The National Marine Biological Analytical Quality Control Scheme
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Macroalgae Biomass Component Report – OMB RT03 2012

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April 2012
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1 Introduction

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

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

- The determination of algal biomass

This is the third year in which biomass of macroalgae has been included as an element of the NMBAQC scheme and was included a single exercise. The format followed that of years one and two of the test (OMB RT01 & RT02). Test material was distributed to participating laboratories from which data forms were completed with algal biomass results and returned for analysis.

Nine laboratories were issued test material. All nine 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. It was possible for each sample to be completed by a different participant; however, this was not recorded within the final results.

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

1.1 Summary of Performance.

This report presents the findings of the macroalgae biomass component for the third year of operation within the National Marine Biological Analytical Quality Control (NMBAQC) Scheme. This component consisted of a single exercise producing a single set of results from each laboratory.

The analytical procedures of the exercise remained consistent with rounds one and two of the scheme (OMB RT01 & RT02). The results for the exercise are presented and discussed with comments provided on the overall participant performance.

A single test consisting of three biomass samples were distributed. Each sample consisted of synthetic mix of j-cloths and wool that are considered to imitate opportunist macroalgae species. Each sample was contaminated with sediment of a sandy muddy nature consistent with the substrate known to result in opportunist algal blooms.

Results for wet weight of biomass varied between laboratories with some laboratories producing much higher measures of biomass compared against the average biomass. The dry weights showed a similar level of inconsistency. Two laboratories deviated significantly from both the average sample wet weight and the actual sample dry weight. Most participating laboratory results were higher than the actual sample weight with the exception of only one laboratory which recorded lower results for two samples.

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 of 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 allow the determination of errors in processing at various stages of the methodology.

One set of three representative samples were distributed to each participating laboratory in January 2012. Participating laboratories were required to submit biomass results for both wet and dry weight. The sample consistency was amended from the second scheme (OMB RT02) to include a greater proportion of wool to further assist with the more accurate imitation of actual macroalgae samples as per OMB RT02.

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 personal requirements. All instructions and additional test material was distributed on CD, within the parcel, to each laboratory. Each disc contained description of methods and data submission forms. Participants were provided a month to complete the test and return the results. Only one set of results could be submitted from each laboratory 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 both j-cloth and wool material that had been cut and finely shredded in order to mimic species of *Ulva* (previously known as *Enteromorpha*). (It was not considered practicable, or reliable, to obtain samples of natural material. The alternative materials were deemed to be the most representative of actual opportunist species and were based on suggestions from OMB RT01 and RT02 feedback). 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.

Three samples were provided and labelled from A to C. Identical weights were provided for all participants.

Sample A – 60g

Sample B – 16g

Sample C – 91g

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 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 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 still be properly squeezed. 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 (over-enthusiastic squeezing of actual algal samples might 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 which may vary between laboratories.

2.4.2 Method for Dry Weight

Once each of the samples has been wet weighed they are laid and spread out on a sorting tray or similar container. By spreading the samples this aided with the drying process. The samples are left to air dry for 24 hours. 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 4 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 original commenced, and the final two digits represent the laboratory. For example, laboratory twelve in scheme year nineteen will be recorded as MA1912.

2.7 Results

2.7.1 General Comments

In total nine laboratories signed up for the biomass component of the macroalgae element for RT03. All nine laboratories returned both wet and dry weight data. A brief extension was given to one laboratory to enable all dry weights to be submitted. The results have been collated and represented in various formats to enable full comparisons between participants and against actual sample weight.

Details of each participating laboratories performance were distributed in OMB RT03 Bulletin Report, which represent a summary of the results for RT03. 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:

$$Z = \frac{X - \mu}{\delta}$$

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.

2.7.2 Returns from Participating Laboratories

Each laboratory returned all 6 algae biomass weights which were compared against other participating laboratories and against actual dry weight of samples as calculated prior to distribution.

The raw data 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 both RT01 and RT02. For wet weight the range of results was 174.7 – 359.1 (Sample A), 44.12 – 289.2 (Sample B) and 108.9 – 490.5 (Sample C). This clearly indicates a high degree of variation in data and lack of consistency between laboratories during the rinsing and squeezing of the samples. These variations in weight could be attributed largely to large outliers from two laboratories (MA1908 and MA1901). The remaining laboratories were more consistent in their wet weight results. The large degree of variation are primarily a result of the non-specific method of squeezing as this is an element of the exercise that cannot be measured successfully and can vary significantly between participants.

The level of variation in dry weight was also much higher than anticipated. Laboratory MA1901 submitted results considerably higher than the sample mean for samples A and C, causing a considerable skew in the overall results and an abnormally high mean and standard deviation. The results from this laboratory indicate significant problems during the processing of the samples. This may be due to procedures used, inadequate rinsing or incomplete drying. The high levels of both wet and dry weights possibly suggest insufficient rinsing of the samples. However, in contrast, sample B was much more comparable in terms of wet weight and also produced an accurate dry weight, indicating the correct procedures were being used, if in part. Therefore it is unclear as to the reason for the production of such outliers. Laboratory MA1908 also produced biomass results that deviated from both the mean laboratory wet weight and the actual dry weight. Dry weight for samples A and C were significantly lower than the actual sample which may indicate loss of material during the rinsing process however for sample C this would be a 73% loss of material which is more difficult to speculate as to the cause of such loss.

The range of results for both the dry and wet weights (as seen in Bulletin OMB RT03) are far more acceptable, and more comparable with the actual dry weights, once the anomalies have been removed from the sample. The range of values after removal of anomalies for Sample A (60g) was 2.1g for Sample B (16g) was 0.4g and for Sample C (91g) was 11.89g. It is evident that as the actual dry weight of the sample increases as does the level of error and total range. This has also been seen in previous exercises.

In total six results were flagged as 'Fail', when using Z-scores based on sample mean. Three of these were for Laboratory MA1908, due to exceptionally high wet and dry weights for sample B and a low dry weight for sample C. The remaining three 'Fails' were for Laboratory MA1901 with exceptionally high wet and dry results for sample A and for dry weight of sample C. All remaining scores were within +/- 1.0.

A second Z-score was calculated based on deviation from the actual known dry weight using the same criteria to flag 'Pass' and 'Fail'. This resulted in a total of three 'Fails'. The Z-scores were all within +/- 0.3, with the exception of the three anomalies, two from laboratory MA1901 and one from MA1908. The high level of deviation from actual dry weight values as submitted by these Labs produced a higher standard deviation for the population mean and has prevented any small deviation from the actual weight becoming evident in this analysis.

With the exception of one laboratory (MA1908) all results were higher than the original sample weight. This is to be expected during the exercise. The two lower dry weights from MA1908 suggest some error during processing.

2.8 Discussion

Of the nine samples distributed, all nine laboratories submitted results of which only one submitted results for the wet weight but was given an extension to submit dry weights as well. Although many laboratories do not routinely measure dry mass for macroalgae, this is still a necessary part of the 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. Seaweed is much harder to rinse especially in the field so may contribute to an overestimation of the levels of biomass present.

Two laboratories provided results for which the dry and wet weights both deviated significantly from the mean and actual biomass values. Excluding these extreme outliers produced, the results do indicate a much higher level of accuracy associated with dry weight than wet weight. This suggests the techniques used between laboratories to rinse and squeeze vary considerable 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. 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.

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, one laboratory produced dry weights less than that of the actual biomass which suggests possible loss of material during the rinsing processing. The significant deviation in results from two laboratories from both the mean and actual weight produced an exceptionally high

standard deviation making it impossible for the analysis to pick up any smaller deviations from mean and actual biomass without removing the outliers.

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.

In general the results were comparable with those from RT01 and RT02 with a good level of agreement between most participating laboratories. 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.

3 Conclusions and Recommendations

A number of observations may be made from the results of the exercise 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 positive 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 artificial material use to mimic algae is an acceptable surrogate for the test. There is still some of questioning over the actual material used with suggestions that a thinner material may be more appropriate or use of plastic bags to mimic Ulva. It is appreciated that the use of wool and J-cloths 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 but at this stage alternative materials have not been tested with the same success rate. However, it was agreed that the use of wool is slightly more representative than the J-cloth, and with new suggestions of material these can also potentially be used for subsequent tests.

3. During this third cycle of the macroalgae biomass exercise the majority of participating submitted results within the designated timescale. However, there are still late submissions which contribute to the delayed production of both bulletins and reports. All laboratories should endeavour to submit results within the requested deadlines as detailed at the beginning of the exercise. In subsequent years reminders will be distributed a week prior to the completion of the exercise to aid with this process.

4. This year all laboratories managed to complete both wet and dry weights for all samples, however there is still a question over the necessity to incorporate dry weights within the ring test. Although many in house field procedures do not incorporate dry weight of algal samples these values are included within NMBAQC scheme to enable analysis 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. During subsequent ring tests, all laboratories should continue to complete the full exercise even if it is not part of their routine monitoring.

5. It was suggested that the mud added to the sample, to enable a more realistic comparison with field procedures, should include more debris. There was a suggestion that shells and hydrobia could be added to the sample as well as thicker and more gloopy mud to reduce the ease with which the samples can currently be rinsed. It has also been commented that the artificial material is also easier to rinse and addition of more debris would be slightly more representative of the usual field conditions.

6. 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.

7. 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. An agreement needs to made on the most applicable number of decimal places, prior to the next exercise, to ensure all laboratories are content with the methodology.

8. 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 a Standard Operating Procedure to be distributed to all laboratories involved in such practices.

If anyone has further thoughts on this, or disagrees with any of the interpretation, please pass forward your comments to Dr Emma Wells (emma@wellsmarine.org) or Dr Clare Scanlan (clare.scanlan@sepa.org.uk). This ring test is still only in its third year and very much in its developmental stage but hopes to be continually refined.