NMBAQC

NE Atlantic Marine Biological Analytical Quality Control Scheme

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Macroalgae Biomass Component Report – OMB RT12 2021

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MACROALGAE BIOMASS COMPONENT REPORT FROM THE CONTRACTOR SCHEME OPERATION –2020-21

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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 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 twelfth 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 – RT11 - see NMBAQC website). Test material was distributed to participating laboratories from which data forms were completed with algal biomass results and returned for analysis.

Four laboratories were issued with test material. All four laboratories completed the macroalgae biomass component of the NMBAQC scheme. All of the participating laboratories were government; no other organisations 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 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. This year each sample consisted of a different synthetic material including j-cloths, wool and synthetic stuffing material. These are

currently considered the most representative materials in terms of imitating the overall look and feel of various opportunist macroalgae species. Cloths and wool were cut to different lengths and sizes to represent different foliose and filiform taxa (e.g., Ulva). The synthetic stuffing is considered to be more representative of finer opportunist algae such as Ectocarpus sp. and Chaetomorpha sp. 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, particularly for the larger sample. The dry weights also showed a high degree of variability between laboratories. All laboratories remained within the Z-score limit of +/- 2.0 for both the dry weight and wet weight against the mean, which may have been due to the high standard deviation caused by the high range of results.

All four laboratories showed significant deviation from the actual dry weight of sample A with a further two 'Fails' against both wet and dry weight from one laboratory. It is worth noting that this means of assessment (against actual weight) is not as accommodating towards outliers hence the higher number of 'Fails'. There was a total of six 'Fails' across all assessments of which five could be attributed to dry weight comparisons. Three laboratories had dry weights lower than that of the actual dry weight for sample B, suggesting minor losses of material during the rinsing process.

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 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 2021. Participating laboratories were required to submit biomass results for both wet and dry weight. The sample material was consistent with that of OMB RT11 including cloths, wool and synthetic stuffing. 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 were distributed via file share to each laboratory. The files 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

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 – 68.5g

Sample B – 24.9g

Sample C – 8.9g

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 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, 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-eight will be recorded as MA2812.

2.7 Results

2.7.1 General Comments

In total four laboratories signed up for the biomass component of the macroalgae element for OMB RT12. All four laboratories returned both wet weight 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 RT12 Preliminary Bulletin Report, which represents a summary of the results for RT12. 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 4 sets of data are 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 degree of variation per sample is usually proportional to the sample size. Sample A had the largest dry weight and resulted in the largest range of results for wet weight (274.1g). However, Sample C, with the lowest dry weight, had the largest range of results for dry weight analysis (14.4g).

Sample A was the largest of the three samples and this was clearly seen through the range of results produced. The wet weight ranged from 211.6g to 485.g, a total difference of 274.1g. The average wet weight (293g) was relatively close to the predicted wet weight (273g) for the sample, suggesting some degree of consistency over the range of results. However, lab MA2811 recorded a wet weight of 485.7 which significantly skewed the data range and raised the average.

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

	Sample A		Sample B		Sample C	
Lab Code	Wet weight 276.3g	Dry Weight 68.5g	Wet weight 100.1g	Dry Weight 24.9g	Wet weight 35.7g	Dry Weight 8.9g
MA2810	249.2	75.45	74.63	24.17	50.32	9.11
MA2802	211.6	74	62.2	24	27.3	9
MA2811	485.7	79.1	254.7	25.1	131.9	23.4
MA2840	227	78	79	24	41	10
Max	485.7	79.1	254.7	25.1	131.9	23.4
Min	211.6	74	62.2	24	27.3	9
Range	274.1	5.1	192.5	1.1	104.6	14.4
Average	293.38	76.64	117.63	24.32	62.63	12.88

Sample A also resulted in dry weights higher than the actual dry weight, this was consistent across all four labs. Comparing the dry weights of Sample A against the mean resulted in no 'Fails'. This was due to the small range of results. However, when comparing against the actual dry weight each of the four labs recorded a 'Fail' for the sample. The average dry weight for Sample A was 8.14g higher than the actual dry weight of 68.5g.

Sample A was wool, and it may be that this sample material is more difficult to dry or rinse. Given that the wet weight was consistent between the labs it may be concluded that the samples were not rinsed efficiently or completed dried to a consistent weigh. Where samples are large it is recommended that the material be spread evenly over a large surface to ensure efficient drying.

Samples B and C also resulted in a higher than usual range of wet weights. This range could also be attributed to higher results submitted from lab MA2811. The large range and high standard deviation for sample B was unable to highlight the outlier produced by MA2811 despite an average difference of approximately 182g. However, the outlier for sample C wet weight was successfully recorded as a 'Fail', albeit with a Z-score only slightly higher (2.041) than the boundary of +2.

Sample B showed very consistent results between labs for the dry weight with a small range of 1.1g. Three labs (MA2810, MA2802 and MA2840) showed minor losses of material albeit no more than 0.9g. This can easily occur during the rinsing phase. Alternatively, it may be as a result of the accuracy of the weighing scales being used. Since the J cloth material is shredded, smaller pieces of material can occasionally be washed away, since the loss was only marginal this is considered acceptable for this test.

Sample C was the smallest of the samples and consisted of synthetic stuffing. The smaller sample usually results in a much smaller range of wet weight results. However, this was not evident from the results which had a range from 9g to 23.4g. The average wet weight was 12.88g compared with the actual dry weight of 8.9g. Three of the four labs recorded the dry weight of Sample C to within 1.1g of the actual weight. The remaining lab recorded a dry weight of 14.4g higher than the actual weight. This outlier was only recorded as a 'Fail' using the z-score analysis when compared against the actual dry weight. The use of mean dry weight to calculate the Z-score was unsuccessful in highlighting the outlier.

It had previous been considered that with such a small sample size removal of debris and drying would be easier thereby resulting in an average dry weight consistent with that of the actual. However, this was not reflected in the data this year and is also slightly inconsistent with previous

years results. Lab MA2811 had a dry weight more than double that of the actual which significantly increased the average and standard deviations.

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. There is clearly still a lack of consistency between laboratories during the rinsing and squeezing of the samples particularly within the much larger sample size (Sample A).

There were no distinct outliers for any of the samples for wet or dry weight against mean, this can be seen in the number of 'Fails' recorded. However, the very wide range of results across all most sample weights produced a high standard deviation, which does not allow the Z-scores to successfully reveal any minor or major outliers.

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 dry and 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 $y = 4.0228x + 0.5844$. The 'expected' wet weight for samples A, B and C were 276.3g, 100.1g and 35.7g respectively.

Comparing wet and dry weights against expected wet weight and actual dry weight is less accommodating and more sensitive to slight deviations in results. As a consequence, all four laboratories 'Failed' at least one of the samples. The wet weight for samples A and B did not result in any 'Fails' possibly due to the slightly larger size of the samples. However, the wet weight for sample C had one 'Fail'. This outlier had a wet weight almost four times the expected. The dry weight for this sample was also much higher than the actual weight of the sample which may be attributed to the presence of excess debris or insufficient drying.

Overall, the range of results for both the dry and wet weights (as seen in Bulletin OMB RT12) when compared against the mean could be considered acceptable with no 'Fails', suggesting a good degree of consistency in practiced methods. The results for comparisons against expected and actual wet and dry weight, respectively, is also consistent with previous years with some 'Fails' recorded using z-scores. As previously seen with this test the high standard deviation prevented some samples from being flagged as significantly deviating from the average wet weight despite the broad range of wet weight results.

2.8 Discussion

Of the four samples distributed, all four laboratories submitted results including both wet and dry weights. 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 remains greater for comparisons of dry weight than for wet weight, for reasons given above. There is also a greater degree of consistency in results for smaller or mid range sample weights e.g., weight from 5g to 40g. The results overall suggest 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, during RT12 three laboratories also produced dry weights less than that of the actual biomass which is likely due to a slight loss of material during rinsing, albeit an insignificant amount. Furthermore, the inflated wet and dry weights produced from one lab resulted in high standard deviations and averages, making it impossible for the analysis to pick up any deviations from average or wet weight deviations from expected.

There was, as in previous years, a distinct trend, whereby the level of deviation from actual biomass increased as the sample biomass increased. It is now suspected the larger biomass may retained more debris and be more difficult to rinse free 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.

It may be considered, in this years test, that the range of wet and dry weight results between samples could again be attributed to the different materials used. This is the fourth year in which the materials have been used separately, as opposed to mixed material samples as in previous years, thus it is possible that some materials are much easy to rinse and squeeze than others leading to more accurate and consistent results between participants. It could also be considered that some materials are also more prone to loss during rinsing than others and may account for slight differences between samples. Given the results from this years test, it is also possible to speculate that it is much more difficult to obtain an accurate dry weight for the wool material which had the highest degree of variation and considerably higher dry weight results. This is consistent with the results from RT11. If the same format is adopted for following years, it will be possible to gather sufficient data to compare the attributes of the different materials used and how they respond to the squeezing and drying. It is hoped that any visible trends can be applied to both the test and to field procedures.

In general, the results were comparable with those from previous years. The ring test can 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 laboratories found the test useful despite the differences between the use of artificial material compared with actual macroalgae samples.

2. All samples arrived in good conditions. Care will continue to be taken to ensure leakage is constantly kept to a minimum during the distribution of the test materials.

3. Three samples arrived in time for the commencement of the test, one sample was late and therefore an extension was given. This year all samples were sent recorded delivery to allow for any undelivered samples to be tracked and prevent loss of tests during delivery.

4. It seems there is now a general agreement that the use of artificial material to mimic algae is an acceptable surrogate for the test. This is the four year in which synthetic stuffing has been used to mimic much finer opportunist algae such as *Pilayella* and *Chaetomorpha* and has been well received and considered at this time the most representative of the three materials. At this time the J-cloths are considered the least representative. It is appreciated that the use of synthetic materials does 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 nine 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 opportunist blooms being experienced in the field.

5. This has been the fifth year in which each sample has consisted of a different artificial material which has enabled a better comparison against actual macroalgae samples. Due to the mixed opinions on which material is the most representative all three materials will continue to be used for future tests or until a more realistic alternative is sourced. However, it was suggested at least one of the samples be a combination of all three materials to represent mixed algal stands in the field and more realistic sampling conditions. This sample may be more difficult to process but its incorporation will allow representation of mixed stands of macroalgae blooms.

6. During this twelfth cycle of the macroalgae biomass exercise four participating laboratories submitted results within the designated timescale. One laboratory was provided an extension due to delayed delivery, however the results were still submitted within the designated time period. 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. If laboratories suspect that they will not be able to submit results within the designated time period prior notice is required (preferably two weeks prior to the deadline and during reminder emails) to allow for this to be factored into the reporting time scales.

7. This year all the four participating laboratories submitted for both wet and dry weights for all samples. Some laboratories 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 remains highly variable. Therefore, the level of squeezing 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 to maximise the usefulness of the ring tests.

8. 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. Larger samples are more difficult to handle and process with higher risk of outliers or loss of material during the rinsing phase but they are a necessary component of the test to allow for a broad range of sample sizes. This will continue to be taken on board when compiling future tests whereby they will be aimed at including a good range of weights.

9. There were a fewer participating laboratories than usual during RT12. This was primarily due to COVID. Larger sample sizes provide more accurate mean values, identify outliers that could skew the data in a smaller sample and provide a smaller margin of error. With such a small data set any outliers have too great a weighting on the overall outcome and are not highlighted as 'Fails'. One laboratory submitted results that were consistently significantly above those of the expected or actual results. However, the Z-scores were unable to clearly indicate this due to the small sample size. Regardless of the outcome, each individual laboratory should take time to assess the methodology employed so as to reach consistent and accurate results.

10. 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.

11. 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 and may result in data discrepancies particularly where there are underlying formulas. It is also requested that only the final dry and wet weight

results be submitted and not the interim results, this is to eliminate error in the transferring of data and these additional results are not required as a part of the test.

12. 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, wool and synthetic stuffing, 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 to highlight minor discrepancies between labs.

13. 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 several 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.

14. 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 twenty eighth year and although proving successful it is still open to continual refinement.