The NE Atlantic Marine Biological Analytical Quality Control Scheme

www.nmbaqcs.org

Macroalgae Biomass

Component Report

Ring Test OMB RT14 2023

Søren Pears &
Georgina Brackenreed-Johnston

APEM Ltd.

28th April, 2023

E-mail: nmbaqc@apemltd.co.uk

Contents

1. Introduction 1

1.1 Background 1

1.2 Participating Laboratories 1

1.3 Introduction 2

1.4 Description 2

1.5 Logistics 2

1.6 Confidentiality 3

1.7 Preparation of the Samples 3

1.7.1 Method for wet weight 3

1.7.2 Method for dry weight 4

1.8 Analysis and data submissions 4

1.9 Z-Scores 4

2. Results 5

2.1 Returns from participating laboratories 5

2.2 Comparisons with expected wet weights and actual dry weights 6

3. Discussion 7

4. Conclusions and Recommendations 8

List of Tables

Table 1 Raw data results from each laboratory including both dry and wet weights 5

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 primarily driven 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 ensure consistency between analysts with improved confidence in ecological quality status.

The Northeast Atlantic Marine Biological Analytical Quality Control (NMBAQC) Scheme addresses several issues relating to macroalgae and seagrass data collection, this report focuses on just one of these:

- The determination of algal biomass

This is the fourteenth year in which biomass of macroalgae has been included as an element of the NMBAQC scheme and was included as a single exercise. The format followed that of previous years of the test (OMB RT01 - RT13). Test material was distributed to participating laboratories along with data forms, which were completed with algal biomass results and returned for analysis.

Graphical representations of the performance of each participating laboratory were distributed in the OMB RT14 Bulletin Report. This bulletin included the z-score based ‘pass’ and ‘fail’ flags assigned to each result to highlight deviation from sample means and actual/expected weights. The current report describes the results in more detail and should be read in conjunction with the OMB RT14 Bulletin.

1.2 Participating Laboratories

Eleven laboratories were issued test material, of which nine laboratories completed the macroalgae biomass component of the NMBAQC scheme. Of those participating, all nine laboratories were government organisations.

Due to the limited number of samples distributed, only a single set of results was permitted per laboratory unless more than one test was requested. It was possible for each sample to be completed by a different participant; however, this was not recorded within the results. Individual laboratories may look at such results internally.

Currently this scheme does not specify a definite qualifying performance level, and NMBAQC ring tests may be treated as training exercises. However, certain targets have been applied to the assessment of the results based on z-scores allowing ‘pass’ or ‘fail’ flags to be assigned; 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.

Samples were synthetic, rather than composed of natural algal material. Natural samples would be subject to deterioration, and it is not feasible to ensure that each participant would receive a truly equivalent sample. This is in line with guidance on general requirements for proficiency testing (BS EN ISO/IEC 17043:2010).

2. Summary of the Biomass exercise

1.3 Introduction

There was one exercise for the assessment of biomass of macroalgae which took the form of three representative artificial samples. This exercise is described in full below to include details of distribution and logistics procedures for determination of biomass, completion of test result forms and full analysis, and comparison of final submitted results.

1.4 Description

This exercise examines the participants' ability to process macroalgae samples to extract values of biomass for wet and dry weight. The exercise assesses the differences in sample processing efficiency and comparability of results using z-scores. Comparison of participating laboratory results can highlight anomalies in processing at various stages of the methodology.

One set of three representative samples was distributed to each participating laboratory in January 2023. Participating laboratories were required to submit biomass results for both wet and dry weight. The samples included material that was consistent with that of OMB RT13 including j-cloths, wool and synthetic stuffing. Based on previous ring tests, these were chosen to be the most representative materials in terms of imitating the overall look and feel of various opportunistic macroalgae species. Cloths and wool were cut to different sizes and lengths to represent different foliose and filiform taxa (e.g. *Ulva* spp.). The synthetic stuffing represents finer algae such as *Chaetomorpha* spp. RT14 also trialled the use of shredded biodegradable food waste bags to represent fronds of the flat, membranous species of *Ulva* and *Porphyra*. Each sample was mixed with sediment of a sandy-muddy nature consistent with the substrate type known to support opportunistic macroalgal blooms to simulate substrates that would be encountered in the field.

1.5 Logistics

Each sample was contained within a plastic sample bucket and distributed via a courier delivery service company. All instructions and additional test forms were distributed via e-mail attachments to each laboratory. The files contained a description of methodology and standardised forms for data submission. Participants were given six weeks to complete the test and return the results. Only one set of results could be submitted per set of samples although it was possible to have up to three participants complete the test analysis.

E-mail has been the primary means of communication for all participating laboratories subsequent to the initial postal distribution of test material.

1.6 Confidentiality

To preserve the confidentiality of participating laboratories, each participant was randomly allocated a four-digit laboratory code, which allowed them to identify their own results. The initial letters (MA) refer to the scheme, this is followed by two digits representing the current NMBAQC scheme year, and the final two digits representing the laboratory. It was noted during the previous scheme year that the macroalgae component was out of synchrony with the rest of the NMBAQC scheme components. Therefore the '29' year prefix has been repeated this year for RT14, to ensure that the macroalgal component is now consistent with the other NMBAQC scheme components.

1.7 Preparation of the Samples

To assess the accuracy of determining biomass of opportunistic macroalgae, samples were prepared using j-cloth, wool, synthetic stuffing material and food waste bags that had been cut and shredded to mimic algal species. These materials were deemed to be the most representative of actual opportunistic species and were based on suggestions from previous ring test feedback forms. Three representative samples were supplied for subsequent processing. Sediments commonly found within areas of opportunistic algal growth were mixed into the samples with small amounts of water. For each sample, wet weight and dry weight had to be ascertained.

The samples were labelled A, B and C with samples of identical original dry weight provided to all participants.

Sample A – 26.4g (mixture of 13.2 g j-cloth and 13.2g wool)

Sample B – 14.0g (mixture of 8.0g synthetic stuffing material and 6.0g wool)

Sample C – 9.7g (food waste bags)

Due to the nature of the samples, they could be kept for several days retaining most of the moisture. However, only enough water was added to thoroughly soak the synthetic materials and liquify the sediments prior to distribution to reduce weight during transportation. It was therefore necessary for participants to add additional water to each of the samples prior to commencement of the tests to enable rehydration of the material and aid with rinsing.

1.7.1 Method for wet weight

The laboratory instructions stipulated that each of the samples required rinsing free of all sediment. The samples should be fully washed in a bucket or sieve to ensure no loss of sample material until the water runs clear and all debris is removed. Once the samples are adequately washed, they are squeezed of excess water. This is achieved by hand, using samples no larger than the size of a tennis ball, to ensure it fits in the palm of the hand, and can still be squeezed properly. Where the sample was large, it should be divided into smaller

clumps for squeezing. The samples are squeezed until no additional running water can be removed by hand (over-enthusiastic squeezing of actual algal samples can damage cell membranes and lose 'genuine' weight). At this stage the whole sample is weighed on a calibrated balance to two decimal places. The exact method used for rinsing and squeezing should be consistent with that used in the field; this may vary between laboratories.

1.7.2 Method for dry weight

Once each of the samples has been wet weighed, they are spread out on a sorting tray or similar container. Spreading the samples in this way aids with the drying process. The samples are left to air dry for at least 24 hours, but this may be longer depending on the size of the sample and the temperature of room. The samples should be checked regularly, and the drying/weighing process is continued until constant mass is achieved, recording weight to 2 decimal places. The unchanged dry weight is the final weight to be submitted.

The same process was required for all 3 samples.

1.8 Analysis and data submissions

A pre-prepared spreadsheet was distributed with the exercise instructions to standardise the format in which the results were submitted. These results will be retained and stored appropriately. Each laboratory was required to submit both a dry weight and wet weight for each of the 3 samples provided within the allocated six-week time period.

1.9 Z-Scores

Values of z-scores were used to apply the 'pass' & 'fail' assessment.

Z-scores were calculated to determine how many standard deviations each participant's weight results deviated from the mean, using the following formula:

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

Where:

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

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

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

A z-score 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 'Fail' or 'Pass' flag on the submitted data.

2. Results

2.1 Returns from participating laboratories

Of the nine laboratories that returned results for OMB RT13, all nine returned both wet and dry weight data. The results have been collated and presented in various formats to enable full comparisons both between laboratories and against actual sample weights.

Details of each participating laboratory's performance were distributed in the OMB RT14 Preliminary Bulletin Report. The Bulletin provided z-score derived 'pass' and 'fail' flags to each result set to highlight deviation from sample mean and actual/expected results.

Table 1 presents the range of wet and dry weights recorded by participating laboratories. Sample A had the largest range of results for wet weight (271.96g) and Sample B had the highest range of dry weights (16.56g).

Table 1 Raw data results from each laboratory including both dry and wet weights

Lab Code	Sample A		Sample B		Sample C	
	Wet Weight (105.75g)	Dry Weight (26.4g)	Wet Weight (58.0g)	Dry Weight (14.0g)	Wet Weight (41.44g)	Dry Weight (9.7g)
MA2901	112.08	26.62	60.51	15.7	21.45	9.64
MA2903	81.4	26.4	51.4	14.4	13.7	9.7
MA2904	106.6	26.88	66.7	14.34	23.22	9.69
MA2905	353.36	27.92	239.2	15.01	187.72	9.88
MA2906	121	31.98	61.81	14.53	16.47	9.69
MA2907	150.3	30	113.7	14.4	20.15	9.7
MA2910	85.96	27.13	59.95	15.35	15.47	9.62
MA2911	108.6	35.4	61.6	30.9	15.4	11.1
MA2912	88.4	31.1	62.7	14.9	12.7	9.9
Max	**353.36**	**35.4**	**239.2**	**30.9**	**187.72**	**11.1**
Min	**81.4**	**26.4**	**51.4**	**14.34**	**12.7**	**9.62**
Range	**271.96**	**9**	**187.8**	**16.56**	**175.02**	**1.48**
Average	**134.19**	**29.27**	**86.40**	**16.61**	**36.25**	**9.88**
St Dev	**84.84**	**3.09**	**60.06**	**5.38**	**56.91**	**0.47**

Sample A consisted of a mixture of equal weights of j-cloths and wool and was the largest of the three samples with an actual dry weight of 26.4g. Participant dry weight results ranged from 26.4g to 35.4g, with an average of 29.27g. This sample had the widest range of wet weights, varying between 81.4g and 353.36g. This large range was primarily due to the exceptionally high weight recorded by laboratory MA2905, which was more than triple the expected weight of 105.75g. The latter sample wet weight was the only 'fail' identified using the z-score of the mean and is so high as to suggest that this laboratory is using different methodology to the other participants or the sample was not squeezed sufficiently to remove excess water prior to weighing.

Sample B consisted of a mixture of stuffing material and wool, with an actual dry weight of 14.0g. Participant dry weight results ranged from 14.34g to 30.9g, with an average of 16.61g. The highest dry weight, recorded by laboratory MA2911 was the only dry weight 'fail' identified using the z-score of the mean participant value. Given that the wet weight for this laboratory was very close to the expected wet weight it can be concluded that this sample had been sufficiently rinsed free of debris but may not have been adequately dried. The wet weights for sample B varied between 51.4g and 239.2g. The large range is again primarily due to the exceptionally high weight recorded by laboratory MA2905, which was more than quadruple the expected weight of 58.0g. As for Sample A, such a high value led to a 'fail' flag identified using the z-score of the mean and indicates either a significant difference in methodology or inadequate squeezing to remove excess water prior to weighing.

Sample C was the smallest of the three samples, consisting only of shredded food waste bags with an actual dry weight of 9.7g. This sample had the lowest range of dry weights, varying between 9.62g and 11.1g. The highest weight resulted in a 'fail' identified using the z-score of the mean and this was again laboratory MA2911. In this case the recorded weight was only 1.4g below the actual dry weight, but the consistency of results between the other laboratories resulted in a low standard deviation and therefore the one outlier had a higher z-score. Four labs (MA2901, MA2904 and MA2906 and MA2910) showed dry weight values slightly below the actual dry weight, albeit no more than 0.1g. This may be due to differences in the accuracy of the weighing scales being used or the loss of smaller pieces of material that can occasionally be washed away. Since the loss was only very marginal this is considered acceptable for this test.

All but one of the wet weights for Sample C were below the expected weight of 41.44g. This is to be expected given that the food waste bag material is much less absorbent than the materials used in previous years, from which the expected weight trendline was derived. The only wet weight higher than the expected wet weight was again laboratory MA2905, which recorded a weight more than triple the expected weight, resulting in a 'fail' flag identified using the z-score of the mean.

2.2 Comparisons with expected wet weights and actual dry weights

The expected wet weight for each sample was calculated using historical NMBAQC biomass ring test participant data combined with the current year's results. As with the RT13 report, the raw data for previous scheme years were not available, but most of the relevant data could be extracted from the previous bulletins available from the NMBAQC website. Again, the only exception was RT04, for which a duplicate of that year's percentage cover bulletin appears to have been erroneously uploaded in place of the biomass bulletin. All other historical data were used to plot measured wet weights against known dry weights to generate a best fit trendline and corresponding linear equation. This linear equation was then applied to the known dry weights for the current year's samples to calculate an 'expected' wet weight for each sample. The linear equation applied to this year's data was $y = 3.8508x + 4.0901$ (where x is the known dry weight and y is the 'expected' wet weight). The resulting expected wet weight for samples A, B and C were 105.75g, 58.0g and 41.44g respectively.

Comparing wet and dry weights using z-scores calculated from the expected wet weight and actual dry weight is usually less accommodating and more sensitive to slight deviations in results than comparisons against the mean. For RT14, the z-scores derived from the expected wet weights and actual dry weights only resulted in one additional 'fail' compared to the z-scores calculated from the mean participant values. This additional 'fail' flag was for the dry weight of Sample A for laboratory MA2911, which consistently recorded higher dry weights than the other laboratories.

Most of the results for both the dry and wet weights (as presented in Bulletin OMB RT14) when compared against the mean values could be considered acceptable. All three 'fails' for the wet weight were for a single laboratory (MA2905) and their weights were so much higher than the other participants to suggest that the instructions were not adhered to and that the samples may possibly have been weighed prior to rinsing and squeezing. All the 'fails' for dry weight were also from a single laboratory (MA2911) and this participant indicated that they do not routinely measure dry weights of macroalgae during surveys and it appears that they may not have allowed sufficient drying time prior to weighing. It is recommended that samples are left to air dry for at least 24 hours prior to weighing. The results for comparisons against expected and actual wet and dry weight, respectively, show fewer additional fails than in previous years. However, one of the limitations of using z-scores is that high standard deviation values can reduce the chance of achieving a 'fail' based on the resulting +/- 2.00 cut-off value.

3. Discussion

Of the eleven sets of samples distributed, nine laboratories submitted results. Although many of these laboratories do not routinely measure dry mass for macroalgae, this is still a necessary part of this exercise as it enables the procedure to be reviewed for inter-laboratory differences. If samples are dried to a level where the mass remains unchanged then a result that lies well above the actual dry weight is a clear indication that the sample has been insufficiently rinsed and it is the additional particles that are adding to this increased weight. This will contribute to an overestimation of both wet and dry weights. Macroalgae is much harder to rinse, especially in the field, which may contribute to an overestimation of the levels of biomass present. Conversely, some laboratories do not measure wet weight and instead only record the final dry weight. Dry weight could be considered a much more accurate measure of biomass since this measure has fewer variables, i.e. it is only dependent upon the removal of debris and not the degree of pressure during squeezing. However, both measurements need to be incorporated into the test to cover the different measurements and procedures utilised.

The level of accuracy remains greater for measurement of dry weight than of wet weight, for reasons given above. In previous years the data have shown a greater degree of consistency in results for smaller or mid-range sample weights, e.g. weight from 5g to 40g and all three tests were within this range for the current year. The results do still suggest variation in the techniques used to rinse and squeeze samples between laboratories and may also vary between participants within the same laboratory. The lack of consistency in wet weight indicates a high level of variation in pressure applied during squeezing of samples. However, this is highly difficult to regulate between field workers. It is the wet weight that is most commonly used during routine monitoring of opportunistic macroalgae and therefore this lack of consistency in technique should be fully addressed within the standard operating procedures, especially in association with areas of high biomass. Each

laboratory should have its own in-house training and competence assessment measures. In the method document distributed with the samples it is recommended that 'where the sample is large it should be divided into smaller clumps for squeezing' and 'this should be achieved by hand using samples no larger than the size of a tennis ball to ensure it fits in the palm of the hand and can be properly squeezed'.

Most laboratories produced dry weights greater than the actual biomass of the sample; this indicates either insufficient drying or rinsing of the sample, some degree of which is to be expected during such a test. After six years of using separate materials for each test, this year re-introduced mixtures of different materials for two of the tests to try and reduce the variance that may be caused by differences in handling properties for the different materials. The test using only food waste bag material had the most consistent dry weights and participant feedback indicated this material was the most easy to process.

In the report for RT12 a trend was identified potentially correlating increasing sample weight with increased deviation from actual/expected weight on the basis that the larger samples may retain more debris and be more difficult to rinse free, squeeze or dry thoroughly. In RT13 this theory was modified to suggest that increased deviation from the actual/expected weight is co-dependent on both sample size and composition material. This led to the mixing of materials for the current year, with the inclusion of a wool component in both Sample A and Sample B to try and reduce the material effect. The standard deviation for wet weight was still highest for the largest sample (Sample A) in the current year, but differences in standard deviation between samples of different sizes were also less pronounced in the current year than in previous years, although they were also heavily skewed by extreme results from one or two laboratories.

In general, the results for the current year were comparable with those from previous years. The ring test can provide evidence of problems in the measuring of biomass samples, such issues may need addressing through workshops and specifically aimed training. The results bulletin also provides those laboratories with outliers an opportunity to review the procedures used during the processing of their samples.

It should be further highlighted that the 'fails' do not necessarily signify poor quality data they merely flag those results which show significant deviation from either the actual sample weights or from the average and should be investigated. These flags have no current bearing on the acceptability of data from such participating laboratories.

4. Conclusions and Recommendations

Observations made from the results of this year's exercise and from participants' feedback are summarised below:

1. Despite the artificial nature of the sample material, the test has been generally well accepted by all laboratories with constructive comments on points of possible improvements. All laboratories found the test useful despite the differences between the processing of artificial material compared with actual macroalgae samples.
2. All samples arrived with participants in good condition. One participant commented that it would be useful to clarify that wet weight of the sample is the weight of rinsed material rather than the starting weight of the sample. Instructions on the

measurement of both wet and dry weight were distributed to all participating laboratories at the same time as the samples were sent out. It is recommended that these instructions are read carefully by all staff that are due to be involved in the biomass estimation process prior to beginning the tests. The text of these instructions will also be reviewed to ensure there can be no ambiguity.

3. After six consecutive years in which each sample consisted of a different artificial material, this year reintroduced mixtures of different materials for two of the samples. This approach was well received by participants as being more representative of the mixtures of different algal types that are often found on the shore. Samples containing mixtures of different materials will therefore be continued in future tests.

4. There is general agreement that whilst synthetic materials cannot fully replicate real algae samples and lack the fragility of real algae, the use of artificial material is an acceptable surrogate for the test. This is the sixth year in which synthetic stuffing has been used to mimic much finer opportunistic algae such as *Pylaiella* and *Chaetomorpha*. As with previous years, this material was most frequently reported to be the most difficult to process due to the difficulty in rinsing free of sediment. The inclusion of food waste bags as a new test material was well-received, although was consistently reported as being the easiest material to process. This material also has the advantage of being biodegradable. Throughout the fourteen years of the OMB ring test there has so far been no consensus on the preferred material of use and can depend on the current opportunistic blooms being experienced in the field. Continued investigation of the viability of alternative materials is ongoing and new materials will be incorporated into future tests if deemed appropriate.

5. During this fourteenth cycle of the macroalgae biomass exercise nine participating laboratories submitted results within an acceptable timescale. No communication was received from the remaining two laboratories that did not submit results. All laboratories should continue to submit results within the requested deadlines as detailed at the beginning of the exercise. Reminders will continue to be distributed one week prior to the completion of the exercise to aid with this process. If laboratories suspect that they will not be able to submit results within the designated timescale prior notice is required (preferably two weeks prior to the deadline) to allow for this to be factored into the reporting time scales.

6. This year all nine of the participating laboratories submitted data for both wet and dry weights for all samples. Although many in-house field procedures do not incorporate dry weight of algal samples these values are included in the NMBAQC scheme to allow comparison of laboratory procedures. The values provide evidence of insufficient rinsing of samples, whereby the laboratory dry weight is considerably higher than the actual dry weight. Also, there is no definitive wet weight from which to compare the individual laboratories submissions, so it is difficult to conclude which results are the most accurate. However, the dry weight can be compared directly with the original weight of the samples which was accurately measured prior to addition of debris. In addition, some laboratories only measure dry weights and therefore, for such an exercise to be appropriate for these laboratories this measure of biomass needs to remain within the test. It is in the interest of all participating laboratories to complete both aspects of the test as submission of partial results may hinder any explanation of outliers and skew statistics due to the relatively small datasets. During future ring tests, it is recommended that all laboratories should continue to complete the full exercise even if it is not part of their routine monitoring to maximise the usefulness of the ring tests.

7. Several participants have requested the inclusion of other materials in the samples to represent items that would usually need to be removed during the rinsing process and this is something that has come up in previous years. Suggestions included wood/twig debris, gravel/stones, material representing non-opportunistic macroalgae, seagrass, *Peringia ulvae* and increased volumes of thicker sediment. Some of these requests are more practicable than others. Since additional macroalgae/seagrass materials will also need to be artificial, there is potential for confusion if materials that are not meant to be weighed are included in the samples. However, these comments will be carefully considered and possible materials and substrata will be investigated for inclusion in future tests.
8. There were an increased number of participating laboratories in the current exercise than in RT12 and RT13, providing a sample size more comparable to pre-pandemic levels. Larger participant sample sizes provide more accurate mean values and help to identify outliers that could easily skew the data in a smaller sample of participants.
9. This year all participants entered their results into the spreadsheets provided. This has made the analysis process smoother and reduced the risk of errors during subsequent calculations. It is requested that participants continue to submit only final dry and wet weight results using the workbooks provided to reduce the risk of transcription errors.
10. There is still some question as to whether the methodology for both wet weight and dry weight is being read and followed consistently across all laboratories. This applies to the appropriate squeezing of samples and the removal of debris. It is clear in the methods that when working with a large biomass this should be split into smaller sizes such as the size of a tennis ball, to ensure they can be squeezed properly. Any attempts to squeeze the sample as a whole will result in too much residual water being retained within the sample and increase the wet weight. This can affect the whole sample weight and increase the average. The length of time required to dry the samples may also vary from sample to sample and from lab to lab and if the samples are not completely dried or thoroughly checked prior to weighing this can result in an estimate of dry weight that is significantly greater than the actual dry weight.

If anyone has further thoughts on this, or disagrees with any of the interpretation, please pass forward your comments to nmbaqc@apemltd.co.uk. The biomass ring test is now in its fourteenth year and although proving successful it is still open to continual refinement.