The NE Atlantic Marine Biological Analytical Quality Control Scheme

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Macroalgae Biomass

Component Report

Ring Test OMB RT16 2025

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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 sixteenth 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 - RT15). 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 RT16 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 RT16 Bulletin.

1.2 Participating Laboratories

Ten laboratories were issued test material, of which nine laboratories returned completed results workbooks. The tenth laboratory decided they no longer wished to participate in the biomass test. Of those participants that submitted results, eight laboratories were government organisations and one was a non-governmental organisation.

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 individual; however, this was not recorded within the results outputs. Individual laboratories may use such results to audit internal processes.

Currently this component of the Scheme does not specify a definite qualifying performance level, and NMBAQC ring tests may be considered 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 both 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 2025. Participating laboratories were required to submit biomass results for both wet and dry weight. The samples were composed of materials that were consistent with those used in previous years including j-cloths, wool, kapok (stuffing material made from natural plant fibres) and shredded biodegradable food waste bags, the latter of which were successfully introduced during OMB RT14. These materials have been deemed to be the most representative of the look and feel of actual opportunistic macroalgae species based on feedback from previous ring tests. Cloths and wool were cut to different sizes and lengths to represent different foliose and filiform taxa (e.g. *Ulva* spp.). The kapok represents finer algae such as *Chaetomorpha* spp. whilst the shredded food waste bags 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 allow different individuals to complete the test analysis of each sample.

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 (31), and the final two digits representing the laboratory.

1.7 Preparation of the Samples

To assess the accuracy of determining biomass of opportunistic macroalgae, samples were prepared using j-cloth, wool, kapok and food waste bags that had been cut and shredded to mimic algal species. 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, both wet weight and dry weights had to be ascertained.

The samples were labelled A, B and C with samples of identical original dry weight provided to all participants. For RT16 all three samples were prepared with the same initial dry weight, but composed of different mixtures of materials:

Sample A – 15.0 g (mixture of 5.0 g wool, 5.0 g j-cloth and 5.0 g kapok)

Sample B – 15.0 g (mixture of 10.0 g j-cloth and 5.0 g kapok)

Sample C – 15.0 g (mixture of 10.0 g wool and 5.0 g food waste bags)

Feedback from participants in previous years has included requests for different substrata and/or fauna that would need to be removed from the samples prior to weighing, so for RT16 empty cockle shells were included with the sediment in Sample A and a synthetic rubber polychaete was added to Sample B and gravel was included with the sediment in Sample C.

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. Larger samples 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 to participants with the exercise instructions to standardise the format in which the results were submitted. The completed results will be retained and stored confidentially. 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 timescale.

1.9 Z-Scores

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

Z-scores were calculated for each submitted weight value 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' submitted weight values;

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

Z-scores were calculated separately using the means of the participants' submitted wet and dry weights for each sample and then using the known dry weights and predicted wet weights (see Section 2.2) for each sample. For consistency with the previous biomass ring tests, 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

All nine of the laboratories that returned results for OMB RT16 provided 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 RT16 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 summarises the range of wet and dry weights recorded by the participating laboratories.

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

Lab Code	Sample A		Sample B		Sample C	
	Wet Weight (66.5 g)	Dry Weight (15.0 g)	Wet Weight (66.5 g)	Dry Weight (15.0 g)	Wet Weight (66.5 g)	Dry Weight (15.0 g)
MA3101	64.02	16.36	65.39	15.79	46.05	16.03
MA3104	94.68	18.56	97.07	17.61	59.08	15.84
MA3105	80.1	17.2	67.5	17.5	42.6	15.5
MA3106	69.1	29.6	101.6	38.7	55.3	18
MA3108	99	16.26	104	21.54	80	24.13
MA3110	92	15	100	15	53	15
MA3111	81	15	77	15	50	15
MA3112	73.48	16.17	55.38	15.79	46.19	17.31
MA3113	83.8	16.4	76.5	16	47	15.4
Max	**99**	**29.6**	**104**	**38.7**	**80**	**24.13**
Min	**64.02**	**15**	**55.38**	**15**	**42.6**	**15**
Range	**34.98**	**14.6**	**48.62**	**23.7**	**37.4**	**9.13**
Average	**81.91**	**17.84**	**82.72**	**19.21**	**53.25**	**16.91**
St Dev	**11.83**	**4.54**	**18.25**	**7.58**	**11.28**	**2.89**

Sample A was composed of equal weights of shredded j-cloths, wool and kapok and had the narrowest range of wet weights, varying between 64.02 g and 99 g. None of the wet weights were flagged as 'fails' based on z-scores. The submitted dry weights ranged from 15 g to 29.6 g. The highest dry weight was recorded by Laboratory MA3106, with a result almost double the known dry weight of 15 g, indicating that the sample was either not completely rinsed of sediment and debris or that insufficient time was allowed for drying prior to

measuring the final dry weight. This outlier was the only Sample A wet or dry weight to be flagged as a 'fail' based on the z-scores calculated from the mean participant results.

Sample B consisted of a mixture of j-cloth and kapok, with an actual dry weight of 15 g. This sample had the largest ranges of both wet and dry weight results. The wet weights ranged from 55.38 g to 104 g but none were flagged as 'fails' based on z-scores calculated from the mean values. The dry weights varied between 15 g and 38.7 g with the highest weight more than double the known dry weight of 15 g, again recorded by laboratory MA3106. As with sample A, this suggests that the sample was either not fully rinsed of sediment and debris or not adequately dried before weighing. This high value was the only Sample B wet or dry weight to be flagged as a 'fail' based on the z-score calculated from the mean participant results.

Sample C comprised a mixture of wool and shredded food waste bags with an actual dry weight of 15.0 g. This sample had generally lower wet weight results than Samples A and B, ranging between 42.6 g and 80 g, with an average of 53.25 g. The highest wet eight was recorded by laboratory MA3108 and was flagged as a 'fail' using z-scores based on mean participant data. Sample C had the narrowest range of dry weights, varying between 15 g and 24.13 g. The highest dry weight was recorded by laboratory MA3108, recording a weight just over 60% higher than the known dry weight of 15 g resulting in a 'fail' flag based on the z-score calculated from mean participant values. As described for Samples A and B, dry weights so high above the known dry weight of the sample suggest either incomplete rinsing of sediment and debris prior or insufficient time allowed for drying prior to measuring the final dry weight.

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. All historical biomass ring test results data were used to plot measured wet weights against known dry weights to establish a best fit trendline and generate a 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 $y = 3.8519x + 8.7189$ (where x is the known dry weight and y is the 'expected' wet weight). As the same dry weight was used for all three samples in RT16, the resulting expected wet weights for samples A, B and C were all 66.5 g.

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 RT16, the z-scores derived from the expected wet weights and actual dry weights resulted in four additional 'fails' compared to the z-scores calculated from the mean participant values. Three of these additional 'fail' flags were for the three highest wet weights for Sample A and the fourth was for the highest wet weight of Sample B.

Most of the results for both the dry and wet weights (as presented in Bulletin OMB RT16) when compared against either mean values or expected/actual weights could be considered acceptable with only four results flagged as 'fails' based on resulting z-scores. However, one of the limitations of using z-scores is that they are directly dependent on the standard deviation values and higher standard deviations can reduce the chance of achieving a 'fail'

based on the resulting +/- 2.00 cut-off value. The results for comparisons against expected and actual wet and dry weight, respectively, are also consistent with previous years with an increased number of 'fails' recorded using z-scores.

3. Discussion

Of the ten 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, although in RT16 each of the samples had a single dry weight outlier that was noticeably higher than the other eight results. In previous years the data have shown the greatest degree of consistency in results for smaller or mid-range sample weights, e.g. weight from 5 g to 40 g and all three tests were within this range for RT16. 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 inconsistencies in wet weight are likely to be at least partly due to variation in pressure applied during squeezing of samples. However, this is highly difficult to regulate between field workers.

Since RT16 used the same dry weight for all three samples, the wet weight results also highlight evident variation in the ranges of wet weights produced for the different materials. Samples A and B both included a mixture j-cloth and kapok; with sample A also including wool. The wet weights for these two samples had overlapping ranges between 55 g and 104 g, although the range of wet weights recorded for Sample A was narrower. Conversely, Sample C was composed of a mixture of wool and food waste bags and had generally lower wet weights, ranging between 42.6 g and 80 g. This disparity is most likely due to the easier processing of the materials in sample C, particularly the non-absorbent nature of the food waste bags. Participant feedback confirms that the food waste bags are easier to process whereas the samples including kapok as part of the mixture are more difficult.

It is the wet weight that is most commonly used during routine monitoring of opportunistic macroalgae and therefore where results suggest inconsistent processing techniques this 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'.

Seven of the nine participating laboratories produced dry weights greater than the known weight of the sample for all three samples; this suggests either insufficient drying or rinsing of the sample, some degree of which is to be expected during such a test. None of the dry weights recorded for RT16 were below known weight of the prepared material.

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 a return to mixing of materials from RT14 to RT16 following six years of using separate materials. The use of the same starting dry weight for each sample in RT16 helps to highlight how differences in processing difficulty between the different materials can contribute to the variance in wet weight results. With the exception of one extreme outlier for each test, the RT16 dry weights were largely consistent with one-another.

In general, the results for the current year were comparable with those from previous years and wet weight ranges were all narrower than those recorded in RT14 and RT15. The ring test can provide evidence of problems in the measuring of biomass samples, such issues may need addressing through workshops or in-house 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 mean participant data and warrant further investigation. These flags have no current bearing on the acceptability of data from 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 continues to be 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. Care will continue to be taken to pack the samples in such a way as to minimise damage and leakage during the distribution of the test materials.
3. This year has been the third to use mixtures of different materials for the samples, following six consecutive years in which each sample consisted of a different artificial material. 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 and helps reduce potential differences caused by the differences in processing difficulty with each of the different synthetic materials. Based on this feedback

samples containing mixtures of different materials will continue to be used 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 eighth year in which kapok has been used to mimic much finer opportunistic algae such as *Pylaiella* and *Chaetomorpha*. The samples including kapok were reported to be the most difficult to process due to the difficulty in rinsing free of sediment, whereas the samples including strips of food waste bags were reported as being the quickest and/or easiest material to process. This material was introduced during RT14 and has the advantage of being biodegradable. Throughout the sixteen years of the OMB ring test there has so far been no consensus on the preferred material of use and opinions can differ depending on the types of opportunistic blooms that are being experienced in the field each year. 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 sixteenth cycle of the macroalgae biomass exercise seven of the nine participating laboratories submitted results within the original deadline, and two laboratories were given an extension. The tenth laboratory decided they no longer wished to participate in the biomass test after the samples had already been distributed. Due to the interdependence of all participant results in calculating z-scores, results submitted outside of this deadline may not be accepted and it may not be possible to include them in the analyses. All laboratories should continue to submit results within the requested deadlines as detailed at the beginning of the exercise. Reminders will 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 timescales.
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. Each year there is mixed feedback from participants regarding the inclusion of other materials in the samples to represent items that would usually need to be removed during processing. Suggestions have included wood/twig debris, gravel/stones, material representing non-opportunistic macroalgae, seagrass and *Peringia ulvae*

shells. Some of these requests are more practicable than others. As has been highlighted previously, any additional macroalgae/seagrass or representative 'fauna' materials would also need to be artificial, which creates potential for confusion if materials that are not meant to be weighed are included in the samples. This year cockle shells were included with Sample A, an artificial polychaete worm was added to Sample B and fine gravel was added Sample C. Inclusion of additional material will be carefully considered and possible materials and substrata will continue to be investigated for inclusion in future tests.

8. 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.
9. 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, suggestions for alternative representative substitutes for algae 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 fifteenth year and although proving successful it is still open to continual refinement.