The National Marine Biological Analytical Quality Control Scheme
www.nmbaqcs.org

Macroalgae Biomass Component Report – OMB RT07 2016

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1 Introduction

To enable correct water quality classification and good management decision-making, quality control of biological data is a high priority. This extends through all biological elements including macroalgae and seagrass. Good quality control ensures consistency of data being reported for management purposes, and for macroalgae and marine angiosperms this has been driven primarily by the requirements of the Water Framework Directive. This QC scheme aims to facilitate improvements in biological assessment whilst maintaining the standard of marine biological data. The scheme should help to ensure consistency between analysts with improved confidence in ecological quality status.

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

- The determination of algal biomass

This is the seventh 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 – RT06 - see NMBAQC website). Test material was distributed to participating laboratories from which data forms were completed with algal biomass results and returned for analysis.

Eight laboratories were issued with test material. All eight laboratories completed the macroalgae biomass component of the NMBAQC scheme. All of the participating laboratories were government; no private consultancy took part in this component of the macroalgae exercises. To ensure consistency between scheme years, each participating laboratory was assigned the same laboratory code as in previous years except where a laboratory was new to the scheme.

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 final 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 accordingly; these may be used by competent monitoring authorities for internal monitoring of performance. These flags have no current bearing on the acceptability of data from such participating laboratories. Ring tests offer a means of assessing personal and laboratory performance from which continued training requirements may be identified, or from which improvements in current field and laboratory procedures may be addressed.

Samples are 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).

1.1 Summary of Performance

A single test consisting of three biomass samples was distributed. Each sample consisted of a synthetic mix of j-cloths, wool and synthetic stuffing material, which are considered to imitate opportunist macroalgae species. Cloths were cut to different sizes to represent different taxa (e.g. laminar or tubular taxa). Each sample was contaminated with debris and sediment of a sandy-muddy nature consistent with the substrate type known to support opportunist macroalgal blooms.

Results for wet weight of biomass varied between laboratories with some laboratories producing high measures of biomass compared against the average biomass and actual/expected biomass. The dry weights showed a similar level of variability. One laboratory failed to remain within the Z-score limit of +/- 2.0 for the average sample dry weight, there were, however, no 'Fails' for wet weight against the mean due to high standard deviation caused by a high range of results.

Three further laboratories showed significant deviation from the actual sample dry weight, this means of assessment is not as accommodating towards outliers. Sample B had a significant number of 'Fails' for wet weight when compared against the 'expected' wet weight, in total 6 out of 8 laboratories 'Failed' this sample assessment. A further one lab 'Failed' the wet weight for sample A. Most participating laboratory results were higher than the actual sample dry weight suggesting no loss of sample material during processing with two marginally lower dry weight results being attributed to limited decimal places.

2 Summary of Macroalgae Biomass Component

2.1 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.

2.2 Description

This exercise examined the participants' ability to process macroalgae samples to extract values for biomass for wet and dry weight. The exercise examines 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 2016. Participating laboratories were required to submit biomass results for both wet and dry weight. The sample material was consistent with that of OMB RT06 including both cloths and wool. However, a new material was introduced, synthetic toy stuffing, to further represent some of the finer opportunist forms. This was suggested by a participant during the previous ring test so was trialled this year for effectiveness in replicating actual macroalgae. Non-biological and non-algal biological material was added to simulate contaminating materials encountered in the field.

2.3 Logistics

Each sample was distributed within an airtight plastic container. Each sample within the container was separately sealed within a zip lock plastic bag to retain moisture. The samples were distributed either via first class mail or recorded delivery, depending upon the recipient's requirements. All instructions

and additional test material was distributed on CD, within the parcel, to each laboratory. Each disc contained a description of methods and data submission forms. 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 sample analysis.

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

2.4 *Preparation of the Samples*

In order to assess the accuracy of determining biomass of opportunistic macroalgae, samples were distributed consisting of j-cloth, wool and synthetic stuffing material that had been cut and finely shredded in order to mimic species of *Ulva*. These alternative materials were deemed to be the most representative of actual opportunist species and were based on suggestions from previous ring test feedback forms. Three representative samples were supplied for subsequent processing. Sediment and debris commonly found within areas of opportunist 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 sample were labelled from A to C. Samples of identical original dry weight were provided for all participants.

Sample A – 17.4g

Sample B – 72g

Sample C – 31.8g

Due to the nature of the samples they could be kept for several days retaining most of the moisture. However, much of the water was removed prior to distribution to reduce weight during transportation therefore it was 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.

2.4.1 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 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 could be removed by hand, but the sample should not run green, as this indicates damage to cell membranes (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.

2.4.2 Method for Dry Weight

Once each of the samples has been wet weighed they are spread out on a sorting tray or similar container. By spreading the samples this 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 is required for all 3 samples.

2.5 Analysis and Data Submissions

A pre-prepared spread sheet 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 a dry weight and a wet weight for each of the 3 samples provided. Laboratories were permitted six weeks to complete the sample analysis and submit results.

2.6 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 originally commenced, and the final two digits represent the laboratory. For example, laboratory twelve in scheme year twenty three will be recorded as MA2312.

2.7 Results

2.7.1 General Comments

In total eight laboratories signed up for the biomass component of the macroalgae element for OMB RT07 and all eight laboratories 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 OMB RT07 Preliminary Bulletin Report, which represents a summary of the results for RT07. The Bulletin provides 'Pass' and 'Fail' flags to each data set to highlight deviation from sample mean and actual results. Values of Z-scores were used to apply the 'Pass' & 'Fail' assessment.

Z-scores, calculated to indicate how much each participant's weight results deviated from the mean, used the following formula:

$$Z = \frac{X - \mu}{\delta}$$ where μ is population mean and δ is the standard deviation

A Z-score of greater than +/- 2.0 was considered to be outside an acceptable limit of deviation from the mean. This value was assigned a 'Fail' or 'Pass' flag on the data. However, it should be noted that 8 sets of data is not considered a large sample size for deriving Z-scores.

2.7.2 Returns from Participating Laboratories

The raw data (Table 1) indicates a wide range of both wet and dry weights. The range of results was greatest for the algae mass of the largest weight from both dry and wet weights. This is consistent with all previous OMB tests. For wet weight the range of results was 70.66 – 127.6 (Sample A), 312.18 – 398.3 (Sample B) and 112.88 – 143 (Sample C). This clearly indicates a degree of variation in data and lack of consistency between laboratories during the rinsing and squeezing of the samples particularly within the larger sample sizes (Samples B and C). The large degree of variation in wet weight results are primarily a result of the non-specific method of squeezing and rinsing as this is an

element of the exercise that cannot be measured successfully and can vary significantly between participants. This is particularly evident with the larger sample sizes where there is a greater chance of error.

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

	Sample A		Sample B		Sample C	
Lab Code	Wet weight	Dry Weight 19.4g	Wet weight	Dry Weight 72g	Wet weight	Dry Weight 31.8g
MA2310	73.421	19.41	355.594	81.381	139.77	32.106
MA2303	91.4	19.5	353.9	72.3	122.3	32.1
MA2302	70.66	19.8	351.44	147.5	125.23	32
MA2309	106	19	369	86	131	34
MA2334	127.6	21.1	398.3	96.7	143	32.6
MA2319	95.71	19.69	337.74	84.61	133.35	34.57
MA2317	115	19	387	105	134	35
MA2311	95.18	21.36	312.18	87.3	112.88	36.35
Max	127.6	21.36	398.3	147.5	143	36.35
Min	70.66	19	312.18	72.3	112.88	32
Range	56.94	2.36	86.12	75.2	30.12	4.35
Average	96.87	19.86	358.14	95.10	130.19	33.59

The level of variation in dry weight was also consistent with previous years. The dry weights results displayed a couple of large outliers, laboratory MA2302 submitted results considerably higher than the sample mean and actual dry weight for sample B and laboratory MA2317 also submitted results from sample B that could be considered significantly higher than the mean and actual dry weight, causing a slight skew in the overall results and a slightly higher mean and standard deviation than would be considered acceptable. The results from these laboratories indicate some problems during the processing of the samples. This may be due to procedures used, inadequate rinsing or incomplete drying. Given that the wet weight values from these labs for sample B were consistent with the average the data suggests that possibly the samples were not dried fully prior to weighing. Sample A shows a much higher range of wet weight values than would be expected for such a small sample, this large range can be attribute to a couple of high wet weights from labs MA2334 and MA2317. The dry weights for these labs were very consistent with the actual dry wet therefore it may be speculated that the sample was not squeezed sufficiently or to the same degree as the other participants.

The range of results for both the dry and wet weights (as seen in Bulletin OMB RT07) when compared against the mean could generally be considered acceptable with only one 'Fail' providing evidence of a good degree of consistency in practiced methods. As with previous years it is evident that the level of error in the results submitted is related to the actual sample size provided with the only 'Fail' recorded for sample B, the largest of the three samples. However the smallest of the samples does not always produce the most consistent results between laboratories either, and in some instances it is the mid range sample sizes that produce the best and most constant sets of data. This may be due to some small biomass samples being harder to squeeze.

In total three results were flagged as 'Fail', when using Z-scores based on actual dry weight of sample. These were for Laboratories MA2311, with a z-score of 2.185 for sample A, Lab 2302 had a Z-score of

3.237 sample B, and lab MA2311 had a Z-score of 2.786 for sample C, all falling just outside of the cut-off value..

The comparison of results against expected wet weight produced a number of 'Fails' for sample B. The expected wet weight was calculated using all historical NMBAQC data including the current years data. The expected wet weight is based upon the known dry wet from which a scatter plot of dryad n wet weight results can be plotted producing a best fit trendline and corresponding linear equation. This linear equation can be applied to the known dry weight to allow an 'expected' wet weight to be calculated from which all wet weights may be compared. The linear equation applied to this years data was $y = 3.9224x + 4.067$.

The 'expected' wet weight for samples A, B and C were 80.16g, 286.48g and 128.80g respectively. Sample A had one 'Fail' for MA2334 with a z-score value of 2.449. However sample B should the greatest degree of deviation from the 'expected' wet weight with a total of 6 of the 8 laboratories 'Failing'. The average wet weight as calculated from all participants was 80g higher than the 'expected' wet weight with those 'Passes' also recording well over the 'expected' wet weight of 286.48.

With the exception of two laboratories (MA2309 and MA2307) all dry weight results were higher than the original sample weight. This is to be expected during the exercise. The two lower dry weights were insignificant and do not detract from their level of accuracy and could probably be attributed to the fact they were not weighed to 2 decimal places thereby recording a dry weight of 19g rather than the actual 19.4g.

2.8 Discussion

Of the eight samples distributed to eight laboratories all 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 both an overestimation of wet and dry weights. Seaweed is much harder to rinse especially in the field so may contribute to an overestimation of the levels of biomass present. Equally some laboratories do not measure wet weight only recording 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 all the different measurements and procedures utilised.

The level of accuracy still remains greater for comparisons of dry weight than for wet weight, for reasons given above. However, this is significantly less for smaller or mid range sample weights e.g. weight from 10g to 40g. This suggests the techniques used between laboratories to rinse and squeeze vary considerably and may also do so 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 opportunist monitoring, therefore this lack of consistency in methodology should be fully addressed within the standard operating procedures especially in association with areas of high biomass. Each lab should have its own in-house training and competence assessment measures. It is recommended within the test methods 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 a dry weight greater than that of the actual biomass of the sample; this would be due to insufficient drying or rinsing of the sample a level of which can be expected during such a test. However, two laboratories produced dry weights less than that of the actual biomass which due to the minor loss of weight may be attributed solely to the lack of use of decimal places in their submitted results. Furthermore the significant deviation in results from one laboratory (MA2302), for Sample B from the actual dry weight produced an exceptionally high standard deviation making it impossible for the analysis to pick up any smaller deviations from actual biomass without removing the outlier.

There was an obvious trend whereby the level of deviation from actual biomass increased as the sample biomass increased. There is no apparent reason for this, the larger biomass may be more difficult to rinse free of debris or possibly it is more difficult to squeeze or dry thoroughly. This is equally something that should be addressed within individual laboratories as well as across standard operating procedures to reduce this level of error. Laboratories may wish to check internal samples for this pattern.

In general the results were comparable with those from previous years. The ring test is able to provide evidence of problems in the measuring of biomass samples, such issues require addressing through workshops and specifically aimed training. Hopefully on receipt of the results bulletin those laboratories with outliers will also be able to review the procedures adopted 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.

3 Conclusions and Recommendations

A number of observations may be made from the results of the exercise and from participants' feedback which have been 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 samples arrived in good condition and apart from some extensive drying times the tests were considered quick and easy.

2. It seems there is now a general agreement that the use of artificial material to mimic algae is an acceptable surrogate for the test albeit less fragile and easier to rinse and squeeze than the real thing. This year saw the addition of synthetic stuffing to mimic much finer opportunist algae such as Pilayella and Chaetomorpha. One lab found this to be less representative of opportunist macroalgae and suggested incorporating more j-cloth where as most other labs thought this to be a good addition. It is appreciated that the use of synthetic materials do not fully represent the conditions experienced within the field. It may be possible in the future to utilise alternative materials that may be more representative of the texture and general nature of opportunist algae but at this stage alternative materials have not been tested with the same success rate. Throughout the seven years of the OMB ring test there has so far been no general consensus on the preferred material of use, therefore all three materials will continue to be used for future tests or until a more realistic alternative is sourced.

3. During this seventh cycle of the macroalgae biomass exercise all participating laboratories submitted results within the designated timescale. 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 two weeks prior to the completion of the exercise to aid with this process.

4. This year all laboratories submitting results managed to complete both wet and dry weights for all samples, however some participants still question the necessity to incorporate both dry and weights within the ring test. Although many in-house field procedures do not incorporate dry weight of algal samples these values are included within the NMBAQC scheme to enable comparison of laboratory procedures. The values provide evidence of insufficient rinsing of samples, whereby the dry weight would be considerably higher than the actual dry weight. Also there is no definite wet weight from which to compare the individual laboratories submissions so it is difficult to conclude which results are the most representative. The dry weight however can be compared directly with the original weight of the samples which was measured very accurately prior to addition of debris. Most laboratories submitted dry weight values that were considered well within an acceptable limit of the actual biomass; however wet weight still remains highly variable. Therefore the level of squeezing still remains an issue within the overall procedure and should be addressed. In addition, some laboratories only measure the dry weight therefore, for such an exercise to be appropriate for such laboratories; this measure of biomass needs to remain within the test. It is in all laboratories' own interest to complete all aspects of the test. Submission of partial results may hinder any explanation of outliers and skew statistics due to the relatively small data sets. During subsequent ring tests, all laboratories should continue to complete the full exercise even if it is not part of their routine monitoring in order to maximise the usefulness of the ring tests.

5. It was suggested that various debris be added to the sample to enable a more realistic comparison with field procedures. There are further suggestions that more *Hydrobia* could be added to the sample or material to mimic Hydrobia. This is definitely something that will be considered and applied for future tests.

6. It is evident that the larger samples create a greater margin of error with far less consistency between laboratories. However, it has been suggested that these samples are more appropriate in terms of representing natural conditions. This will be taken on board when compiling future tests whereby they will be aimed at including a good range of weights but focusing on some much larger biomass weights.

7. There may be future requirements to include biomass analysis within a workshop to further discuss processing procedures and levels of intensity for manual removal of debris and water. This has been suggested by some participating laboratories and may be considered a more realistic measure of quality assurance. This is something that requires further discussion as to the nature of the approach.

8. A number of laboratories submitted results to a lesser degree of accuracy than others. It is stipulated that both wet and dry weights be provided to 2 decimal places where possible. This will highlight smaller variations in weight as the samples are relatively small compared with some field samples. However if this is not feasible for some laboratories then measurements to the nearest gram are also acceptable but it needs to be recognised by participating laboratories that such measurements will be less accurate particularly with smaller sample sizes. In the instance where the dry weight recorded is less than the actual weight this may be an indication of loss of

material but may also be linked to the accuracy of the scales. It is recommended that all laboratories use calibrated scales so as to reduce such minor discrepancies.

9. It is requested that all laboratories fill out the result spreadsheets provided and include *all* the required information. Data presented in Word files or within emails is very inconvenient when collating and storing the results and will not be accepted in subsequent years. If this does occur a request will be sent for the data to be completed in the correct format. Not complying with instructions can create significant extra work.

10. There is 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 and increase the average. It is also clearly stated that the material used to mimic the algae is J-cloth and wool, any other material within the sample may be considered debris and should be removed during the washing phase. Failure to remove the debris will result in much higher wet and dry weights. 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 a dry weight significantly greater than the actual dry weight. These points will be made clearer in future methodologies. In future tests extreme outliers may also be removed from the analysis so as to highlight minor discrepancies between labs.

11. The differences in sample processes have become evident through the degree of variation in the results submitted. There needs to be a greater level of consistency in the methodology utilised for both rinsing and squeezing of samples and documented in guidance procedures to be distributed to all laboratories involved in such practices. There are often a number of outliers which significantly skew the results and affect the average weight which is used to compare all other results. If this average is abnormally high or low it will affect the outcome of some laboratories results which might otherwise be considered acceptable.

12. It has also been questioned whether the procedures of the test should be followed or those of the individual laboratory. The two methods may vary in terms of the amount of squeezing pressure applied to the sample. It is important that an individual laboratory has consistent results that are comparable from year to year. However if they are consistently higher of lower than other labs they may be under or overestimating the actual biomass, particularly with regards to wet weight, which may then be reflected in the overall classification of a water body when applying the WFD blooming tool or any other quality status assessment.

If anyone has further thoughts on this, or disagrees with any of the interpretation, please pass forward your comments to Dr Emma Wells (emma@wellsmarine.org). This ring test is now in its seventh year and although proving successful it is still open to continual refinement.