



NMQC

NE Atlantic Marine Biological Analytical Quality Control Scheme

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**Macroalgae Component - Algal Identification
Module Report – RM RT12 2018**

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The logo for Wells Marine, featuring a stylized blue wave above the text 'wells marine' in a lowercase, sans-serif font.

wells marine

**MACROALGAL IDENTIFICATION MODULE REPORT FROM THE
CONTRACTOR SCHEME OPERATION -2017-18**

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

- The identification of macroalgae species

This is the twelfth year in which the identification of intertidal macroalgae has been included as an element of the NMBAQC scheme, with the format following that of previous years. Test material was labelled and distributed to participating laboratories using previously employed procedures, from which species identification forms were completed and returned for analysis.

Five laboratories subscribed to the macroalgae ring test with all five laboratories submitting results with a total of ten participants. Three of the subscribing laboratories were government organisations and two were independent consultancies. 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. Individual codes may, however, change slightly due to variations in individual participants. Due to the nature of the exercise there was no limit on the number of participants per lab.

Currently this scheme does not specify a definite qualifying performance level, and NMBAQC ring tests may be treated as training exercises. However, a pass rate of 80% is suggested as an indicator of good performance, which may be used by competent monitoring authorities for internal monitoring of performance. 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.

1.1 Summary of Performance.

This report presents the findings of the macroalgae identification component for the twelfth year of operation within the National Marine Biological Analytical Quality Control (NMBAQC) Scheme. This component consisted of a single macroalgae exercise the analytical procedures of which remained consistent with round eleven of the scheme (RM RT12). The results for the exercise are presented and discussed with comments provided on the overall participant performance.

Images of twenty macroalgae specimens were distributed to the five subscribing laboratories. Round twelve of the ring test produced a good degree of agreement between identifications made by participating laboratories and initial identification as made by Wells Marine. The ring test tried to incorporate a variety of common and more challenging species including some microscopic and epiphytic species.

The level of performance between laboratories and participants varied, with scores ranging from 30, with 4 incorrect genus names and 6 incorrect species names, to 39, with just one incorrect species name. All participants correctly identified eleven species. Most incorrect species identification were

made at the species level with four species showing considerably difficulty at both genus and species levels. Overall the level of identification was greater than the previous year with a high level of knowledge of the common species and increased knowledge of the more challenging and unusual species.

2 Summary of Macroalgae Component

2.1 Introduction

There was one module for the macroalgae identification component for scheme year eleven. This module is described in full below to include details of distribution and logistics, completion of test result forms and full analysis and comparison of final submitted results.

2.2.1 Logistics

The test material was distributed on CD to each laboratory with labelling and distribution procedures following those of previous years. Each disc contained the full identification module including photos and additional habitat, geographical, textural, and size details from which to identify specimens as well as description of methods and data submission forms. Participants were given six weeks to complete the test and return the results. There were no restrictions on the number of participants per laboratory.

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

2.2.2 Analysis and Data Submissions

A prepared results sheet was distributed with the exercise instructions to standardise the format in which the results were submitted as per previous years. All returned data was done so in Excel and has been stored and analysed in this format. In this and previous scheme years slow or missing returns for exercises lead to delays in data processing data, reporting and feedback of results, therefore reminders were distributed two weeks before the exercise deadline.

2.2.3 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, the final two digits represent the laboratory. For those laboratories where multiple submissions were provided the four-digit code is followed by a letter allocated to each participant of that laboratory. For example, participant c from laboratory twelve in scheme year twenty-five will be recorded as MA2512c.

2.3 Macroalgae Ring Test (RM RT09) Module

2.3.1 Description

This training module enables the inter-laboratory comparisons of participants' ability to correctly identify macroalgae taxa and whether errors may be attributed to inadequate keys, lack of reference material or incorrect use of satisfactory keys.

One set of photographs for twenty specimens was distributed in January 2018. The specimens included a range of Chlorophyta, Rhodophyta and Phaeophyta and a mix of macroscopic and

microscopic specimens from a variety of habitats including epilithic, epiphytic and endozoic species. There were several photographs per taxon showing different aspects of the alga and its habitat. Some supplementary information on habitat, zonation, geographical location, general size, texture, and any additional information considered vital for correct identification, was included.

2.3.1.1 Preparation of the Sample

Each specimen was to be identified through several in-situ, macroscopic and microscopic photographs. In total a minimum of five photographs was used for each specimen collected by Wells Marine for this exercise. Specimen photographs were obtained from a range of surveys from around the coast of the UK. Photographs were selected to sufficiently represent each specimen including in-situ (where possible), overall structure, branching patterns, cellular arrangements and cell contents making sure to include key characteristics for accurate identification. Scale bars were included where appropriate. Attempts were also made to ensure a high quality of photographs primarily focusing on clean specimens with sharp photographs.

Using a photographic test is considered a more practical means of testing macroalgal identification skills than preserved samples. These are known to lose colour rapidly and cell contents may become distorted making key characteristics more difficult to distinguish. Equally, fresh samples would not last a sufficient period to enable identification. It may also be difficult to obtain sufficient numbers of more unusual taxa for distribution to all laboratories.

2.3.1.2 Analysis Required

The participating laboratories were required to identify each of the macroalgae specimens from the photographs provided. Additional information should also be submitted including brief notes, information on keys used or possible problems with identification or quality of photograph provided. Expressing the level of confidence of identification should also be detailed, as this can aid in results of any disputes and in the preparation of reports. Participating laboratories were permitted to submit multiple data entries for each exercise to maximise results and allow sufficient comparisons of data entries. The protocol for circulating and completing the module followed that of previous years with six weeks allowed for the identification and submission of results.

2.3.2 Results

2.3.2.1 General Comments

The scheme has taken on the same format as previous years; this includes the format of the test and method of data analysis and scoring. The macroalgae ring test can act as a training aid in the identification of species allowing those difficult taxa to be revealed and further identifying problematic areas.

For this current round of the scheme (RM RT12) specimen photographs were circulated to a total of five laboratories. All five of the laboratories returned data entries with a total of ten individual data sets.

Results were distributed to each of the participating laboratories four weeks after data submission. These results are documented in the preliminary results bulletin (RM RT12) which detailed individual scores and highlighted incorrect identifications, miss-spellings and use of synonyms. The bulletin also outlined reasons for identification discrepancies by comparing incorrect species and genus names with those of the AQC with the aid of photographs to pick out key characteristics.

2.3.2.2 Analysis and Scoring of Data Returns

Laboratories returned lists of their species identifications within the format provided; these were compared against AQC identification as determined by Wells Marine to assess the number of differences. The method of data comparison was achieved by comparing both the genus and species names and identifying where these differed with the AQC names. Such comparison included differences in spelling or use of a valid synonym for example:

- Use of different synonym for a taxon, e.g. *Enteromorpha prolifera* for *Ulva prolifera*
- Mis-spelling of taxa name, e.g. *Halydris siliquosa* for *Halidrys siliquosa*

Such differences are highlighted, but not considered during calculation of the total number of differences in identification.

Data entries were tabulated (as seen in RM RT12 Preliminary Results Bulletin, Table 2) in order of specimen number and laboratory. The individuals' data entries are only given where they differ from the AQC identification. This includes those entries for which species are spelled incorrectly or where an appropriate synonym is provided, as well as those instances in which the specimen has been identified incorrectly. For those entries in which the participant recorded a synonym or mis-spelling, but for which the identification was consistent with that of the AQC, the name was presented in brackets [species name]. Those entries in which the identification was considered different to the AQC the species or genus name that did not correspond to the AQC was provided in the table. If part or the entire species name entered was correct this was indicated by a dash "-" any incorrect name was included in the table e.g. where *Prasiola stipitata* was identified as *Prasiola furfuracea* this would be entered as "-furfuracea".

The data entries for an individual scored one point where the entry was consistent with that of the AQC. For instance, where text other than a dash "-" or a bracketed name [name] is provided no score was given. This includes differences at both genus and species level, although species can be considered a largely independent value (where the generic identification was incorrect then the species identification would also be incorrect). Therefore, where the full genus and species name was correct a score of two would be given; where either genus or species name was incorrect a score of one would be given. The method of scoring applied to those species in which a correct identification was provided and included those instances where synonyms were used, or species/genus names spelled incorrectly.

2.3.2.3 Ring Test Results

RM RT12 contained twenty specimens for identification for which there was a good, albeit varied, level of agreement through all ten participants. At the generic level, there were a total of twenty-five differences (from a potential two hundred) across the ten sets of data received from the five participating laboratories (12.5%). At the specific level, there were a total of thirty-nine differences (19.5%). Although the total number of differences was much lower than the previous year the overall % of incorrect species identification did not change due to the lower number of participants in the current ring test.

The differences in species identifications could be attributed primarily to four taxa which showed the highest number of incorrect identifications at both the genus and species level. The four species were *Antithamnionella ternifolia* (RT1207) with 5 generic and 5 species differences, *Halopteris filicine*

(RT1208), *Derbesia marina* (RT1211) and *Capsosiphon fulvescens* (RT1216) all of which had 6 generic and 6 species differences recorded. These four species accounted for 72% of differences. *Vertebrata nigra* and *Ulothrix flacca* contributed to a further 5 and 8 differences, respectively, albeit only at the species level. Of the remaining three species where a misidentification was recorded none had more than 1 incorrect genus or species. These results indicate most of incorrect identifications could be attributed to a few species. Incorrect identifications could not be attributed to one specific phylum with Chlorophyta, Rhodophyta and Phaeophyta species proving equally problematic. In total eleven specimens were identified correctly across all participants which is significantly higher than in previous years.

There were a few alternative synonyms used, mainly attributed to very recent changes in nomenclature, these included *Acrosorium venulosum* currently known as *A. ciliolatum*, *Leathesia difformis*, currently known as *L. marina* and *Vertebrata nigra* in which *Polysiphonia nigra* was also used as a correct synonym. *Bangia atropurpurea* was also accepted as an alternative for *B. fuscopurpurea* this was due to confusion in its current status. It has been proposed (Muller et al, 2003) that *B. atropurpurea* should be re-recognized as a distinct freshwater species, and that marine populations should be recognized as *B. fuscopurpurea*. However, several descriptions still identify *B. atropurpurea* as being present at high tide and occasionally subtidally, on exposed coasts. All synonyms are accepted for the ring test and receive no scoring penalty. *Halidrys* also had one incorrect spelling but this did not affect the scoring.

The difference between participants' entries and AQC identifications was generally well distributed with all participants identifying at least one species incorrectly and no participants correctly identifying all genera. The overall scores and number of incorrect identifications ranged from one to ten which is much lower than in the previous year. A pass rate of 80% (which equates to a total score no lower than 32) is suggested as an indicator of good performance, which may be used by competent monitoring authorities for internal monitoring of performance, two participants failed to achieve this pass rate scoring 75% and 77.5% (Table 1).

Table 1: Participants final scores and overall pass mark.

Lab Code	Total Score	Pass Mark
MA2503c	39	97.5
MA2503d	37	92.5
MA2512a	34	85
MA2535	33	82.5
MA2510	33	82.5
MA2507	33	82.5
MA2503a	33	82.5
MA2503b	33	82.5
MA2512b	31	77.5
MA2512c	30	75

2.4 Discussion

This is the twelfth macroalgae identification ring test as circulated through the NMBAQC scheme, with early exercises being essentially trials of the methodology. Although the results were broadly comparable with those of previous years (RT010 and RT11) there was a noticeable increase in the level of agreement between participating laboratories and the AQC. As per previous years the test included several cryptic and taxonomically challenging species as well as those considered more common. Such genera included *Antithamnionella sp.* and *Ulothrix sp.* which are notoriously difficult to identify to species level. *Halopteris filicine* can also be easily misidentified due to confusions with other morphologically similar genera such as *Sphacelaria sp.* and it can be very difficult to tell these species apart from each other. These genera require an increased depth of knowledge on the cellular attributes, which can be remarkably similar between species, as well as other characteristics, such as overall texture, which can be used to separate such species. Other challenging species included *Derbesia marina* and *Capsosiphon fulvescens*, these two species are less commonly found and can also be easily confused with other morphologically similar species such as *Bryopsis sp.* and *Ulva sp.* respectively. As such participants are often unfamiliar with these species and despite having their own unique characteristics can be easily confused with other similar species. As intended by the scheme these tests aim to challenge participants and assist with training by stimulating the use of various keys and increasing familiarity with taxonomic terminology. Further, it allows problem taxa to be identified stimulating areas for inclusion in workshops and targeting such taxa within future exercises. Photographs used within the ring tests may be retained within the participating laboratories for future reference, with some descriptions allowing the comparison of taxonomically similar species.

No one participant managed to identify all species and genera correctly but there were eleven species for which all laboratories were successful in their identification (Table 2 and Figure 1) 6 more than for RT11. The most problematic species were *Antithamnionella ternifolia*, *Halopteris filicine*, *Derbesia marina* and *Capsosiphon fulvescens* which may be considered relatively difficult to identify due to the occurrence of morphologically similar species and genera or their microscopic nature, making them less commonly found and identified. Although fewer misidentifications were recorded within the present test it is unclear if this can be attributed to the improved level of competency of participants or the degree of difficulty of the test. The overall range of results was consistent with previous year also indicating a range of abilities between the participants.

Table 2: Summary of differences in identification.

Specimen	Genera	Species	Total differences for 10 returns	
			Genus	Species
RT1201	<i>Dictyota</i>	<i>dichotoma</i>	0	0
RT1202	<i>Ceramium</i>	<i>echionotum</i>	0	0
RT1203	<i>Ulva</i>	<i>clathrata</i>	0	0
RT1204	<i>Calliblepharis</i>	<i>jubata</i>	0	0
RT1205	<i>Undaria</i>	<i>pinnatifida</i>	1	1
RT1206	<i>Blidingia</i>	<i>marginata</i>	0	1
RT1207	<i>Antithamnionella</i>	<i>ternifolia</i>	5	5
RT1208	<i>Halopteris</i>	<i>filicine</i>	6	6
RT1209	<i>Mastocarpus</i>	<i>stellatus</i>	0	0
RT1210	<i>Bangia</i>	<i>fuscopurpurea</i>	0	0
RT1211	<i>Derbesia</i>	<i>marina</i>	6	6
RT1212	<i>Halurus</i>	<i>equisetifolius</i>	0	0
RT1213	<i>Myrionema</i>	<i>strangulans</i>	0	0
RT1214	<i>Vertebrata</i>	<i>nigra</i>	0	5
RT1215	<i>Laminaria</i>	<i>digitata</i>	0	0
RT1216	<i>Capsosiphon</i>	<i>fulvescens</i>	6	6
RT1217	<i>Acrosorium</i>	<i>ciliolatum</i>	1	1
RT1218	<i>Halidrys</i>	<i>siliquosa</i>	0	0
RT1219	<i>Ulothrix</i>	<i>flacca</i>	0	8
RT1220	<i>Leathesia</i>	<i>marina</i>	0	0
Total differences			25	39
Average differences per Genus/ species			1.250	1.950

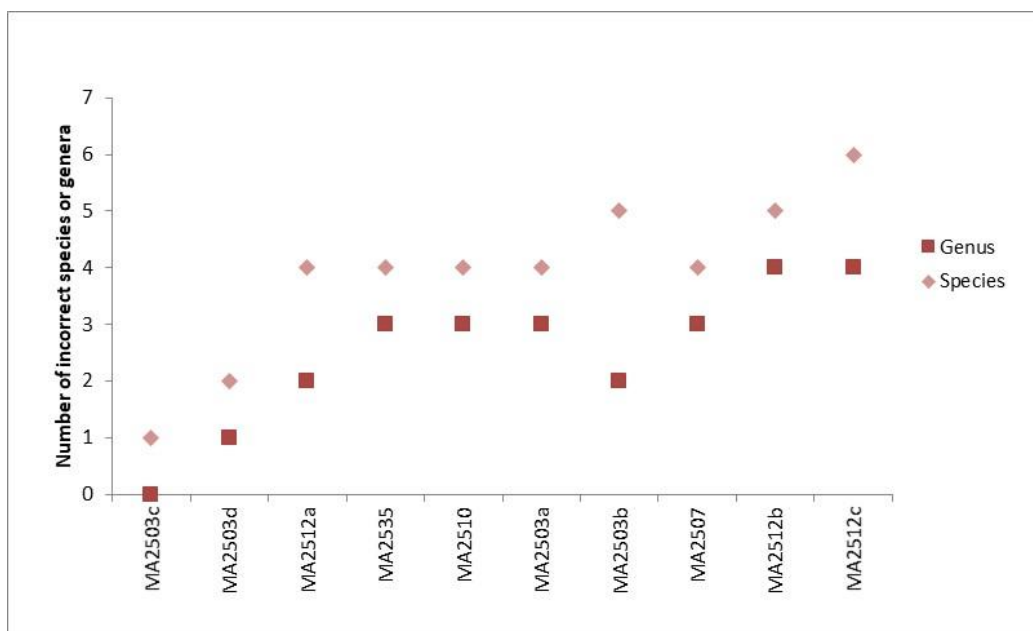


Figure 1: The number of differences from the AQC identification of intertidal macroalgae specimens, for each of the participating laboratories for RT12, arranged in order of increasing number of differences.

Certain issues arose with a few species. *Halopteris filicine* was unidentified by a couple of participants while other misidentifications could be attributed to both incorrect genera and species. Although relatively common in the intertidal it bears close resemblance to several other species, however in this instance could be distinguished by its branching pattern. Similarly, *Antithamnionella ternifolia* can be considered morphologically similar to many other small Rhodophyta species. However, this species is not so commonly recorded in routine monitoring due to its epiphytic nature and may be easily confused with other microscopic epiphytic red algae. Its main distinguishing feature includes the presence of a gland cell. *Derbesia marina* is also not so commonly recorded and bears close resemblance to *Bryopsis sp.* due to the coenocytic nature of its cells, however it lacks the regular feather like plumose branching of *Bryopsis sp.* *Capsosiphon fulvescens* was confused for various species of *Ulva*, all *Ulva sp.* lack the distinct grouping (in packets of 2 or 4) of the cells of *Capsosiphon sp.* and the golden colour of its frond. *Ulothrix flacca* was misidentified by 8 laboratories as *U. speciosa*. The species within this genus are all morphologically similar and with such overlapping characteristics it was necessary to look closely at the cell size and outer cell wall. In the case of *U. flacca* one of the most distinguishing features is the presence of particles in its outer cell wall giving it a rough appearance and although both species generally have cells broader than long this is less so with *U. flacca* where by cells can often be seen to be the same cell length as width. *Vertebrata* was also misidentified by 5 participants as *Vertebrata fucoides*. These two species can be distinguished by the terminal branches, which are multiple, dense and corymbose with *V. fucoides* and tend to be sparse and short with *V. nigra*.

In some instances, it was unclear which keys or guides were used to identify the species, making them impossible to compare although many appear to be consulting with photos from algaebase. This information is vital to determine if the guide descriptions were insufficient to correctly identify the species or if the photographs provided were insufficient. Additionally, it is recognised that some keys require revision, but this is not within the scope of NMBAQC.

At this time the use of a photographic test is considered the most effective means of testing macroalgal identification skills. Preserved samples are known to rapidly lose colour with cells becoming distorted making key characteristics more difficult to distinguish. Equally, fresh samples would not last a sufficient period to enable identification. However, it is possible that some photographs were not considered to be of sufficient quality or contain sufficient characteristics to correctly identify the specimens despite all efforts. This may have attributed to some misidentifications with some of the more cryptic species.

It is accepted that using fresh samples can be much easier to identify than photographs, however it must also be appreciated that even when using fresh specimens, it is not always possible to see certain characteristics, such as unique branching patterns and cell contents or perhaps it was not possible to retain the holdfast. Some features may be masked by excessive debris or diatoms or the specimen may be too small or partly deteriorated. Other issues arise where species show high degrees of morphological variation. All these factors would have to be considered in the field as well as within such ring tests as this and while all attempts are made to ensure perfect specimen material this is not always possible. It is equally difficult to find microscopic epiphytes and endophytes, much less be able to clearly see the cell contents and branching patterns and capture a still of such fundamental characteristics. However, it is considered important for the personal development of participants to be challenged with such species.

3 Conclusions and Recommendations

1. The twelfth macroalgae ring test exercise was implemented successfully and completed by all participating laboratories with a general agreement of the format. All feedback has been reviewed and will be considered for subsequent exercises; such feedback is encouraged to enable the protocols to be refined.
2. The tests are distributed with a spreadsheet of additional species information such as geographic location of species, height found on the shore and habitat preferences. This year there was better uniformity in terms of habitat, morphological or textural information being provided. A more detailed spreadsheet was provided during the current ring test to include such information for all species in a clear and concise manner and included the following characteristics:
 - i. Specimen number
 - ii. Geographic location from where species was collected
 - iii. Zonation/height at which the species was located
 - iv. Habitat preferences
 - v. Overall texture e.g. gelatinous, cartilaginous, hairy
 - vi. General size of species
 - vii. Host species where relevant
 - viii. Number of photos provided and magnification levels
 - ix. Any relevant additional information

It is unclear if this additional information provided significant assistance with the identification, but it is hoped that it aided with eliminating possible confusions between potential species identifications and will continue to be included in the future.

3. The high range of performance levels within this ring test provided evidence of a high range of proficiency but with the number of cryptic and microscopic species included within the test this does not necessarily indicate a reduced level of competence within and between laboratories. There are, naturally, several problematic areas but this is to be expected, as some taxa are inherently more difficult than others. The errors occurring were at both the generic and specific level and within all three divisions, Rhodophyta, Phaeophyta and Chlorophyta. Many of these errors occurred due to confusions with taxonomically and morphologically similar species which share similar characteristics and are therefore hard to separate. Such species will be noted for possible future workshops and will be targeted in future exercises.
4. There were still several incorrect spellings; therefore, participants are urged to take more care prior to submitting results to ensure all names are spelled correctly. It is also important that only one genus and one species name is to be entered per specimen, where more than one name is recorded it becomes difficult to assess whether the species has been correctly identified. Where there is limited confidence in the final identification it should be remembered that this scheme does not specify a definite qualifying performance level, and NMBAQC ring tests should be treated as training exercises. Ring tests offer a means of assessing personal and laboratory performance from which continued training requirements may be identified. In practice, it is likely that additional expertise would be consulted where the level of confidence in species identification is questionable.
5. Several data spreadsheets were also not fully completed, often missing out the keys or guides that were used. This may seem trivial information but can help identify where the participant has been misled with the keys or help explain how or why an alternative identification was reached.

For future ring tests it is requested that the data spreadsheets be completed in full, including level of confidence in the identification. Participants should include the authority alongside taxon names, as this also aids in the analysis of returns.

6. All laboratories are encouraged to keep all test photographs within a reference collection. This has several benefits particularly with regards to improving identification ability, training new staff and maintaining consistency of identification between surveys and staff. This reference collection should also be extended through to literature to ensure current keys are used with up to date nomenclature. A list of identification works will be given on the NMBAQC website. However, this is not exhaustive, and does not necessarily include unpublished keys provided at workshops unless specifically authorised by the key's author.
7. During this twelfth cycle of the macroalgae identification exercise all participants submitted results within the designated timescale except where ring tests were not received by the commencement date. In future ring tests 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 and in the case of very late submissions at the deadline. Emails will also be distributed to inform laboratories that the ring test material has been posted and expected date of arrival although this may be difficult with some laboratories outside of the UK. However, all attempts will be made to ensure all laboratories receive the material by the test commencement date.
8. This year one participating laboratory received a corrupt CD from which the test material could not be viewed. This was quickly rectified to ensure the laboratory did not lose time in which to identify and submit the results. All attempts will be made to ensure that this does not occur in the future, however, it is important that all laboratories check the CD on receipt so that should a problem arise it can be dealt with early on during the test period and limit disruption with reporting later.
9. There is now good consensus over the time of year for the test with the slightly earlier distribution of this years' test allowing the results bulletin and final report to be distributed before the sampling season. There has also been no further comment on the amount of time provided for the test, so this has been taken as acceptable.
10. There was a general agreement from participants that this years test was considered reasonably difficult there was less agreement on the overall quality, detail and use of photographs with most participants. It is unclear as to where such problems lay as no further comments were provided. However, all attempts will be made to ensure more clarity in subsequent tests. It is hoped that recommendations from previous tests have been taken on board and that for most species enough photos and key characteristics were provided for correct and confident identification. However, it must be recognised that even when looking at fresh specimens not all such characteristics may be present, e.g. reproductive structures. No staining is currently used, and this shall remain for the following test. All attempts will be made in the future to ensure that sufficient material is provided, allowing correct identification to species level.

If anyone has further comments 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 continually being refined to ensure it provides the best opportunity to test macroalgae identification skills, so all suggestions and comments are welcomed.

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