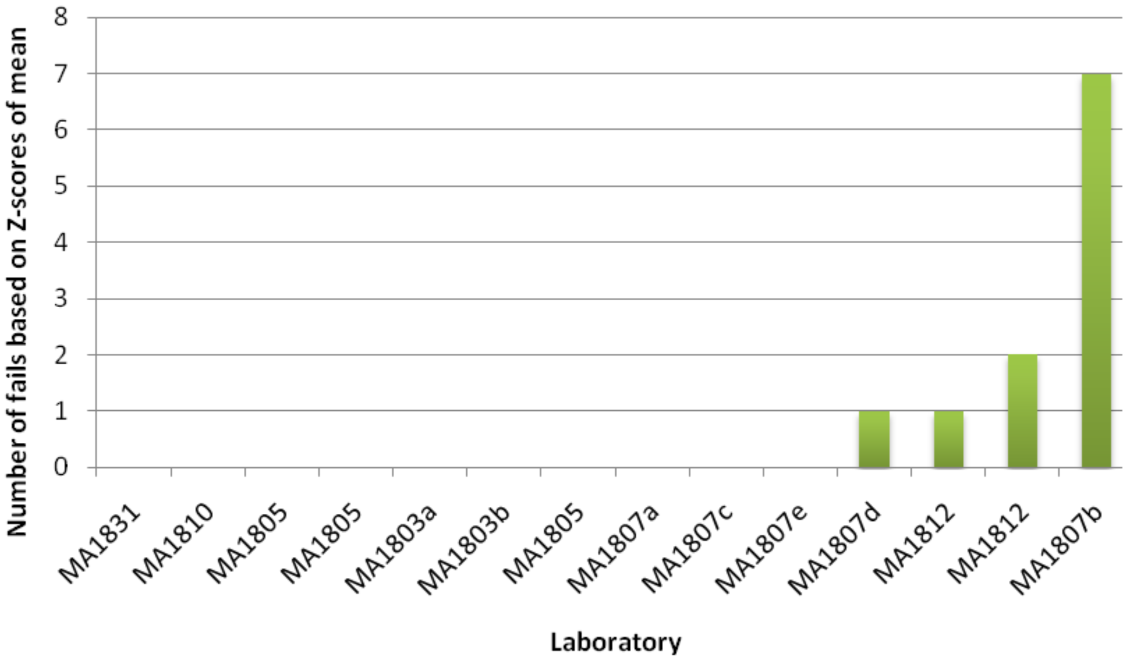


Figure 8. Number of quadrat fails per participant based on z-scores derived from mean % cover estimates for test C.

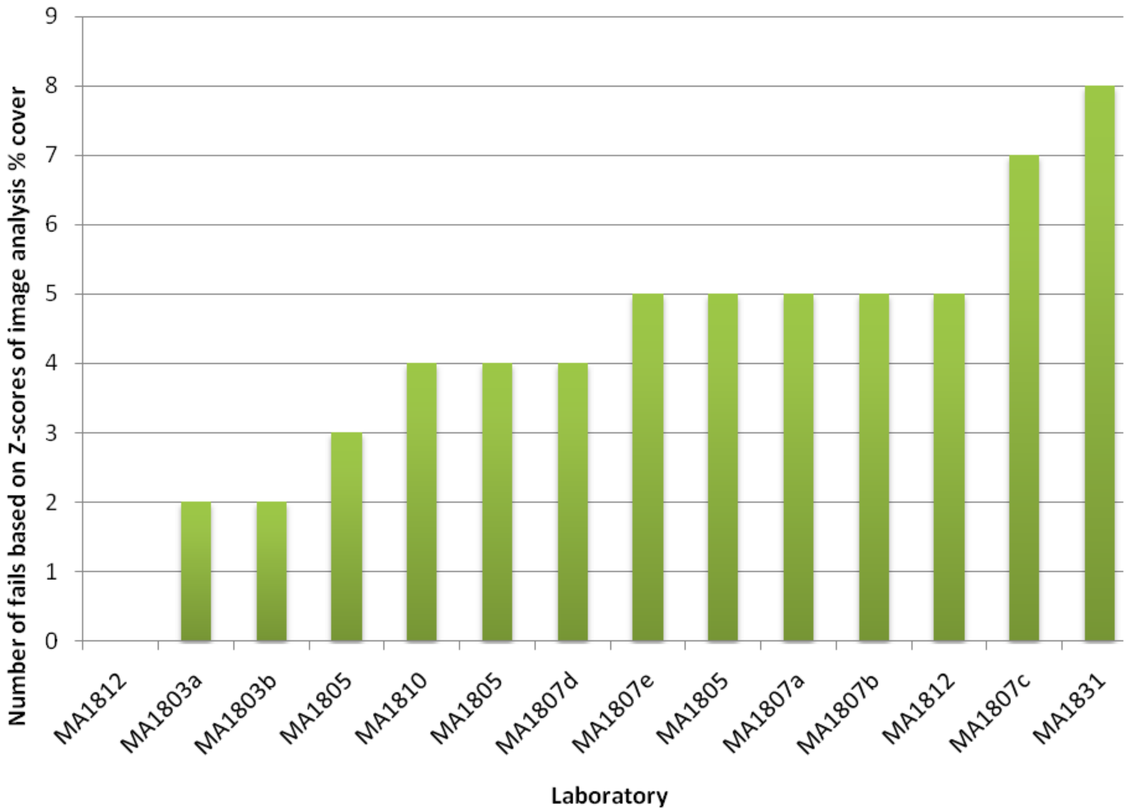


Figure 9. Number of quadrat fails per participant based on z-scores derived from % cover as calculated using image analysis for test C.

2.7.4 Results Summary and Comparison

For each of the tests the results were presented as raw data. The raw data allowed results to be compared between participants and against the image analysis results using the Z-scores (see below for description) which were displayed as the number of failures. These comparisons were further used to calculate deviation from the image analysis results.

Z-scores indicate how much each value deviates from the mean. It uses 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.

2.7.4.1 Test A Results (open quadrat)

Test A consisted of 17 participants with varying levels of deviation from the population mean. The range of results per quadrat varied considerably with the largest range of results produced for quadrat 12 from 65% cover to 95% cover and the smallest range for quadrat 7 from 5% to 12%. Z-scores calculated against the population mean resulted in 41% of laboratories failing at least one quadrat with between 1 and 3 failures per lab. In total there was a 96.47% pass rate for test A when using Z-scores derived from the mean.

Most participants showed an average % cover deviation from image analysis % cover ranging between 4% and 12%. The pass rate was much lower using Z-scores derived from image analysis estimates of % cover with 100% of labs failing at least one quadrat. The overall pass rate was lower at 80.29%. Quadrat 12 also showed the highest degree of deviation from % cover as calculated from image analysis.

2.7.4.2 Test B Results (9 x 9 crosshairs quadrat)

Test B had the greatest number of participants with 27. As with test A there was a greater degree of correlation of % cover against population mean compared with the image analysis. A total of 74.5% of labs (20 out of the 27) produced a Z-score of less than 2.0, which is considered a 'pass'. The remaining 7 labs failed between 1 and 9 quadrats. The largest range of % covers per quadrat was a range of 38% cover from 25% to 62.96% cover recorded in quadrat 4. Other Quadrats that had eaully large % cover ranges were 2, 3, 11 16 and 18 all of which showed a range of at least 30%.

Consistent with test A, test B also showed a higher degree of deviation from between the results and the image analysis results with 25 laboratories failing at least one quadrat and an overall pass rate of

79.07 compared with a pass rate of 95.55% using Z-score from the population mean. Although this method is thought to provide a less subjective means of estimating % cover the average level of deviation from % cover as calculated from image analysis was the highest of the three tests.

2.7.4.3 Test C Results (5 x 5 gridded quadrat)

A total of 14 participants opted to complete Test C using the 25 square method which was also the least popular method. 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 population mean.

The average range of percent covers per quadrat was 20% with quadrats 1 and 2 producing the highest range of 36% and 35% respectively. Four laboratories failed at least one quadrat using Z-scores from the mean with each of these resulting in between 1 and 7 failures and an overall pass rate of 96%. There were also more 'Fails' using Z-score from image analysis with between 2 and 8 'Fail' per lab and an overall pass rate of 79%.

3 Conclusions and Recommendations

The % cover of opportunist algae in a ¼ m^2 quadrat is usually estimated based on a skilled eye observation using either an open quadrat or gridded quadrat with +/- 5% agreement. It is highly unlikely that this method of % cover estimation is 100% accurate due to the subjectivity of individuals. OMC RT02 has used the population mean and an image analysis method to calculate a more precise % cover for comparison with individual participants' records. There are difficulties in obtaining 100% accuracy for % cover of opportunist algae, 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, identifying either the green colour of the algae or the brown/red underlying substrate (usually the most dominant of the two). Each of the photographs was enhanced prior to analysis to ensure maximum contrast in colour. Once the two distinct colours have been identified within the program it is able to calculate the total area covered thus reducing the degree of subjectivity experienced with killed eye evaluations. During this second round of the macroalgae scheme photographs were also ground truthed against actual presence of algae within the field to ensure each area of algae could be accurately identified 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. The results show a high level of consistency between participants when comparing with the population mean. There were a greater number of Z-scores failures when comparing the image analysis % cover with the population mean of the quadrats. This was consistent across all three tests. The degree of deviation from the image analysis % cover value depended significantly upon the quadrat. Some quadrats were more problematic than others (Figure 10) and this was evident in the range of % covers and could be partly attributed to the more patchy coverage of opportunist algae in some quadrats which is much harder to accurately estimate. It was also evident that those quadrats with a mid % cover from between 25% and 75% resulted in a greater range of results. Those quadrats with either a very high or low percent cover appeared much easier to accurately estimate total cover.

There was little difference in the levels of pass rate and number of 'Fails' between tests using both Z-scores from the population mean and Z-scores from image analysis. Test C produced the lowest levels of deviation from image analysis results and also the smallest range of estimation and Test B had the highest deviation. However, this difference between the three tests was very marginal and much more consistent than seen in RT01. The standard deviation per quadrat also varied considerably between tests with the different tests producing dissimilar results.

There is evidently a high degree of error between tests as well as between participants and this has prompted the need for a specific workshop where methods may be discussed and possibly % cover estimations compared in the field. It is impossible 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 Test B is the most favoured method.

The image analysis method used during RT02 differed from RT02 as it was considered more objective and likely to produce a more accurate results, RT02 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 although aims to be subjective to more rigorous testing 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.

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) or Dr Clare Scanlan (clare.scanlan@sepa.org.uk). This ring test is still only in its second year and very much in its developmental stage but hopes to be continually refined.

During this second cycle of the macroalgae scheme there were slow and missing returns from some laboratories which have lead to some delays in processing and subsequent reporting and feedback of results. In subsequent years reminders will be distributed prior to the completion deadline for the exercise.

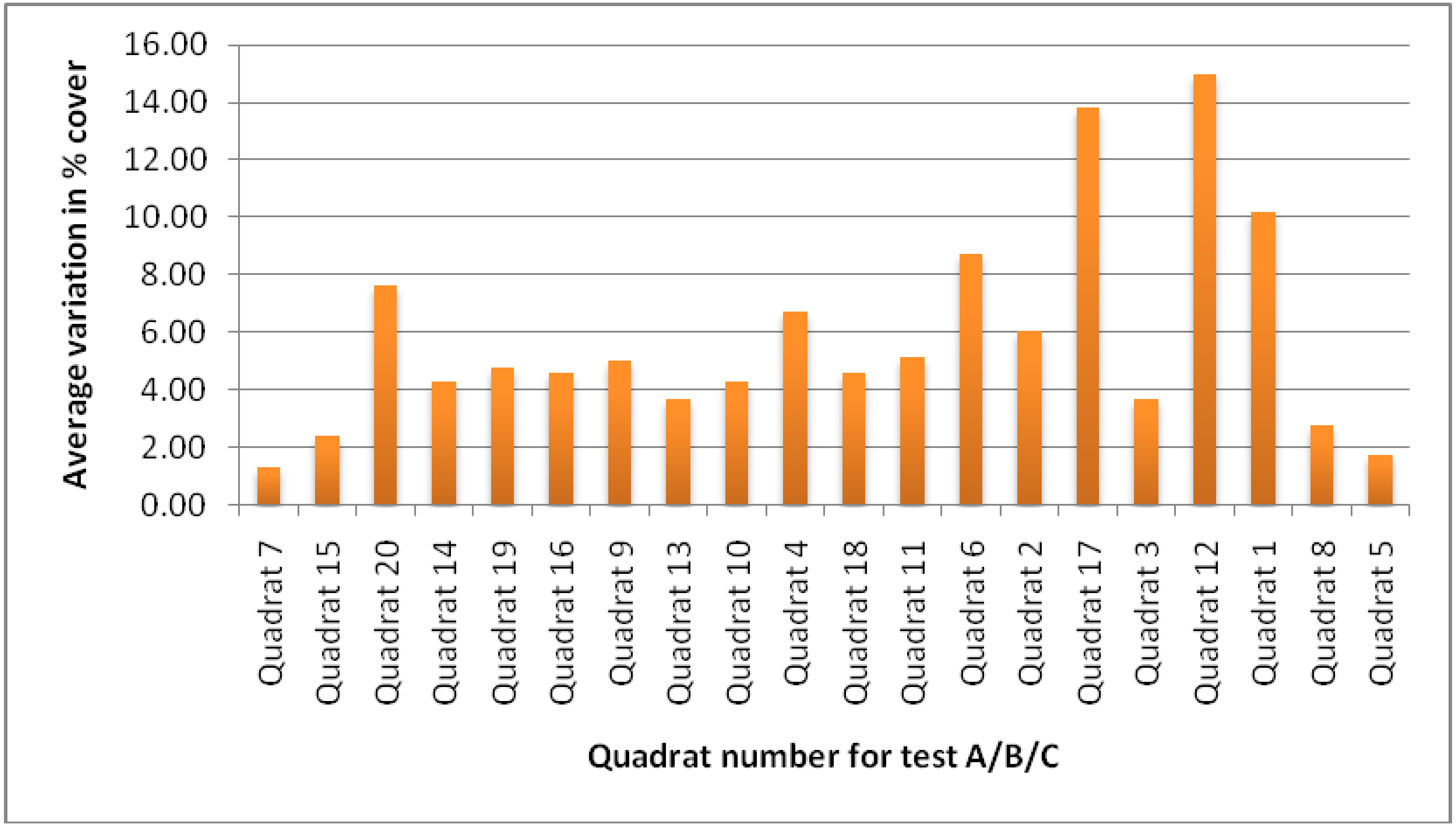
Table 4. Summary of results from tests A, B and C including minimum values recorded, maximum, mean and range per quadrat across all participants

		Results from Test A						Results from Test B						Results from Test C					
	image analysis algal coverage %	Min	Max	Mean	Deviation mean from actual	StDev	Range	Min	Max	Mean	Deviation mean from actual	StDev	Range	Min	Max	Mean	Deviation mean from actual	StDev	Range
Quadrat 1	74.55	55	75	64.24	10.32	4.99	20	54.0	80.25	69	5.06	7.2	26.2	44	80	59.4	15.2	10.21	36
Quadrat 2	58.20	45	75	61.12	2.92	6.71	30	51.7	83.95	73	15.25	8.1	32.2	45	80	58.2	0.0	10.96	35
Quadrat 3	63.10	50	80	65.18	2.08	8.13	30	53.0	88.89	72	8.77	8.1	35.9	46	75	62.9	0.2	9.34	29
Quadrat 4	34.16	20	40	27.00	7.16	6.88	20	25.0	62.96	42	7.92	10.9	38.0	20	41	29.1	5.0	5.42	21
Quadrat 5	82.61	75	90	82.53	0.08	4.78	15	70.1	93.83	86	3.21	5.7	23.7	68	88	80.7	1.9	5.93	20
Quadrat 6	44.84	20	45	32.00	12.84	6.27	25	31.0	60.49	48	2.72	8.0	29.5	26	45	34.2	10.6	6.13	19
Quadrat 7	7.74	5	12	8.65	0.91	2.57	7	6.0	15	10	2.66	2.9	9.0	4	15	8.1	0.4	2.80	11
Quadrat 8	79.17	70	90	80.53	1.35	6.41	20	72.4	93	84	4.42	6.2	20.6	60	85	76.6	2.5	8.49	25
Quadrat 9	30.61	10	35	20.94	9.67	6.68	25	20.0	49.38	30	0.24	7.5	29.4	18	35	25.4	5.2	4.50	17
Quadrat 10	33.98	15	40	27.76	6.22	7.40	25	32.1	41	38	3.78	2.3	8.9	24	36	31.2	2.8	4.10	12
Quadrat 11	38.62	20	45	33.41	5.21	8.16	25	29.9	64.2	45	6.03	9.2	34.3	26	46	34.4	4.2	5.71	20
Quadrat 12	69.21	65	95	84.53	15.32	8.20	30	72.0	96.3	87	17.66	6.6	24.3	68	90	81.2	12.0	6.29	22
Quadrat 13	31.11	15	40	31.18	0.06	7.96	25	30.0	59.26	40	8.85	7.1	29.3	25	42	33.2	2.1	5.86	17
Quadrat 14	20.95	10	30	17.15	3.80	5.37	20	11.0	38.27	25	3.98	6.1	27.3	12	22	15.9	5.1	3.74	10
Quadrat 15	9.98	5	20	9.74	0.25	4.56	15	7.0	25.93	16	6.13	4.4	18.9	4	20	9.1	0.9	4.07	16
Quadrat 16	28.97	10	40	23.47	5.50	7.65	30	17.0	50.62	31	1.88	7.5	33.6	15	30	22.6	6.3	4.67	15
Quadrat 17	59.23	60	92	74.24	15.00	8.02	32	59.0	85.19	75	15.70	7.6	26.2	60	77	70.1	10.8	5.70	17
Quadrat 18	38.15	27	55	39.00	0.85	7.91	28	35.0	65.43	49	10.83	8.1	30.4	28	55	36.0	2.2	6.56	27
Quadrat 19	25.83	15	30	21.59	4.24	5.43	15	22.0	41.98	31	5.59	5.1	20.0	15	35	21.3	4.5	5.03	20
Quadrat 20	17.68	5	13	8.53	9.15	2.81	8	6.9	27.16	13	4.29	5.3	20.3	4	15	8.3	9.4	3.47	11
		average range			5.65	6.35	22.25	average range			6.75	6.7	25.90	average range			5.1	5.95	20

Table 5. Comparison of estimated results from tests A, B and C with image analysis % cover results. The value represents the average deviation from image analysis % cover value for each participant.

Lab Code	Test A	Test B	Test C
MA1801		5.51	
MA1802	4.85	6.17	
MA1802	5.10	6.65	
MA1802		4.79	
MA1802	5.15	3.67	
MA1803a	7.18	11.49	4.86
MA1803b	7.11	12.91	4.91
MA1803c		17.37	
MA1805	6.77	6.04	7.48
MA1805	12.03	6.41	5.66
MA1805	9.40	12.34	6.57
MA1806	11.68	15.59	
MA1809		7.16	
MA1810			6.85
MA1811		7.57	
MA1812	4.77	8.34	4.11
MA1812	7.06	5.97	8.57
MA1817		11.68	
MA1817		14.61	
MA1818		9.37	
MA1830		5.02	
MA1830		7.34	
MA1830		4.84	
MA1831	9.56	5.70	9.42
MA1807a	8.68	6.69	8.20
MA1807b	8.15	10.14	7.47
MA1807c	8.58		8.78
MA1807d	6.40	7.06	7.26
MA1807e	10.42	7.79	8.97
Average	7.82	8.45	7.08

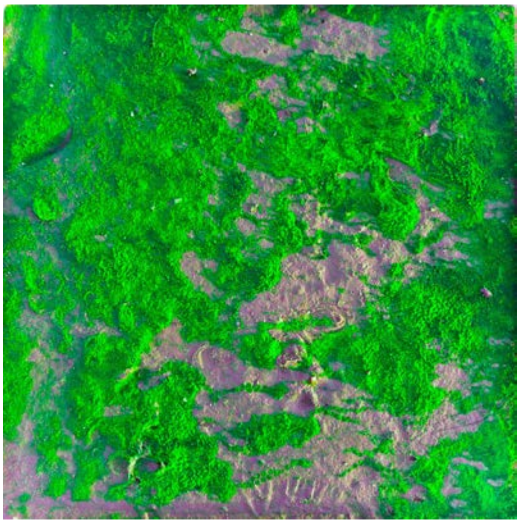
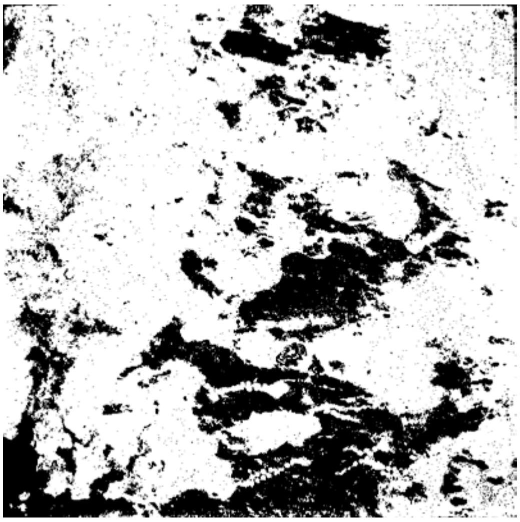
Figure 10. Comparison of deviation in image analysis % cover from estimated % cover between quadrats highlighting those problematic quadrats. (Quadrats are arranged in order of lowest to highest % cover).



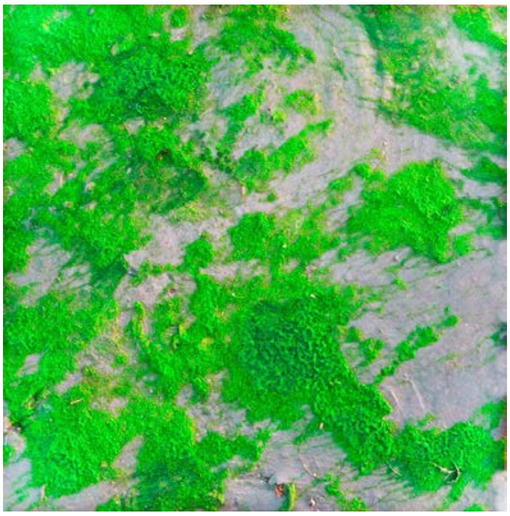
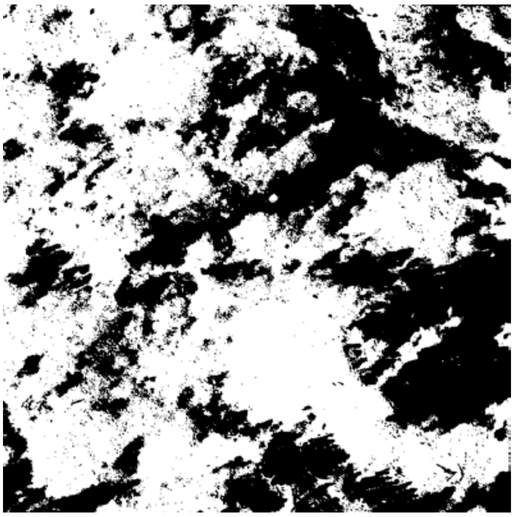
4 Image Analysis Results

Areas of algal coverage as calculated using imageJ analysis. Summary results for each quadrat include the total number of failures across all three tests as calculated using Z-scores based on both the mean % cover across all participants and the actual % cover as determined by image analysis.

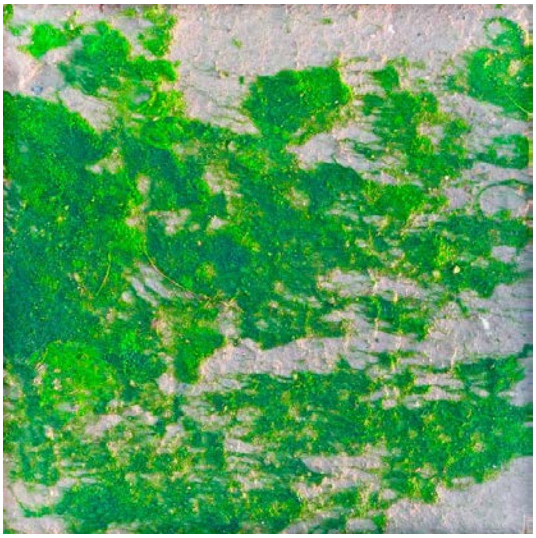
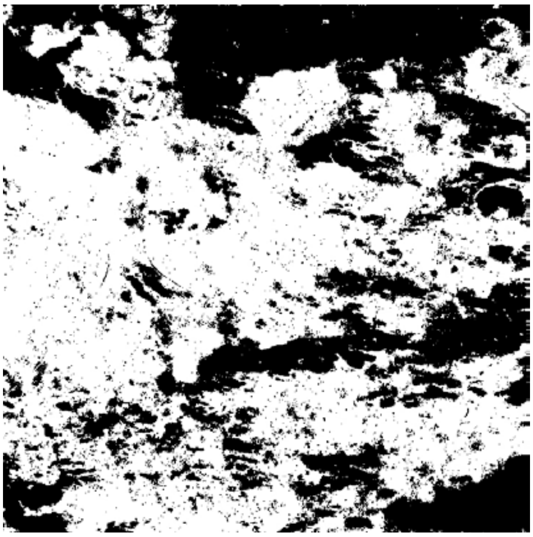
Quadrat: **1**
Actual % cover: **74.55**
Total No. of fails from Z-score of mean: **2**
Total No. of fails from Z-score of actual % cover: **16**
Average deviation from mean: **10.19**

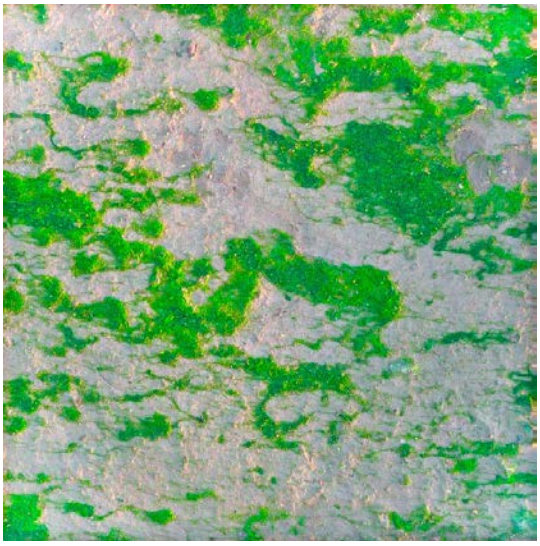
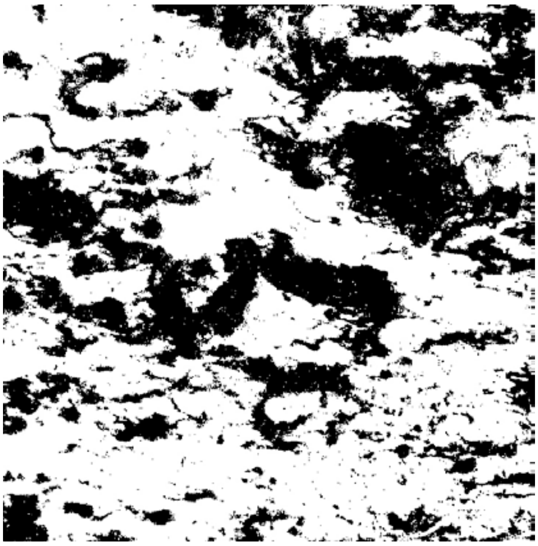
Quadrat: **2**
Actual % cover: **58.20**
Total No. of fails from Z-score of mean: **6**
Total No. of fails from Z-score of actual % cover: **14**
Average deviation from mean: **6.06**

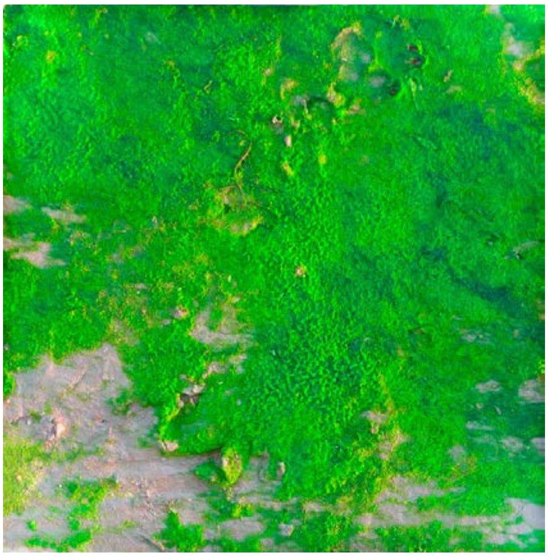
Quadrat: **3**
Actual % cover: **63.10**
Total No. of fails from Z-score of mean: **3**
Total No. of fails from Z-score of actual % cover: **6**
Average deviation from mean: **3.67**

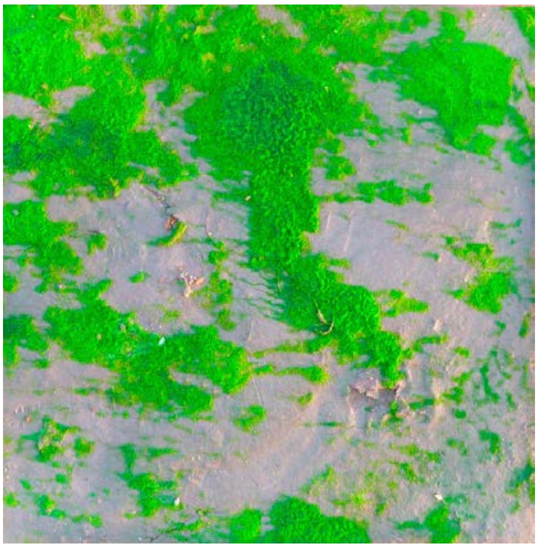
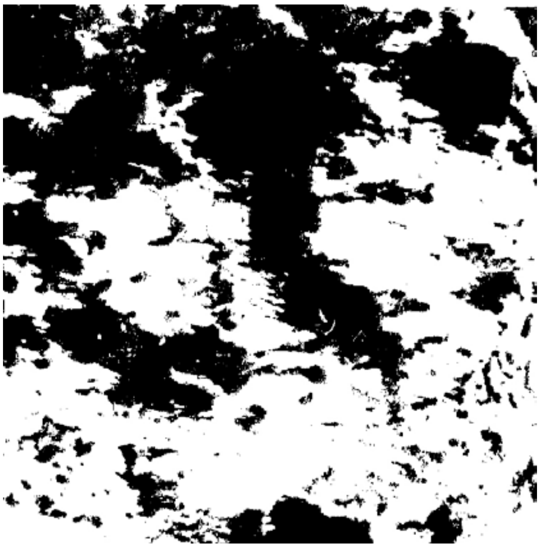
Quadrat: **4**
Actual % cover: **34.16**
Total No. of fails from Z-score of mean: **1**
Total No. of fails from Z-score of actual % cover: **11**
Average deviation from mean: **6.70**

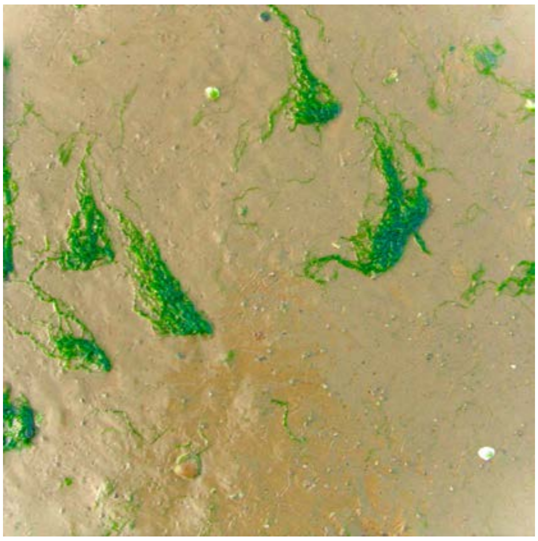
Quadrat: **5**
Actual % cover: **82.61**
Total No. of fails from Z-score of mean: **2**
Total No. of fails from Z-score of actual % cover: **2**
Average deviation from mean: **1.73**

Quadrat: **6**
Actual % cover: **44.84**
Total No. of fails from Z-score of mean: **2**
Total No. of fails from Z-score of actual % cover: **16**
Average deviation from mean: **8.73**

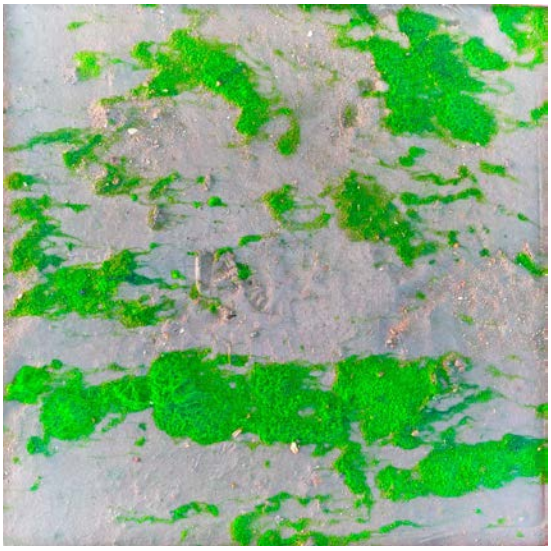
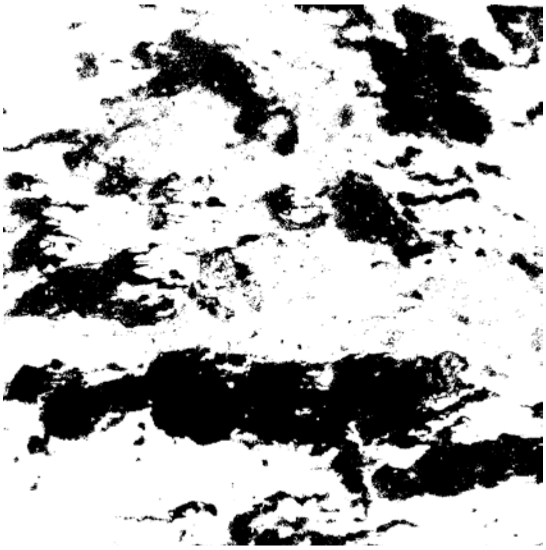
 

Quadrat: **7**
Actual % cover: **7.74**
Total No. of fails from Z-score of mean: **1**
Total No. of fails from Z-score of actual % cover: **7**
Average deviation from mean: **1.33**

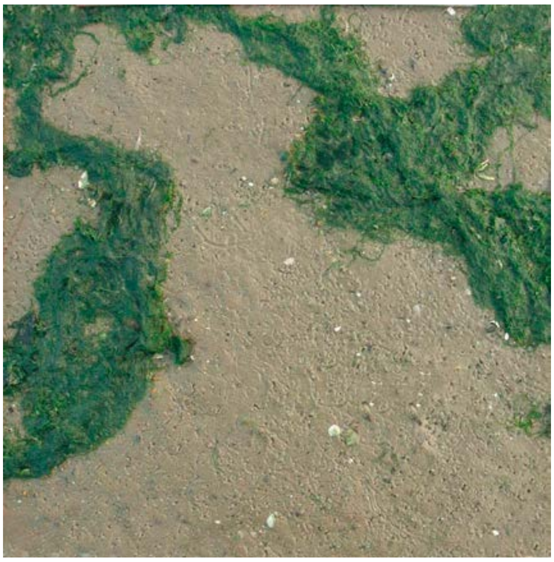
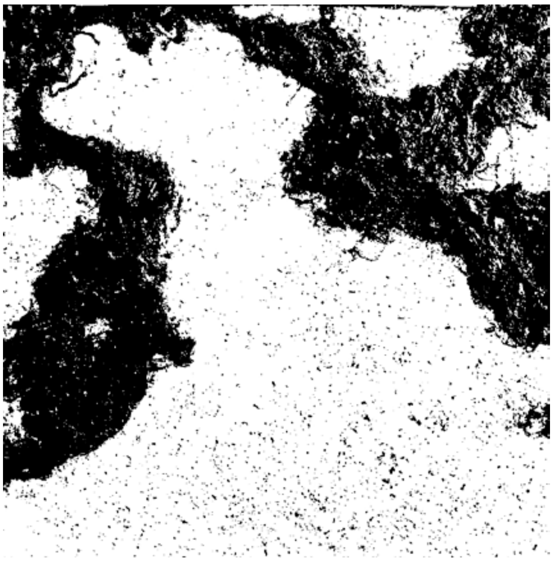



Quadrat: **8**
Actual % cover: **79.17**
Total No. of fails from Z-score of mean: **0**
Total No. of fails from Z-score of actual % cover: **3**
Average deviation from mean: **2.77**

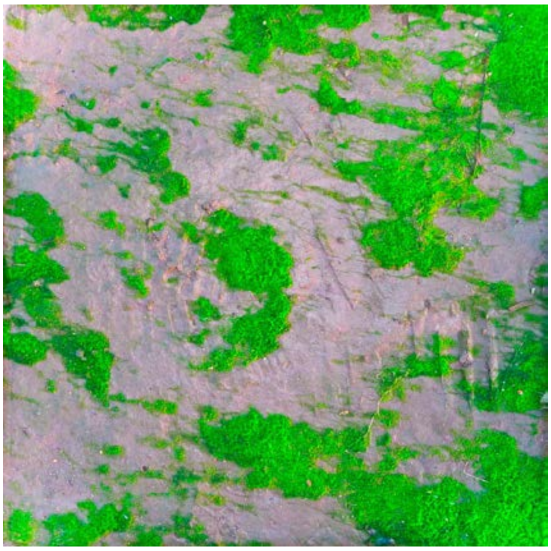
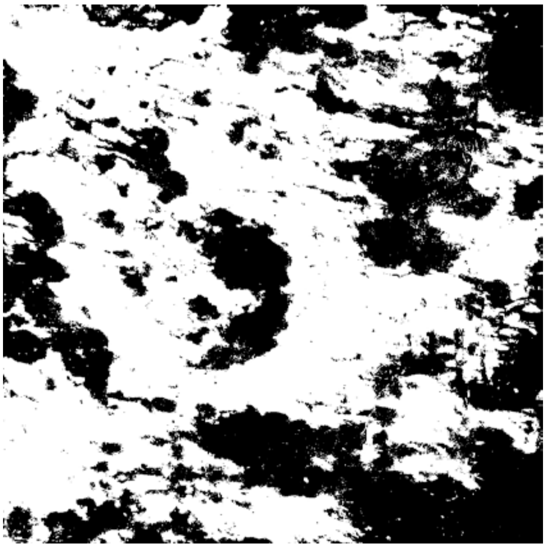

Quadrat: **9**
Actual % cover: **30.61**
Total No. of fails from Z-score of mean: **3**
Total No. of fails from Z-score of actual % cover: **9**
Average deviation from mean: **5.03**

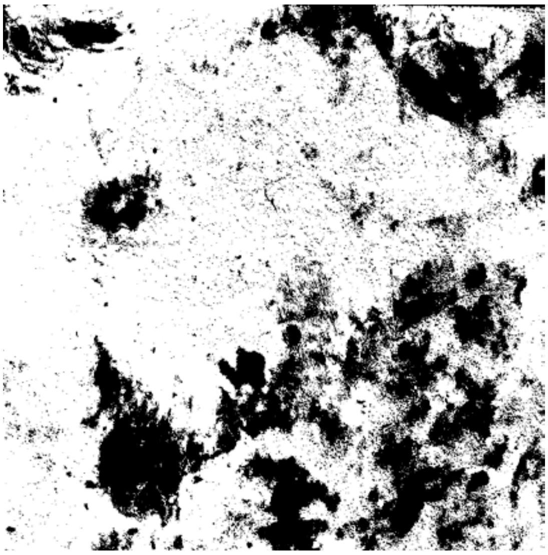
Quadrat: **10**
Actual % cover: **33.98**
Total No. of fails from Z-score of mean: **2**
Total No. of fails from Z-score of actual % cover: **13**
Average deviation from mean: **4.26**

Quadrat: **11**
Actual % cover: **38.62**
Total No. of fails from Z-score of mean: **2**
Total No. of fails from Z-score of actual % cover: **4**
Average deviation from mean: **5.14**

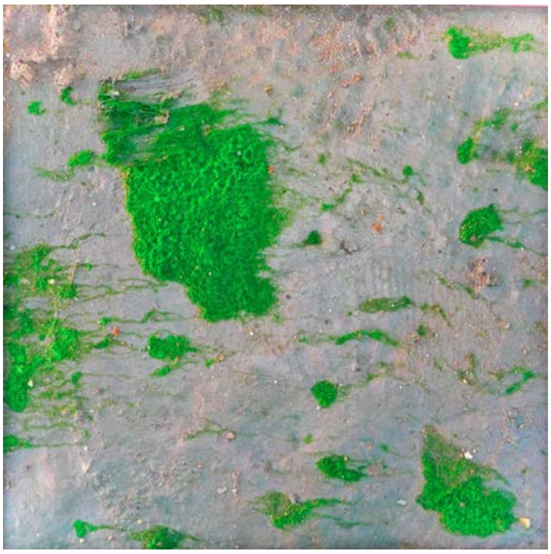
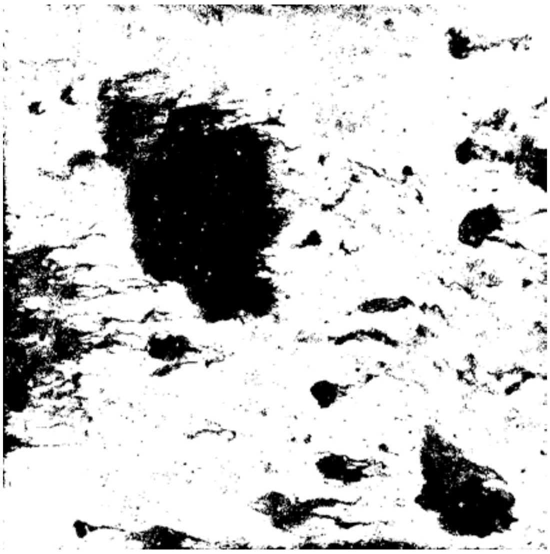



Quadrat: **12**
Actual % cover: **69.21**
Total No. of fails from Z-score of mean: **3**
Total No. of fails from Z-score of actual % cover: **34**
Average deviation from mean: **15**

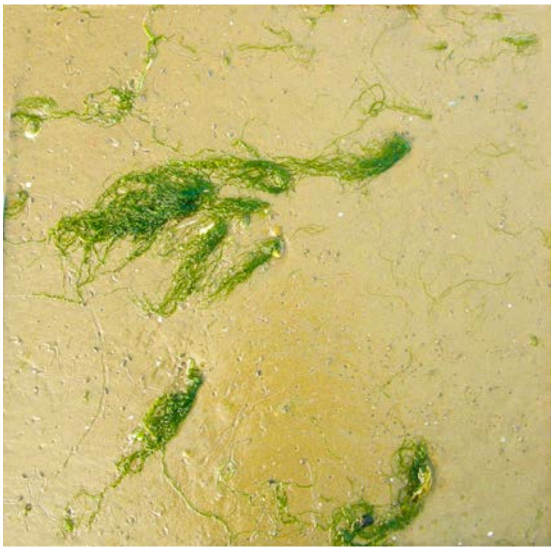



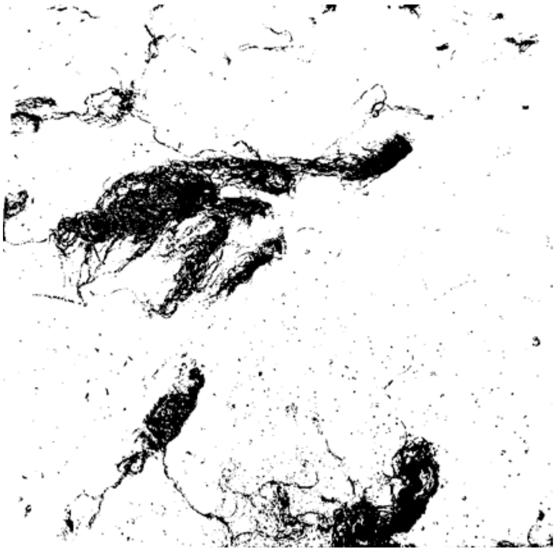
Quadrat: **13**
Actual % cover: **31.11**
Total No. of fails from Z-score of mean: **2**
Total No. of fails from Z-score of actual % cover: **6**
Average deviation from mean: **3.67**


Quadrat: **14**
Actual % cover: **20.95**
Total No. of fails from Z-score of mean: **3**
Total No. of fails from Z-score of actual % cover: **11**
Average deviation from mean: **4.29**

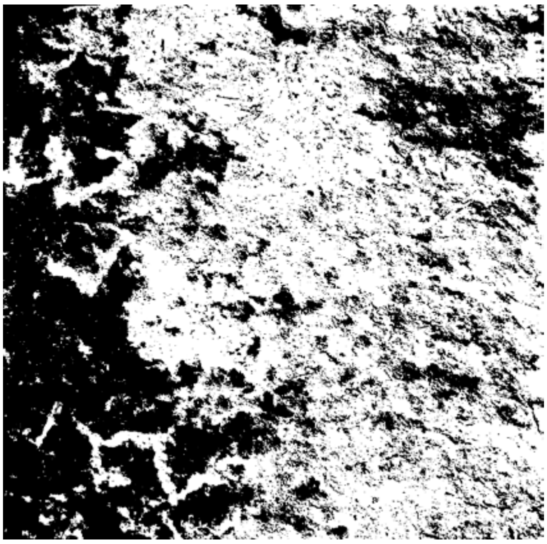
Quadrat: **15**
Actual % cover: **9.98**
Total No. of fails from Z-score of mean: **4**
Total No. of fails from Z-score of actual % cover: **8**
Average deviation from mean: **2.42**



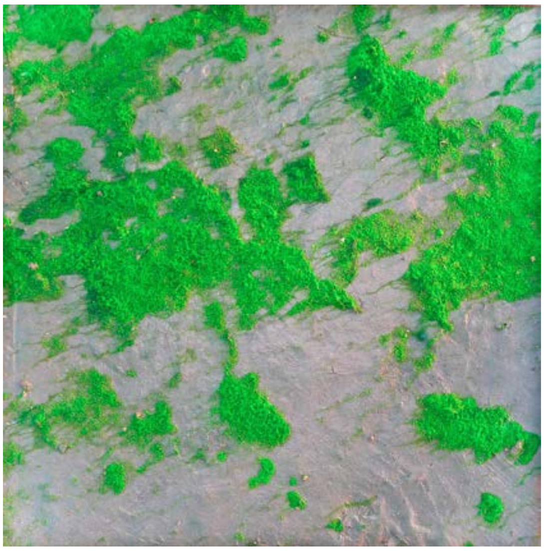
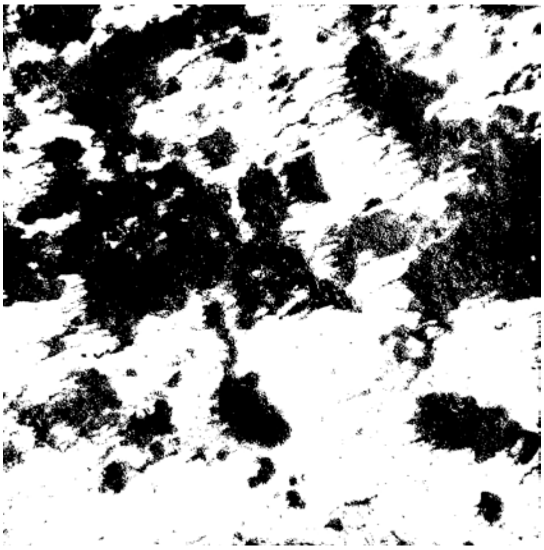


Quadrat: **16**
Actual % cover: **28.97**
Total No. of fails from Z-score of mean: **3**
Total No. of fails from Z-score of actual % cover: **6**
Average deviation from mean: **4.57**

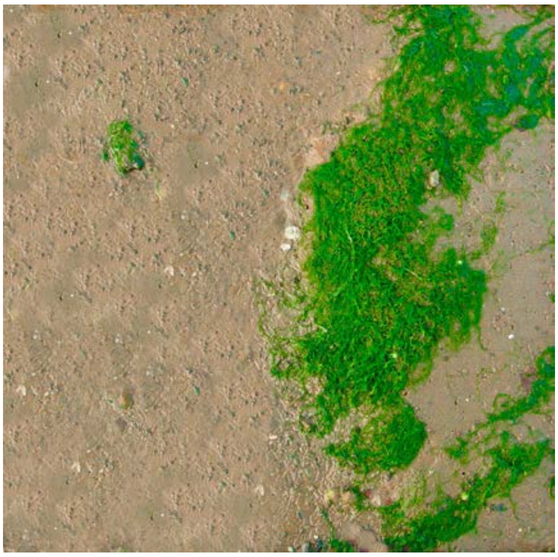
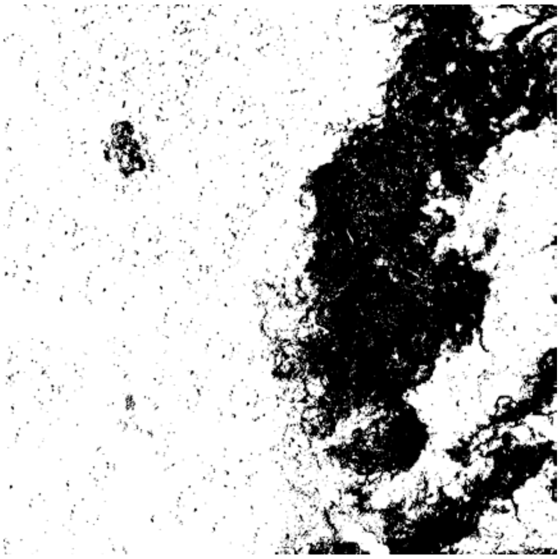
Quadrat: **17**
Actual % cover: **59.23**
Total No. of fails from Z-score of mean: **2**
Total No. of fails from Z-score of actual % cover: **28**
Average deviation from mean: **13.85**

Quadrat: **18**
Actual % cover: **38.15**
Total No. of fails from Z-score of mean: **3**
Total No. of fails from Z-score of actual % cover: **8**
Average deviation from mean: **4.61**

Quadrat: **19**
Actual % cover: **25.83**
Total No. of fails from Z-score of mean: **2**
Total No. of fails from Z-score of actual % cover: **5**
Average deviation from mean: **4.79**

Quadrat: **20**
Actual % cover: **17.68**
Total No. of fails from Z-score of mean: **1**
Total No. of fails from Z-score of actual % cover: **32**
Average deviation from mean: **7.61**