The National Marine Biological Analytical Quality Control Scheme
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Macroalgae Component - Algal Identification Module Report – RM RT09 2015

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MACROALGAL IDENTIFICATION MODULE REPORT FROM THE CONTRACTOR SCHEME OPERATION –2014-15

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. Good quality control ensures consistency of data being reported for management purposes, and for macroalgae this has been driven primarily by the requirements of the Water Framework Directive. The Healthy, Biologically Diverse Seas Evidence Group (HBDSEG), part of UK Marine Monitoring and Assessment Strategy, sets the key areas for UK agencies in which this external quality control is particularly needed. For 2015/2016 the components are: Benthic invertebrates, Fish, Particle Size Analysis, Macroalgae/Seagrass, Phytoplankton, Epibiota and Zooplankton. This QC scheme aims to facilitate improvements in biological assessment whilst maintaining the standard of marine biological data. The scheme should help to ensure consistency between analysts with improved confidence in ecological quality status.

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

- The identification of macroalgae species

This is the ninth 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.

Seven laboratories subscribed to the macroalgae ring test with six laboratories submitting results with a total of fifteen participants. One laboratory failed to submit results due to time restrictions. Five of the subscribing laboratories were government organisations and two were private 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.

Data for macroalgal blooming and seagrass are currently used in relation to WFD classification and assessments for other Directives/purposes. In the UK at present they are not reported through a national database such as Merman; consequently they do not have definite national qualifying performance levels. They may be treated as training exercises. However, certain indicative targets have been applied to the assessments of the results based on a pass rate of 80% as an indicator of good 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.

2 Summary of Performance.

This report presents the findings of the macroalgae identification component for the ninth 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 eight of the scheme (RM RT08). 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 seven subscribing laboratories. Round nine of the ring test produced a high 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. RT09 generally resulted in a greater number of correct identifications than seen in earlier years suggesting an increase in competence.

3 Summary of Macroalgae Component

3.1 Introduction

There was one module for the macroalgae identification component for scheme year nine. 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.

3.2 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 2015. 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 a number of photographs per taxon showing different aspects of the alga and its habitat. Some supplementary information on habitat was included.

3.3 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 habitat details from which to identify specimens, description of methods and data submission forms. Participants were given six weeks to complete the test and return the results. One laboratory was given an extension of time due to late receipt of the test material. 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.

3.4 Preparation of the test

Each specimen was to be identified through a number of in-situ, macroscopic and microscopic photographs. In total a minimum of five photographs was used for each specimen collected by Wells Marine for the purpose of this exercise. Specimen photographs were obtained from the UK coast. Photographs were selected to represent sufficiently 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.

3.5 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 were in Excel and have been stored and analysed in this format. In this and previous scheme years slow or missing returns for exercises led to delays in processing data, reporting and feedback of results, therefore reminders were distributed shortly before the exercise deadline.

3.6 Analysis

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.

3.7 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 two will be recorded as MA2212c.

4 Results

4.1 General Comments

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.

Results were distributed to each of the participating laboratories four weeks after data submission. These results are documented in the preliminary results bulletin (RM RT09) 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.

4.2 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 were highlighted, but not taken into account during calculation of the total number of differences in identification.

Data entries were tabulated (as seen in RM RT09 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 a synonym or mis-spelling was supplied by the participant but for which the identification was consistent with that of the AQC, the name was presented in brackets [genus/species name]. For those entries where 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 *Ulva prolifera* was identified as *Ulva clathrata* this would be entered as " – *clathrata*". Due to the difficulty in definitively distinguishing between *Gracilaria gracilis* and *Gracilariopsis longissima* using available literature, both names were accepted for the identification of species RT0920. *Gracilaria verrucosa* was also accepted as a taxonomic synonym of *Gracilariopsis longissima.*

The data entries for an individual were scored as one 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 the species name was incorrect a score of one would be given; where genus (and hence also species) was incorrect, a score of two (one plus 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 were spelled incorrectly.

4.3 Ring Test Results

RM RT09 contained twenty specimens for identification for which there was an excellent level of agreement through all fifteen participants. At the generic level there were a total of fourteen differences (from a potential three hundred) across the fifteen sets of data received from the six participating laboratories (4.7%). At the specific level there were a total of thirty two differences (10.7%), which is considerably higher than the previous years' results with 18.18% and 30.91% error at the generic and specific level respectively.

These differences could be attributed primarily to four taxa. A total of 35% of all errors were from one species (*Helminthocladia calvadosii*) contributing to 57% of all generic differences and 25% of all specific differences. *Ulva prolifera, Cladophora sericea* and *Ulva flexuosa* contributed to a further 13%, 22% and 30%, respectively, of differences. Therefore a total of 65% of incorrect identifications were attributed solely to the Chlorophyta division most of which were incorrectly identified at the species level (80%). All other specimens were identified correctly.

There were a number of incorrect spellings mainly attributed to changes in nomenclature such as *Polyides sp.* which was previous named as *P. rotundus* but is now recorded within algaebase as *P. rotunda* and *Pilayella littoralis* now recorded as *Pylaiella littoralis.* However, both current names and synonyms were accepted for the ring test.

The difference between participants' entries and AQC identifications was generally evenly distributed between participants with all but one identifying at least one species incorrectly. Only one participant (MA2203e) correctly identified all species. The overall scores and number of incorrect identifications ranged from one to six which is much lower than in previous years. 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, all participants achieved this pass rate with all participants scoring at least 85% (Table 1).

Table 1: Participants final scores and overall pass mark.

Lab Code	Total Score	Pass Mark
MA2203e	40	100
MA2212	39	97.5
MA2235c	38	95
MA2235b	38	95
MA2232b	38	95
MA2221	38	95
MA2203a	38	95
MA2232a	37	92.5
MA2232d	37	92.5
MA2232c	36	90
MA2210	36	90
MA2203b	35	87.5
MA2203c	35	87.5
MA2203d	35	87.5
MA2235a	34	85

5 Discussion

This is the ninth 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 (RT07 and RT08) there is a noticeable increase in the level of agreement between participating laboratories and the AQC. As per previous years the test included a number of cryptic and taxonomically challenging species as well as those considered more common. Such genera included *Ulva sp*. and *Cladophora sp*. which are notoriously difficult to identify to species level. *Helminthocladia calvadosii* can also been easily misidentified due to confusions with other morphologically similar species. These genera require an increased depth of knowledge on the cellular attributes and other characteristics, which can be remarkably similar between 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.

One individual managed to identify all species and genera correctly and there were 16 species (80%) for which all laboratories were successful in their identification (Table 2 and Figure 1). The most problematic species was *Helminthocladia calvadosii* which may be considered relatively difficult to identify due to the occurrence of morphologically similar species such as *Dumontia contorta*. Those characteristics which are considered more specific and may be used to distinguish such species were

detailed within the Bulletin. As the largest portion of incorrect identifications could be attributed to just four species, it may be considered that there were too few cryptic species included within the test. However comments within the feedback clearly indicate that the difficulty level of the test was considered acceptable, therefore it could be concluded that the level of competency is increasing.

Table 2: Summary of differences in identification.

			Total differences for 15 returns	
Specimen	Genera	Species	Genus	Species
RT0901	*Catenella*	*caespitosa*	0	0
RT0902	*Halidrys*	*siliquosa*	0	0
RT0903	*Chaetomorpha*	*melagonium*	0	0
RT0904	*Ahnfeltia*	*plicata*	0	0
RT0905	*Cladophora*	*sericea*	0	10
RT0906	*Laminaria*	*digitata*	0	0
RT0907	*Corallina*	*officinalis*	0	0
RT0908	*Helminthocladia*	*calvadosii*	8	8
RT0909	*Himanthalia*	*elongata*	0	0
RT0910	*Elachista*	*fucicola*	0	0
RT0911	*Ulva*	*prolifera*	0	6
RT0912	*Gigartina*	*pistilata*	0	0
RT0913	*Lomentaria*	*articulata*	0	0
RT0914	*Fucus*	*spiralis*	0	0
RT0915	*Polyides*	*rotunda*	0	0
RT0916	*Percusaria*	*percursa*	0	0
RT0917	*Membranoptera*	*alata*	0	0
RT0918	*Pylaiella*	*littoralis*	0	0
RT0919	*Ulva*	*flexuosa*	6	8
RT0920	*Graciliaria*	*gracilis*	0	0
		Total differences	14	32
		Average differences per Genus/ species	0.700	1.600

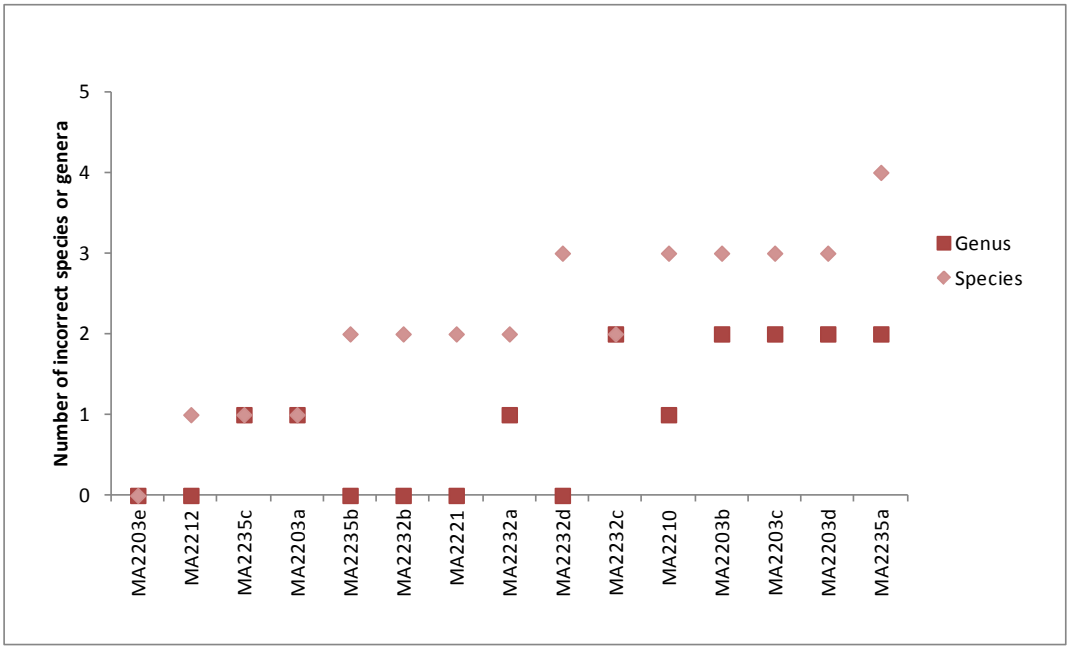

Figure 1: The number of differences from the AQC identification of intertidal macroalgae specimens, for each of the participating laboratories for RT08, arranged in order of increasing number of differences.

Another issue arose with *Gracilaria gracilis*. This is a fairly common species, but with limited distribution and highly variable morphology. Similar species have overlapping characteristics, and it was considered that this overlap between *Gracilaria gracilis and Gracilariopsis longissima* was sufficient to justify accepting both names on this occasion. In this instance it was also unclear which keys or guides were used by all individuals to identify the species making them impossible to compare. 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. Participants are reminded that they should complete all parts of the return form including keys used and level of confidence of assessment.

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 to 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.

6 Conclusions and Recommendations

1. The ninth ring test exercise was implemented successfully and completed by most participants 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 relatively good level of agreement within this test provides evidence that macroalgae identification skills are increasing; however there are still a number of problematic areas. This is to be expected, as some taxa are inherently more difficult than others. The errors occurring were generally at the specific level and within the Chlorophyta division, however where generic errors occurred these were most often with taxonomically 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.

3. There were still a number of incorrect spellings; therefore participants are urged to take more care prior to submitting results to ensure all names are spelled correctly. This is equally important when submitting data records or reports where scientific names are incorporated. It should also be noted that a number of data spreadsheets were 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 to 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.

4. As with some previous tests there was some disagreement as to the correct identification of some species. Descriptions of some species have recently changed; some have resulted in nomenclatural changes or use of more specific characteristics that were previously considered more generic. New studies in species taxonomy are regularly highlighting previously unidentified (cryptic) species, splitting one species into two based on a previously unknown characteristic. In these instances both species identification have been accepted such as *Gracilaria gracilis* and *Gracilariopsis longissima.* Keying out the two species shows very little difference except for some basic morphological differences, or at the microscopic level which was not fully evident through the photos provided. This problem highlights the need for more definitive photos, specimens and descriptions to be provided in future exercises so as to save confusion. However, it is not always possible to obtain specimens showing certain features if they are not in the correct reproductive phase.

5. All laboratories are encouraged to keep all test photographs within a reference collection. This has a number of 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.

6. During this ninth cycle of the macroalgae identification exercise all participants submitted results within the designated timescale; one lab was granted an extension due to late receipt of the test. In future exercises all laboratories should continue to submit results within the requested deadlines as detailed at the beginning of the exercise. In subsequent years reminders will

continue to be distributed two weeks prior to the completion of the exercise. All participants are encouraged to contact the contract manager as soon as possible if they think there will be difficulties in meeting deadlines, but the presumption is against extensions.

7. Although there was general approval on the quality, detail and use of photographs with most participants agreeing on the levels of difficulty, there were some areas which require some improvement. Some more specific cellular information was also requested within the photos, and where possible this will be achieved such as cross sections of filamentous species such as *Polysiphonia* or some terete species or inclusion of basal cells which may be the defining feature. However, 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) or Dr Clare Scanlan (clare.scanlan@sepa.org.uk). 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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