The National Marine Biological Analytical Quality Control Scheme
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Macroalgae Component - Algal Identification Module Report – RM RT10 2016

Emma Wells
Wells Marine Surveys
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Email: emma@wellsmarine.org

MACROALGAL IDENTIFICATION MODULE REPORT FROM THE CONTRACTOR SCHEME OPERATION –2015-16

1 Introduction

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

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

- The identification of macroalgae species

This is the tenth 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.

Four laboratories subscribed to the macroalgae ring test with all four laboratories submitting results with a total of ten participants. Three of the subscribing laboratories were government organisations and one was a private consultancy. 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 tenth 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 nine of the scheme (RM RT09). 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 four subscribing laboratories. Round ten 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 which was reflected in the number of correct identifications which was slightly fewer than seen in the previous year.

2 Summary of Macroalgae Component

2.1 Introduction

There was one module for the macroalgae identification component for scheme year ten. 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 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. 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 shortly 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 three will be recorded as MA2312c.

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 2016. 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.

2.3.1.1 Preparation of the Sample

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 a range of surveys from around the coast of the UK. 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.

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 RT10) specimen photographs were circulated to a total of four laboratories. All four 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 RT10) 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 taken into account during calculation of the total number of differences in identification.

Data entries were tabulated (as seen in RM RT10 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 [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 were scored by 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 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 RT10 contained twenty specimens for identification for which there was a good level of agreement through all ten participants. At the generic level there were a total of thirty two differences (from a potential two hundred) across the ten sets of data received from the four participating laboratories (16%). At the specific level there were a total of thirty eight differences (19%), which is slightly higher than the previous years' results. These differences could be attributed primarily to five taxa. The greatest number of misidentifications could be attributed to two species, *Mesogloia vermiculata* (RT1007) with 7 generic and 9 specific differences, and *Colpomenia peregrina* (RT1019) with 8 generic and 8 specific differences recorded, accounting for 46% of differences. *Cruoria pellita, Blidingia minim*a and *Ulva pseudocurvata* contributed to a further 14%, 14% and 10% of differences, respectively. A further 6 species contributed to between 1% and 6% of all differences with a total of eleven species having a misidentification at either the specific or generic level. A total of 52% of incorrect identifications could be attributed to the Phaeophyta division most of which were incorrectly identified at both the genus and species level, 34% of incorrect identifications lay within the Chlorophyta division and the final 14% were from the Rhodophyta division. The remaining 9 specimens were identified correctly.

There were a couple of incorrect spellings mainly attributed to changes in nomenclature such as *Hincksia granulosa* which was previously known as *Giffordia granulosa* and *Mesogloia vermiculata*, previously known under its Generic synonym *Liebmannia*. However, both current names and synonyms were accepted for the ring test.

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 five to ten which is much higher than in the previous year. 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, 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
MA2310	35	87.5
MA2303f	35	87.5
MA2303a	34	85
MA2303b	34	85
MA2303e	34	85
MA2303d	33	82.5
MA2303c	32	80
MA2303g	32	80
MA2312	31	77.5
MA2321	30	75

2.4 Discussion

This is the tenth 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 (RT08 and RT09) there is a noticeable decrease 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 *Hincksia sp*. which are notoriously difficult to identify to species level. *Mesogloia vermiculata* can also been easily misidentified due to confusions with other morphologically similar species such as *Liebmannia sp*. and in general it is 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. 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 and there were only 9 species for which all laboratories were successful in their identification (Table 2 and Figure 1). The most problematic species were *Mesogloia vermiculata* and *Colpomenia peregrina* which may be considered relatively difficult to identify due to the occurrence of morphologically similar species such as *Liebmannia sp.* and *Leathesia difformis,* respectively. Those characteristics which are considered more

specific and may be used to distinguish such species were detailed within the Bulletin. With an increased number of species with misidentification it could be concluded that this test was slightly more difficult than previous tests so has little reflection on the level of competency of the participants since the pass rate was lower across all participants.

Table 2: Summary of differences in identification.

			Total differences for 10 returns	
Specimen	Genera	Species	Genus	Species
RT1001	*Dilsea*	*Carnosa*	0	0
RT1002	*Litosiphon*	*laminariae*	0	1
RT1003	*Prasiola*	*stipitata*	0	1
RT1004	*Phyllophora*	*pseudoceranoides*	0	0
RT1005	*Chaetomorpha*	*ligustica*	0	0
RT1006	*Fucus*	*vesiculosus*	0	0
RT1007	*Mesogloia*	*vermiculata*	7	9
RT1008	*Cruoria*	*pellita*	5	5
RT1009	*Saccharina*	*latissima*	1	0
RT1010	*Codium*	*fragile subsp. fragile*	0	2
RT1011	*Odonthalia*	*dentata*	0	0
RT1012	*Acrosiphonia*	*acrta*	2	2
RT1013	*Bostrychia*	*scorpioides*	0	0
RT1014	*Crytopleura*	*ramosa*	0	0
RT1015	*Hincksia*	*granulosa*	1	1
RT1016	*Blidingia*	*minima*	5	5
RT1017	*Phycodrys*	*rubens*	0	0
RT1018	*Ulva*	*pseudocurvata*	3	4
RT1019	*Colpomenia*	*peregrina*	8	8
RT1020	*Palmaria*	*palmata*	0	0
		Total differences	32	38
		Average differences per Genus/ species	1.600	1.900

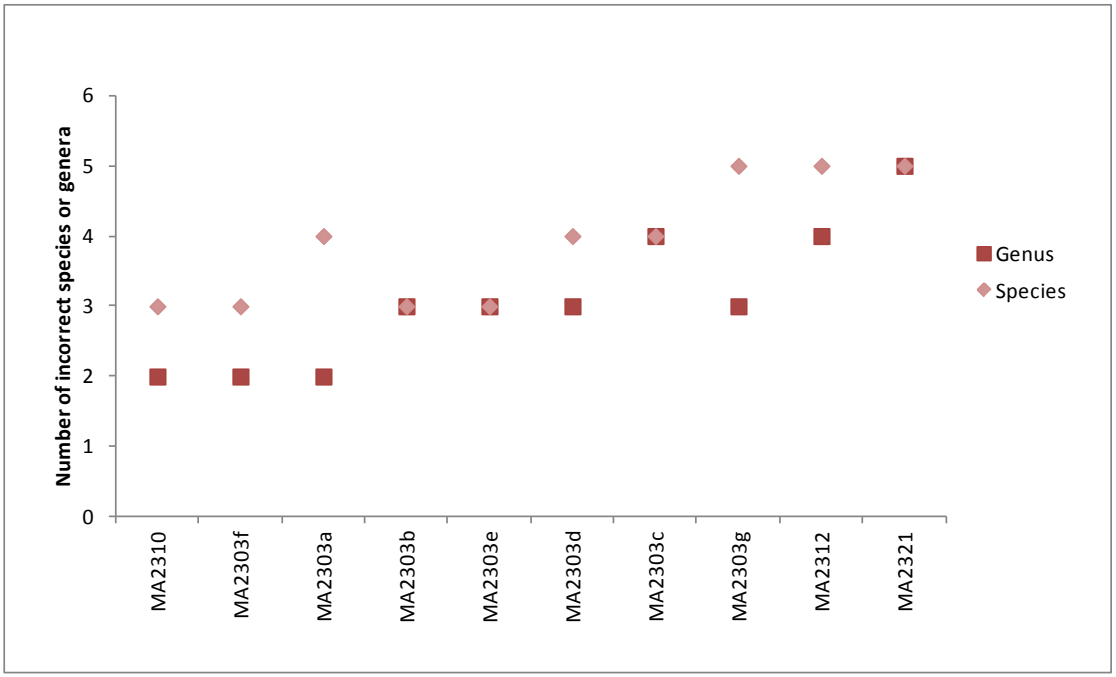

Figure 1: The number of differences from the AQC identification of intertidal macroalgae specimens, for each of the participating laboratories for RT10, arranged in order of increasing number of differences.

Certain issues arose with a couple of species. Firstly *Mesogloia vermiculata* was confused for various *Liebmannia sp. Liebmannia* is less common than *Mesogloia* but morphologically incredibly similar and with such overlapping characteristics it was necessary to consider the texture of the specimen which was supplied in the test information. It was considered and decided that the difference in texture was enough to conclude the species was indeed *Mesogloia* and only this name was accepted. A similar problem was encountered with *Colpomenia peregrina* which was misidentified as *Leathesia difformis*. No details on its texture were provided and it was felt this was necessary however it was agreed that the cellular photographs provided were sufficient to distinguish the two with "the internal portion of *Leathesia* being composed of radiating dichotomous filaments unlike the truely parenchymatous form of *Colpomenia* which is membranous and consists of just two layers of tissue; when a portion of the frond is squashed no separable filaments can be seen". In these instances it was also 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 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. However, it is possible that some photographs were not considered to be of sufficient quality to correctly identify the specimens despite all efforts. This may have attributed to some confusion over the identification of some more cryptic species.

3 Conclusions and Recommendations

1. The tenth 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. There were a couple of species for which there was some dispute as to the correct identification, any such problems are discussed in full with the contract manager before final results are distributed to ensure agreement. However, any feedback or disagreement in the final results is encouraged to ensure all participants are satisfied with the structure of the test.

3. In this particular test where a subsp. name was included there was no column to include such additional names causing some confusion. In future tests the results spreadsheet will be clearly labelled so that all species names can be included and the correct scoring can be allocated.

4. 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. However there is no uniformity of in terms of morphological or textural information being provided. A more detailed spreadsheet will be provided in subsequent ring tests to include such information for all species in a clear and concise manner. The exact contents of the table will be discussed prior to the next ring test but would hope to include, but not be restricted, to the following attributes:

    i. Specimen number
    ii. Geographic location from where species was collected
    iii. Number of photos provided and magnification levels
    iv. Zonation/height at which the species was located
    v. Habitat preferences
    vi. Overall texture e.g. gelatinous, cartilaginous, hairy
    vii. Host species where relevant
    viii. General size of species

5. 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 at both the generic and specific level and mainly within the Phaeophyta and Chlorophyta divisions. Many of these error 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.

6. 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 mislead 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.

7. 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. Keying out such species often 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.

8. 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.

9. During this tenth cycle of the macroalgae identification exercise all participants' submitted results within the designated timescale. 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.

10. 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.

11. 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 e.g. the photos for specimens 7 and 16 were considered a little fuzzy with cell content difficult to distinguish. Therefore all attempts will be made to ensure a greater degree of clarity in subsequent tests. It is also requested that for in-situ photos that the species in question be clearly identified using an arrow particularly where several different species may be present. 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 *Ceramium* or *Polysiphonia* 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). 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.

4. References

Bunker, F.StP.D., Brodie, J.A., Maggs, C.A. & Bunker, A.R. (2010). *Seasearch guide to seaweeds of Britain and Ireland*. pp. [1] 5-224, many colour photographs. Ross-on-Wye: Marine Conservation Society.

Brodie, J. & Irvine, L.M., 2003. Seaweeds of the British Isles. Volume 1. Rhodophyta. Part 3B. Bangiophycidae. Pp. i-xiii, 1 – 167, map. Andover: Intercept.

Dixon, P.S. and Irvine, L.M., 1977. Seaweeds of the British Isles. Vol. 1 Rhodophyta. Part 1. Introduction, Nemaliales, Gigartinales. British Museum (Natural History), London.

Guiry, M.D., 1997. Benthic red, brown and green algae. In: Howson, C.M. and Picton, B.E. The species directory of the marine fauna and flora of the British Isles and surrounding seas. The Ulster Museum and the Marine Conservation Society, Belfast and Ross-on-Wye.

Hiscock, S., 1979. A field guide to the British brown seaweeds (Phaeophyta). Field Studies. **5**, 1 – 44.

Hiscock, S., 1986. A Field Guide to the British Red Seaweeds (Rhodophyta). Field Studies Council Occasional Publications no. 13.

Irvine, L.M., 1983. Seaweeds of the British Isles. Vol. 1 Rhodophyta. Part 2a. Cryptonemiales (sensu stricto), Palmariales, Rhodymeniales. British Museum (Natural History), London.

Irvine, L.M. and Chamberlain, Y., 1993. Seaweeds of the British Isles. Vol. 1. Rhodophyta. Part 2b. Corallinales. British Museum (Natural History), London.

Kornmann, P. and Sahling, P.H., 1983. Meeresalgen von Helgoland: Erganzung. Helgoländer Wissenschaftliche. Meeresuntersuchungen. **36**, 1 – 65.

Maggs, C.A. and Hommersand, M., 1993. Seaweeds of the British Isles. Vol. 1. Rhodophyta. Part 3a. Ceramiales.

Wells, E. 2007. Water Framework Directive – coastal water rocky shore monitoring: Field guide to British seaweeds. Environment Agency, Bristol.