NATIONAL MARINE BIOLOGICAL
ANALYTICAL QUALITY CONTROL SCHEME
Annual Report - Year 20 - 2013/2014

A report prepared by the NMBAQC Coordinating Committee – January 2016

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This Year 20 Annual Report provides synopsis of the scheme year's activities over 2013/2014. Detailed information about each of the scheme components is now available as separate reports or bulletins on the scheme's website. The relevant documents are all cited here and the reader is directed via hyperlinks to the NMBAQC website as appropriate.

The NMBAQC coordinating committee held 4 meetings during the scheme Year 20 on 2nd July 2013, 1st October 2013, 24th January 2014 and 23rd April 2014.

Committee Membership for Year 20 is shown in Appendix 1.

1 Scheme Review

The scope of the NMBAQC scheme continued to develop in Year 20 to encompass the requirement to provide quality assurance for assessments under the Water Framework Directive (WFD), for which monitoring commenced in the UK in 2007. The scheme still maintains its role to provide Analytical Quality Control for Invertebrate and Particle Size data collected for UK CSEMP (Clean Seas Environment Monitoring Programme). Under the UK Marine Monitoring and Assessment Strategy (UKMMAS) the NMBAQC scheme coordinating committee now reports to the Healthy and Biologically Diverse Seas Evidence Group (HBDSEG).

Year 20 of the scheme followed a similar format to the previous year and involved training and testing exercises for the Invertebrate, Particle Size, Fish, Phytoplankton, Marine angiosperms and Macroalgae components. Year 20 saw the first developments of a new Epibiota Best Practice Guide, and Natural England hosted a workshop in September to assess which are the critical elements for this component. Following on from the Zooplankton report, SAHFOS will take on a trial zooplankton ring test next year, and has used this year to put the mechanisms into place.

In April 2014, at the end of year 20, the four year contracts for the Benthic Invertebrate, Particle Size Analysis (PSA) and Fish components were up for renewal. The Fish Component contract remained with Thomson Unicomarine Ltd. while the Benthic Invertebrate and and PSA contacts were awarded to APEM Ltd. This is the first time these latter components have switched to a different contractor.

The Year 20 participation level in the NMBAQC was similar to the previous year (see Appendix 2).

Summaries of all the component activities are provided below:

2 Invertebrate component

Contract Manager: Myles O'Reilly, Scottish Environment Protection Agency.
Component Administrator: Richard Arnold & Ruth Barnich, Thomson Unicomarine Ltd.

2.1 Summary of activities

Forty-one laboratories participated in the benthic invertebrate component in Year 20 (2013/2014). Fifteen participants were Competent Monitoring Authorities (CMAs) and twenty-six were private consultancies. One of the participants was a consortium of sole traders. Thirteen of the CMA participants were responsible for the Clean Seas Environment Monitoring Programme (CSEMP) or Water Framework Directive (WFD) sample analysis. Laboratory Codes were assigned in a single series for all laboratories participating in the benthic invertebrate components of the NMBAQC Scheme.

2.2 Summary of exercise results

This component consisted of four modules (each with one or more exercises):

- Macrobenthic Sample module (MB) - analysis of a single natural estuarine macrobenthic sample;
- Own Sample module (OS) - re-analysis by APEM Ltd. of three own samples supplied by each of the participating laboratories;
- Invertebrate Ring Test module (RT) - identification of two sets of twenty-five invertebrate specimens; and
- Laboratory Reference module (LR) - re-identification by APEM Ltd. of a set of twenty-five specimens supplied by each of the participating laboratories.

The analytical procedures of the various modules were the same as for Year 19 of the Scheme, which includes the specification that the Macrobenthic Sample module and Clean Seas Environment Monitoring Programme (CSEMP 2010; formerly NMMP) or Water Framework Directive (WFD) samples within the Own Sample module should be conducted using the NMBAQC guidance for macrobenthic invertebrate sample analysis (Worsfold, Hall & O'Reilly (Ed.) 2010).

Two Ring Tests (RT) of 25 specimens were distributed (RT45 and RT46). Both sets contained 25 invertebrate specimens, the first (RT45) was targeted at Crustacea.

For RT45 each participating laboratory (a total of 19 laboratories with 24 participants) recorded on average 1.08 generic differences and 2.04 specific differences. Four taxa (an isopod, a mysid, a tanaid and a cumacean) were responsible for over half (53%) of the specific differences.

For RT46 each participating laboratory (a total of 20 laboratories with 24 participants) recorded on average 1.1 generic differences and 2.6 specific differences. Four taxa (two polychaetes, a crustacean and an echinoderm) were responsible for almost half (49%) of the specific differences.

Laboratory Reference (LR): APEM received specimens for confirmation from seven laboratories. Most misidentifications were found to be for Polychaeta, and bivalve and gastropod Molluscs, belonging to genera which are either speciose, or for which the taxonomy has yet to be finalized and keys are inadequate. The majority of taxonomic errors could be attributed to the submitted polychaetes (55%) and molluscs (23%).

Eight laboratories participated in the **Macrobenthic module (MB)**. Analysis of the sample by the eight participating laboratories and subsequent re-analysis by the previous contractor provided information on the efficiency of extraction of the fauna, accuracy of enumeration and identification and the reproducibility of biomass estimations. For MB21, natural marine samples from the south east coast of England were distributed. Results for this macrobenthic exercise showed an extraction efficiency (of individuals) was on average 97.55%. Comparison of the results from the laboratories with those from analysis by previous contractor was made using the Bray-Curtis similarity index (BCSI) (untransformed). The value of the index varied between 93.58% and 100% meaning all laboratories passed when Own Sample standards were applied. The average BCSI was 97.89%. Only one taxonomic error was recorded across all eight laboratories.

The revised protocols of Scheme Year 10 for 'blind' **Own Sample (OS)** audits were continued in this Scheme year. Laboratories were asked to submit full completed data matrices from their previous year's CSEMP/WFD or similar alternative sampling programmes (if not responsible for CSEMP/WFD samples). The OS 'Pass/Fail' flagging system, introduced in Scheme Year 8, was continued (see Description of the Scheme Standards for the Benthic Invertebrate Component). Extraction efficiency was better than 90% in 85% of the comparisons and better than 95% in 75% of all comparisons. 100% of countable taxa were extracted from the sample residues in 35% of samples. The Bray-Curtis similarity index ranged from 16% to 100% with an average figure of 90%. The Bray-Curtis similarity index was greater than 95% in 54% of comparisons and in 72% of cases the value of the index was greater than 90% and, therefore, achieved 'Pass' flags. Fourteen samples (14%) achieved 'Pass-Excellent' flags with Bray-Curtis similarity scores of 100%.

2.3 Issues and recommendations

In year 20 some participants generated late returns, and due to a contractor change between year 20 and 21 of the Benthic Invertebrate component, there were some legal problems clarifying contractor requirements for completing the Own Sample and Lab Reference modules. After some considerable delays the Lab Reference and Own Samples were transferred from the Thomson Unicomarine Ltd to the new contractor APEM Ltd. To prevent a situation like this happening in the future, from year 21 onwards deadlines for all modules have been brought forward to ensure that each component should be finalised within the financial year.

2.4 Reports & Taxonomic literature

Benthic Invertebrate Component Annual Report, Year 20 (2013/14)
Milner, C., Hall, D. and O'Reilly, M. (Ed.) 2015. National Marine Biological Analytical Quality Control Scheme. Benthic Invertebrate Component Annual Report: Year 20 - 2013/2014. Report to the NMBAQC Scheme participants. 30pp, January 2016.

Own Sample Module Summary Report OS53, 54 & 55 - September 2015
Milner, C., Hall, D. and O'Reilly, M. (Ed.) 2015. National Marine Biological Analytical Quality Control Scheme. Own Sample Module Summary Report OS53, 54 & 55. Report to the NMBAQC Scheme participants. 22pp, September 2015.

RTB 46 - Oct 2014
Freeston, T., Barnich, R. and Wolffe, C., 2014. National Marine Biological Analytical Quality Control Scheme. Ring Test Bulletin: RTB#46. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQC RTB#46, 32pp, Oct 2014.

RTB 45 - July 2014

Freeston, T., Barnich, R. and Wolffe, C., 2014. National Marine Biological Analytical Quality Control Scheme. Ring Test Bulletin: RTB#45. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQC RTB#45, 36pp, July 2014.

MB 21- May 2014
Barnich, R. and Wolff, C. 2014. National Marine Biological Analytical Quality Control Scheme. Macrobenthic Exercise Results - MB21 (Year 20). Report to the NMBAQC Scheme participants. 17pp, May 2014.

For further taxonomic literature, see the NMBAQC web site, Literature and Taxonomic Keys for the invertebrate component.

3 Particle Size Analysis component

Contract Manager: .Myles O'Reilly, Scottish Environment Protection Agency.
Component Administrator: Richard Arnold & Ruth Barnich, Thomson Unicomarine Ltd.

3.1 Summary of activities

The PSA component consisted of one module with four exercises: Analysis of four sediment samples (PS48, PS49, PS50 and PS51) for physical description.
PS48 – Muddy Sand combination of natural sediment from estuary and from offshore.
PS49 – Gravelly Sand combination of commercial aggregates and natural offshore sand
PS50 – Sand combination of commercial sand and natural offshore sand
PS51 – Gravel derived from commercial aggregate materials

The analytical procedures of this module were the same as for the nineteenth year of the Scheme. The results for the four exercises are presented and discussed. Comments are provided on the performance for each of the participating laboratories in each of the exercises.

In previous years the Particle Size exercises (PS) 'Pass/ fail' criteria were based upon z-scores from the major derived statistics with an acceptable range of ±2 standard deviations (see Description of the Scheme Standards for the Particle Size Analysis Component). The annual report for Scheme Year 16 deemed the use of z-scores inappropriate for such a low number of data returns where two erroneous results can significantly alter the 'Pass/ fail' criteria.

The z-score method also assumes that the majority of respondents are correct and raised genuine concerns regarding technique and method bias. Following this, the 'Pass/ fail' criteria are currently under review and alternative flagging criteria are being trialled. Therefore, Scheme Year 20 continues the use of z-scores calculated for each half-phi interval, and multivariate analysis using Euclidean distance matrices (dendrograms and non-metric multidimensional scaling plots) as trialled from Years 17 - 19.

The variation within the ten replicate results produced for Thomson Unicomarine Ltd in-house analysis (using the NMBAQC PSA statements of performance) was minimal for each of the four exercises; this is partly attributable to the use of only Malvern laser instruments and some standardised protocols, i.e. no use of chemical dispersants or hydrogen-peroxide pre-treatment. In most cases there was reasonably good agreement between participant laboratories for all four PS exercises. All four particle size exercises of Scheme year 20 received fourteen data returns.

3.2 Summary of results

The samples distributed, from an analysis of replicates, appear to be good with very little variance. Results from participating laboratories also showed a generally good similarity. Cluster analysis using Euclidean distance was performed to see how many laboratories were grouped apart in each of the exercises and possible causes for this were identified.

Participating laboratories were asked to provide the sediment description using the Folk triangle post analysis. Data was provided by all fourteen participating laboratories for PS48, PS49, PS50 and PS51. Two laboratories failed to provide the post analysis description for PS48. For PS48, eight laboratories had post-analysis sediment descriptions of Muddy Sand; four laboratories had a post-analysis description of Sand. Two laboratories failed to provide the post analysis description for PS49. For PS49, eleven participating laboratories recorded the post-analysis sediment description as Gravelly Sand. The remaining laboratory recorded Sandy Gravel. Four laboratories failed to provide the post analysis description for PS50. All other post-analysis sediment descriptions for PS50 were Sand. Four laboratories failed to provide the post analysis description for PS51. All other post-analysis sediment descriptions for PS50 were Gravel.

It is essential that analytical methods, including pre-treatment, are stated when reporting or attempting to compare results. The situation is further complicated by the fact that the difference between the techniques and the effects of the pre-treatment also varies with the nature of the sediment sample. As demonstrated in these and previous PS exercises, possible variations in equipment and methods can result in variable data. In order to eliminate as much variation as possible the NMBAQC's Best Practice Guide was devised for use in Scheme Year 17. Although most laboratories used the methods detailed in this document, a few laboratories still used in-house methodologies. All laboratories involved in CSEMP sample analysis used the NMBAQC PSA SOP for supporting biological data.

The workbook format introduced in Scheme Year 19 is continued in Scheme Year 20, the aim of this is to standardise the way in which laboratories provide data. Over the four exercises most laboratories completed the forms correctly.

One of the issues that came to light during the analysis stage was that the half-phi percentage proportions in the 'PS_Final Merged Data' tab did not match the data from participant laser and sieve entries. This was due to an auto-calculation error in the distributed workbook. Therefore, participant results were calculated independently by using the provided sieve and laser data, where appropriate, supplied and re-calculated using the in-house PSA analysis excel workbook. The results contained in each report are based upon the independently merged data rather than those presented by the results from the 'PS_Final Merged Data tab' of each participant.

It is important for laboratories using the NMBAQC Scheme SOP for particle size analysis to adhere to it. There have been instances where some laboratories have modified how they analysed the sediment due to the apparatus (i.e. laser analysis equipment) used. Altering the SOP on the basis of laser equipment capabilities can change the final results derived from analysis.

The main issue with the workbook trialled in Scheme Year 18 was with the laser replicates section, where sediment ≥1mm was being passed through laser diffraction. If following the NMBAQC methodology, laser subsamples should be passed through a 1mm sieve before laser diffraction. Although this has not occurred in Scheme Year 20, it remains noteworthy if standardisation of submitted results is to be maintained.

PS51 was distributed as a sieve only exercise and therefore analysis below 1mm was not a requirement. However, some laboratories recorded results below 1mm. These results had to be discounted. Although there were trace amounts of sediment below 1mm, if following NMBAQC SOP, there was insufficient material (< 5%) to analyse the below 1mm portion. It is acknowledged, however, that those participants using other methods may not know of the NMBAQC SOP. This serves an example showing the need for participants to follow the same protocols during analysis to make the results more comparable.

3.3 Issues and recommendations

1. Laboratories should endeavour to report their PS results in the requested format, *e.g.* at half phi intervals. This would enable the direct comparison of data from all participants and simplify the creation of cumulative curve figures. Participants should review their data prior to submission; zeros should only appear in submitted data where no material was present; dashes, '-', should appear where analysis has not been conducted.
2. Laboratories involved in CSEMP data submission should endeavour to return data on **ALL** necessary components of the Scheme in the format requested. This will be required to allow the setting of performance "flags". Non-return of data will result in assignment of a "Fail" flag. For CSEMP laboratories this deemed "Fail" for no submitted data is to be perceived as far worse than a participatory "Fail" flag.
3. Particle size exercises (PS) over the years have shown differences in the results obtained by different techniques (laser and sieve / pipette), in-house methods (*e.g.* pre-treatment) and also differences between equipment (*e.g.* Malvern Mastersizer 2000, Mastersizer X and Coulter LS230 lasers). PS data indicates that the variance between laser and sieve results is further emphasised by certain sediments characteristics. The overall range of these variances needs to be determined if combining data sets derived from differing methods. The NMBAQC's Best Practice Guide has been developed for use in Scheme Year 17; this has helped to reduce the amount of variation between methods. It is essential that particle size data are presented with a clear description of the method of analysis and equipment used.
4. An improved learning structure to the Scheme through detailed individual exercise reports has been successfully implemented and was continued in this Scheme year. For the PS exercises, detailed results have been forwarded to each participating laboratory as soon as possible after the exercise deadlines. Participants that submit significantly incorrect data are contacted immediately to ensure that in-house checks can be implemented to ensure future quality assurance. The PS48, PS49, PS50 and PS51 reports included the data submission sheets received from all participants as an appendix; Participants are encouraged

to review their exercise reports and provide feedback concerning content and format wherever appropriate.

5. The current NMBAQC Scheme standards for PSA are under review. The alternative use of z-scores for each phi-interval, trialled in Scheme Year 17 appears inappropriate for such a low number of data returns where two erroneous results can significantly alter the pass/fail criteria. For example, this can occur if laboratories do not have the representative sieves to analyse the whole range of sediment fractions. The z-score method also assumes that the majority of respondents are correct and raised genuine concerns regarding technique and method bias. Scheme Year 20 (2013/14) follows Year 19 in that z-score analysis was run alongside cluster analysis using Euclidean distance matrices.
6. Future reports could include a reverse ring test whereby benchmark samples are tested by a randomly selected laboratory to ensure representative results analysed for the ring test are satisfactory. This could also be achieved by the responsible laboratory providing raw data (with file extension *.mea) to external verifiers to assess in-house quality. It is also possible to add Similarity Profile Analysis (SIMPROF) testing of own samples to show minimal inter-sample variation in interim reports.

3.4 Reports

PSA Component Annual Report, Year 20 (2013/14)

Proctor, A., 2014. Particle Size component - Report from the contractor. Scheme Operation - Year 20 2013/14. A report to the NMBAQC Scheme co-ordinating committee. 15pp, Sept 2014

PS51 May 2014

Procter, A., 2014. National Marine Biological Analytical Quality Control Scheme. Particle Size Results: PS51. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQCps51, 32pp, May 2014.

PS50 May 2014

Procter, A., 2014. National Marine Biological Analytical Quality Control Scheme. Particle Size Results: PS50. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQCps50, 35pp, May 2014.

PS49 Feb 2014

Procter, A., 2014. National Marine Biological Analytical Quality Control Scheme. Particle Size Results: PS49. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQCps49, 33pp, Feb 2014.

PS48 Feb 2014

Procter, A., 2014. National Marine Biological Analytical Quality Control Scheme. Particle Size Results: PS48. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQCps48, 32pp, Feb 2014.

4 Fish component

Contract Manager: Jim Ellis, Centre for Environment, Fisheries and Aquaculture Science (CEFAS) from June 2013.
Component Administrator: Richard Arnold, Thomson Unicomarine Ltd.

4.1 *Summary of activities*

Year twenty of the National Marine Biological Analytical Quality Control (NMBAQC) Scheme (2013/14) followed the format of the nineteenth year. A series of exercises involved the distribution of test materials to participating laboratories and the centralised examination of returned data and samples.

The Fish component of the scheme commenced in its twelfth year (2005/06). Twenty eight laboratories / fish teams participated in the Fish component of the Year 20 NMBAQC Scheme. Twenty four participants were government laboratories / fish teams, and four were private consultancies. Although some fish are sampled under the Clean Seas Environment Monitoring Programme (CSEMP) the number of target species is relatively few. However the requirement to monitor fish assemblages in transitional waters for the Water Framework Directive (WFD) provides the major impetus for fish component exercises.

This component consisted of two modules, each with a single exercise:

- Re-identification of a set of fifteen fish specimens supplied by each of the participating laboratories (Fish Reverse Ring Test module).
- Identification of one set of fifteen fish specimens circulated by the scheme contractor (Fish Ring Test module).

The analytical procedures of both modules were the same as for the nineteenth year of the Scheme.

Fish Reverse Ring Test (F_RRT05): The identification of a set of fifteen fish species selected and supplied by the participating laboratories was relatively accurate (30 errors for 320 specimens submitted). The majority of specimens were collected by fish teams during their 2013 autumn monitoring surveys. Two recurring errors highlighted by this exercise concerned the identification of the Gobies (several species), with nine incorrectly identified and the Grey Mullets with six individuals incorrectly identified. Other recurring errors included Pipefishes.

However, there were differences in the approach to this exercise by the individual laboratories; some laboratories used this as a test for confirming voucher specimens whilst others sought a means of having uncertain or unknowns identified making it difficult to directly compare results.

Fish Ring Test (F_RT07): Fifteen fish specimens were distributed by the contractor. This fish ring test produced good agreement between the identifications made by the participating laboratories and those made by the contractor. On average each laboratory recorded 1.2 generic differences and 1.5 specific differences.

4.2 *Summary of results*

In the majority of instances for the Fish Reverse Ring Test, identifications made by the contractor were in agreement with those made by the participating laboratories with thirty errors occurring from a potential three hundred and twenty. Most identification issues were associated with Gobies, with misidentifications amongst the following species: *Gobius niger; Gobius paganellus; Pomatoschistus microps; Pomatoschistus minutus* and *Pomatoschistus pictus*. Seven out of the 41 specimens submitted by participating laboratories were identified incorrectly and two were labelled as unidentified. The grey mullets were another taxonomic group with which identification issues were associated (*Liza aurata*; *Chelon labrosus* and *Liza ramada*). Similar errors were noted in the previous two reports F_RRT04 and F_RRT03.

There were also discrepancies for Greater Pipefish (*Syngnathus acus*) and Lesser / Nilsson's Pipefish (*Syngnathus rostellatus*). Potentially difficult taxa such as the gobies could be specifically targeted in future fish ring tests (F_RT exercises) to quantify and resolve problems via the circulation of standardised specimens.

This is the seventh fish ring test circulated through the NMBAQC Scheme and the results were comparable with those from the six previous exercises (RT28 (F_RT01), RT31 (F_RT02), RT33 (F_RT03), F_RT04, F_RT05 and F_RT06) with a high level of agreement between participating laboratories for the majority of distributed species. The F_RT component is considered to provide a valuable training mechanism and be an indicator of problematic groups and possible areas for further targeted exercises or inclusion at taxonomic workshops. Multiple data entries from some laboratories and the inclusion of images in the ring test bulletins (RTB) have further emphasised the learning aspect of these exercises. F_RT07 indicated that the majority of laboratories are using the same literature to identify most specimens; Wheeler 1969, Wheeler 1978 and Maitland & Herdson 2009. However, only five of the participating laboratories provided information as to the literature used for identification.
Two participants mis-identified a sandeel that is perceived to be readily identifiable (*Hyperoplus lanceolatus*). Deterioration of ring test material may have contributed to some mis-identifications; reasons for this include fin and scale damage due to repeated examination which could result in inaccurate fin ray and scale counts.

4.3 Issues and recommendations

The Indian Goatfish (*Parupeneus indicus*) was included in the F_RT07 for identification by participating laboratories. The inclusion of a non-indigenous species illustrates how important it is for every specimen to be inspected rather than solely relying on habitat and geographical location when determining their identification. *Parupeneus indicus* was selected for inclusion due to its similar appearance to the Striped Red Mullet (*Mullus surmuletus*) on first glance. It is deemed important for non-native invasive species and migrant fish to be included as part of ring tests as sightings and oddities are becoming more prevalent around UK shores. However, future ring tests will only use species that are known to be invasive in the region of the participating laboratories, or Atlantic species for which occasional vagrants are known from north-west Europe.

4.4 Reports

Fish Component Annual Report, Year 20 (2013/14)
Hussey, S., 2014. Fish component - Report from the contractor. Scheme Operation - Year 20 - 2013/14. A report to the NMBAQC Scheme co-ordinating committee. 15pp, July 2014.

RRT 05 - March 2014
Hussey,S., 2014. National Marine Biological Analytical Quality Control Scheme. Fish Reverse Ring Test: FRRT05. Final report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQC FRRT05, 34pp, March 2014.

FRT 07 May 2014

Hussey, S., 2014. National Marine Biological Analytical Quality Control Scheme. Fish Ring Test Bulletin: FRT#07. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQCfrtb#07, 15pp, May 2014.

5 Phytoplankton component

Scheme Administrator: Joe Silke, Marine Institute, Republic of Ireland.

5.1 Summary of activities

The Phytoplankton BEQUALM inter-comparison study in 2013 was designed to test the ability of analysts to identify and enumerate correctly marine phytoplankton species in preserved water samples. As in previous years, samples have been designed using laboratory cultures. There were four species of interest in this inter-comparison exercise. These were: *Chaetoceros diadema* (Ehrenberg) Gran, *Coscinodiscus granii* Gough, *Gyrodinium instriatum* Freudenthal & J.J.Lee and *Heterosigma akashiwo* (Y.Hada) Y.Hada ex Y.Hada & M.Chihara. Also, we asked participants to return cell counts on three replicate samples as part of a homogeneity test.

Collaboration between the Marine Institute in Ireland and the IOC UNESCO Centre for Science and Communication of Harmful algae in Denmark on the BEQUALM inter-comparison exercise commenced in 2011. This collaboration involves the use of algal cultures from the Scandinavian Culture Collection of Algae and Protozoa in Copenhagen and also includes the elaboration of a marine phytoplankton taxonomy quiz using an online platform called ‘Ocean Teacher’. This HAB quiz was designed by Jacob Larsen (IOC) and Rafael Salas (MI).

This year, 49 analysts from 34 laboratories signed up for this inter-comparison. 47 analysts and 32 laboratories returned results. Laboratories from the USA, Singapore, Uruguay, France, Italy and Iceland took part in this exercise for the first time. Most laboratories are based in Europe (29): Ireland (3), Northern Ireland (1), Scotland (2), England (7), France (6), Netherlands (2), Sweden (1), Spain (3), Croatia (1), Iceland (1), Italy (1) and Greece (1). A small number of laboratories come from the USA (1), Australia (2), Singapore (1) and Uruguay (1).

This inter-comparison exercise has been coded in accordance with defined protocols in the Marine Institute, for the purposes of quality traceability and auditing. The code assigned to the current study is PHY-ICN-13- MI1. PHY standing for phytoplankton, ICN for inter-comparison, 13 refers to the year 2013, MI refers to the Marine Institute and 1 is a sequential number of inter-comparisons for the year. So, 1 indicates the first inter-comparison for the year 2013.

5.2 Summary of results

- 49 analysts from 34 laboratories took part in this inter-comparison. 47 analysts and 32 laboratories returned results. This year, new laboratories have joined the scheme from France, Iceland, Italy, Singapore, Uruguay, USA and Australia
- Most participating laboratories are based in Europe (29): Ireland (3), Northern Ireland (1), Scotland (2), England (7), France (6), Netherlands (2), Sweden (1), Spain (3), Croatia (1), Iceland (1), Italy (1) and Greece (1). A small number come from different continents: USA (1), Australia (2), Singapore (1) and Uruguay (1).

- There were four species of interest in this intercomparison exercise. These were: *Chaetoceros diadema, Coscinodiscus granii, Gyrodinium instriatum* and *Heterosigma akashiwo*.
- The average and confidence limit for each test item was calculated using the robust algorithm in annex C of ISO13528 which takes into account the heterogeneity of the samples and the between samples standard deviation from the homogeneity test. ISO 13528 is only valid for quantitative data. We have used the consensus values from the participants.
- The homogeneity and stability test show that samples do not meet the assessment criteria set out in the standard. The number of replicates needed for the samples to meet the criteria would be impractical. So instead the between-sample Standard deviation is taken into account for the final confidence limits. Outliers do not affect test result as robust analysis is being used.
- The assigned values standard uncertainty was found to be negligible for all test items, so there is no bias in the method.
  The laboratory bias plot indicates that results are normally distributed around zero for all test items.
- The percentage difference plots show that only a few analysts are outside the warning (2SD) and action (3SD) limits. The % rank using probability plots gives an indication of the most extreme values.
- The Z-scores were calculated using the robust mean and standard deviation for each test item. There was one warning signal on the *C.diadema* count, two warning signals on the *H.akashiwo* count and two warning and two action signals in the *G.instriatum* count. A total of seven signals from 184 results. Also, four analysts failed to identify one of the species in the samples, two analysts failed to identify *C.diadema* and two others *H.akashiwo*.
- The bar plot shows bias across all levels (test items) for three analysts which have tended to underestimate all counts. This could point to methodology issues. The plots of repeatability standard deviation assume that there is no difference between laboratories means and standard deviations. The plots showed unusual results for two out of the four counts with extreme values found on the *C.diadema* count and on the *H.akashiwo* count. Some counts look implausible as the variation in the counts exceeds normal statistical distributions.
- Sample composition results show that the easiest items for identification were *C.granii* and *H.akashiwo*, with near perfect scores for all analysts; *G.instriatum* proved the most difficult item for identification, with ten incorrect answers and *C.diadema* proved difficult at species level but all correct to genus.
- The Ocean teacher online HAB quiz results suggests a high rate of proficiency. 45 analysts returned results and 27 analysts achieved 100% scores with another 12 analysts over 90% mark.
- Most questions average above 90%. The worst answered question was Q8 (planozygote) with 73% on average.
- Problems arose from ‘short answer’ questions where grammar errors, punctuation or similar answers were given. In this case, where the answer was correct, notwithstanding these grammar issues, it was given as a valid answer and the scores should reflect this change.
- Issues arose regarding naming authority and use of synonyms in answers as in *Preperidinium* (*Zygabikodinium*). Either of these answers was given as correct.

5.3 *Reports*

[Phytoplankton Enumeration And Identification Ring Test, 2013]

Salas, R.G., Larsen, J., 2013. BEQUALM Phytoplankton proficiency test in the abundance and composition of marine microalgae 2013 report. PHY-ICN-13-MI1 VR 1.0. 78pp.

6 Macroalgae/Marine Angiosperms component

Contract Manager: Clare Scanlan, Scottish Environment Protection Agency.
Component Administrator: Emma Wells, Wells Marine.

6.1 *Summary of activities*

6.1.1 Marine Macroalgae Identification Ring Test (RM-RT08)

Eight laboratories subscribed to the macroalgae ring test with six laboratories submitting results with an overall total of eleven participants. Two laboratories failed to submit results; no reasons were provided. Four of the submitting laboratories were government organisations and two private consultancies.

6.1.2 Opportunistic Macroalgae Biomass - Ring Test (OMB - RT05)

The format followed that of previous years of the test (OMB RT01 – RT04 - see NMBAQC website). Ten laboratories were issued with test material. Ten laboratories completed the macroalgae biomass module with a single laboratory submitting two sets of results. All of the participating laboratories were government; no private consultancy took part.

6.1.3 Macroalgae/Seagrass Cover - Ring Test (OMC-RT05)

This included a single unit for macroalgae and one for seagrass both of which had three test options based on individual laboratories' methodologies. The format followed that of previous years (OMC RT01 – OMC RT04). Thirteen laboratories were issued test material. Twelve laboratories completed the % cover macroalgae/seagrass module with up to 24 participants; one laboratory failed to submit any results. Participation in each option varied. Of those laboratories submitting results, eleven were government organisations and one was a private consultancy.

6.2 *Summary of exercise results*

This component consisted of three modules with participants taking part in some or all:

- Marine macroalgae identification ring test (RM-RT08) – up to 5 images each of twenty macroalgae specimens were distributed on disc
- Opportunistic macroalgae biomass (OMC-RT05) – three synthetic samples of different weights for washing and drying to both wet and dry weights
- % cover estimation of Opportunistic macroalgae and seagrass - 15 photographs of quadrats of each (seagrass and macroalgae) using any or all of three quadrat options

Procedures were the same as for previous years.

6.2.1 Marine Macroalgae Identification Ring Test (RM-RT08)

Participant pass rates ranged from 62.5% to 95% with four of the eleven participants scoring >80% and eight scoring >70% overall. Across all participants 18% of all genus determinations were incorrect, i.e. 82% were correct and 30% were incorrect at species

level, i.e. 70% of species level determinations were correct. This was lower than in previous years, but this ring test incorporated more challenging species than in some previous tests. >70% is deemed “acceptable”, while >80% is deemed “good” and >90% is “proficient”. There are no absolute pass/fail criteria, and these levels are for guidance only. They may be subject to review.
The most misidentified taxon was *Boergeseniella thuyoides*, with no-one correct at both genus and species level. Five taxa accounted for 65% of the errors.

6.2.2 Opportunistic Macroalgae Biomass - Ring Test (OMB - RT05)

This year all laboratories submitted results for both wet and dry weights for all samples. Results for wet weight of biomass varied among laboratories with some producing high measures of biomass compared with the average. Dry weights showed a similar level of variability. Two laboratories exceeded the Z-score target range of +/- 2.0 for the average sample wet weight. Two other laboratories exceeded the target Z-scores for dry weight, but no Z-scores >3 were recorded. Z-scores ranged from -2.399 to +1.671 for wet weight and from -2.856 to +1.257 for dry weights. Most laboratories submitted dry weight values that were considered well within an acceptable limit of the actual biomass; however wet weight still remains highly variable.

6.2.3 Opportunistic Macroalgae/Seagrass Cover - Ring Test (OMC-RT05)

The sets of quadrat photos differed by the use of grid squares of varying quantities; open quadrat (A), 10 x 10 squares (B) grid and 5 x 5 squares (C) grid. Z-scores were derived using the mean of participants’ results and also using the image analysis results as reference values.

There were considerably more exceedances using image analysis derived Z-scores than those from the means of participants’ results. This is consistent with previous years’ results; reasons for this are being considered. Seagrass produced more Z-score exceedances than macroalgae, and are considered to be inherently more difficult to assess due to their patchiness. However, for macroalgae the overall pass rate was 94% for Test A, 96% for Test B and 97% for Test C when using Z-scores based on the population mean. For seagrass the comparable figures were 94% for Test A, 97% for Test B and 95% for Test C. Patterns across the options are not entirely consistent, but Option 2 (10 x 10 squares grid) appears to lead to over-estimation of percentage cover. However, this varies among participants, and it is therefore difficult to justify recommending one method over another at present.

6.3 *Issues and recommendations*

General

Participants have not all followed instructions correctly, which presented problems for the contractor. This included miss-spelling of taxon names (not checked properly); not including authority for taxon name; not completing spreadsheets properly; including information in email and formats other than the specified one. Participants will be reminded for future exercises that they must return information in the correct formats, otherwise data may not be accepted.

Participants were consulted on the timing of exercises and the great majority of respondents preferred early in the year. Consequently all exercises will be sent out at the start of January, with a six week period for return of results. Reports will then be available in good time for the start of the sampling season, so that key training areas can be addressed.

Marine algae identification

In some instances photographs would have benefitted from a scale and additional details of habitat, etc. than were provided. This additional information will be included in subsequent tests where necessary. Some more specific cellular information was also requested, and where possible this will be included where relevant, e.g. cross-sections of filamentous species such as *Ceramium* or *Polysiphonia*. However, even fresh specimens may not show all important characteristics e.g. reproductive structures. No staining is currently used and this shall remain for the next test.

Opportunistic macroalgal biomass

Some participants still question the necessity to incorporate both dry and wet weights within the ring test as dry weight is not part of the WFD tool. However, the data provide evidence of insufficient rinsing of samples, whereby the dried weight could be considerably higher than the original dry weight. Also there is no definitive wet weight with which to compare the individual laboratories submissions so it is difficult to conclude which results are the most representative. Dry weight will remain a required parameter.

Larger samples create a greater margin of error with far less consistency between laboratories, but are often representative of natural conditions. Future tests will cover a good range of weights, but include some much larger biomass weights.

Synthetic materials are used to mimic natural algae, but the addition of further natural material such as more sediment and *Hydrobia* will be considered. Differences in processing samples between operators was evident, and these could usefully be explored in a workshop.

% cover opportunistic macroalgae and seagrass

The accuracy of assessing % cover from quadrats versus in the field has been raised as an issue by some participants, due both to some perceived artefacts of photography and the inability to probe the surface as is possible in the field. However, field conditions are not always optimum either, but this could be explored within a workshop setting.

Labs should review their data before submission to minimise the likelihood of submitting incorrect data. Some apparently anomalous data were submitted and these skew the results.

Not all participants do all three quadrat options. This is recommended as, by completing all three methods for both seagrass and macroalgae, it is possible to compare results with other laboratories in order to gauge individual lab's/participant's level of accuracy.

As many laboratories take quadrat photos whilst estimating % cover for in house quality control, it has been suggested that a reverse ring test could be included in the % cover component. This would enable laboratories to submit their own quadrat photos for validation. This will be considered.

6.4 Taxonomic literature & reports

Identification of intertidal macroalgae

RM RT08 Final report May 2014

Wells, E., 2014. National Marine Biological Analytical Quality Control Scheme- Macroalgae Identification Component Report -RM RT08 2014 Year 20. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

RM RT08 Final Preliminary report April 2014
Wells, E., 2014. National Marine Biological Analytical Quality Control Scheme- Macroalgae Identification Component Report -RM RT08 2014 Year 20. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

Biomass of opportunistic macroalgae

OMB RT05 Final Report May 2014
Wells, E., 2014. National Marine Biological Analytical Quality Control Scheme- Macroalgae Biomass Component Report -OMB RT05 2014. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

OMB RT05 Preliminary Report April 2014
Wells, E., 2014. National Marine Biological Analytical Quality Control Scheme- Macroalgae Biomass Component Report -OMB RT05 2014. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

Percentage cover of macroalgae & seagrass

OMC Macroalgae & Seagrass RT05 Final Results Bulletin May 2014
Wells, E., 2014. National Marine Biological Analytical Quality Control Scheme- Macroalgae and Seagrass % Cover Component Report - OMC RT05 2014. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

OMC Macroalgae RT05 Preliminary Results Bulletin April 2014
Wells, E., 2014. National Marine Biological Analytical Quality Control Scheme- Macroalgae and Seagrass % Cover Component Report - OMC RT05 2014. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

OMC Seagrass RT05 Preliminary Results Bulletin year April 2014
Wells, E., 2014. National Marine Biological Analytical Quality Control Scheme- Seagrass % Cover Component Report - OMC RT05 2014. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

7 Epibiota component

Component Administrator: Dan Bayley, JNCC.

7.1 Summary of activities

The NMBAQC committee held a teleconference meeting on 17th April 2013 to reinitiate proceedings towards an Epibiota Best Practice guide. During the meeting, the possible interpretation problems of the current BS (British standards) were discussed as well as matters that were considered important for quality purposes of epibiota imaging.

A workshop organised by Natural England was held in Plymouth in September to come up with identifying a more unified approach to detecting chance using video techniques. Many epibiota stakeholders were present, including competent monitoring agencies, research institutions and contractors. Some valuable discussions were held and a report on the workshop has subsequently been produced by Cefas. The report, compiled by Sue Ware, July 2014 is available from the NMBAQC web site: Epibiota Video Workshop Summary Recommendations, 2014

As a result from the workshop the NMBAQC committee set up a googlegroup for discussion, and a discussion document as a startup for the Best Practise guide was produced. Subsequently, JNCC took it upon themselves to progress this further and make it into a workable guide, with input from all stakeholders.

8 Zooplankton component

Component Administrator: David Johns & Astrid Fischer, SAHFOS.

8.1 Summary of activities

The National Marine Biological Analytical Quality Control (NMBAQC) and the Sir Alister Hardy Foundation for Ocean Science (SAHFOS, http://www.sahfos.ac.uk) are developing a quality control scheme for the analysis of zooplankton samples. In 2014 NMBAQC on behalf of SAHFOS sent out a call of interest for a UK Trial Zooplankton Ring Test, to be held in 2014-2015.

8.2 Summary of results

Six UK laboratories expressed an interest in the UK trial ring test, and will be informed once a format for the test has been decided.

Appendix 1 - NMBAQC Co-ordinating Committee – Year 20 - 2013/2014

Name	Organisation	Position
David Johns	Sir Alister Hardy Foundation for Ocean Science (SAHFOS)	Chair
Tim Mackie	Northern Ireland Environment Agency (NIEA)	CMA Representative
Amanda Prior	Environment Agency (EA)	Finance Manager
Myles O'Reilly	Scottish Environment Protection Agency (SEPA)	Invertebrate Contract Manager
Joe Silke/ Rafael Salas	Marine Institute, Ireland (MI)	Phytoplankton Contract Manager
Clare Scanlan	Scottish Environment Protection Agency (SEPA)	Macroalgae Contract Manager
Carol Milner	APEM Ltd	Contractors Representative
Dan Bayley	Joint Nature Conservation Committee (JNCC)	Epibiota Contract Manager
Keith Cooper/ Claire Mason/ Jim Ellis	Centre for Environment, Fisheries & Aquaculture Science (Cefas)	CMA Representative//CMA Representative/Fish Contract Manager
Matthew Green	Natural Resources Wales (NRW)	CMA Representative
Astrid Fischer	SAHFOS	Technical Secretary

Appendix 2 - NMBAQC scheme participation for Year 20

ORGANISATION	BENTHIC INVERTS	PARTICLE SIZE	FISH	MACROALGAE/ SEAGRASS	PHYTO
AFBI, UK	✓	✓	✓	✓	✓
APEM Ltd, UK	✓				✓
Australian Shellfish Quality Assurance Program (SASQAP), Australia					✓
Benthic Solutions Limited, UK	✓	✓			
Biopol Sjávarlíftæknisetur / Marine Biotechnology, Iceland					✓
Biotikos Limited, UK	✓				
Cefas, UK	✓	✓	✓		✓
CLS, Ireland					✓
CMACS Ltd, UK	✓	✓			
CNRS, France					✓
Corben Ltd, UK					✓
Cyfoeth Naturiol Cymru / Natural Resources Wales, UK		✓	✓	✓	
DHI Laboratory, Singapore					✓
eCoast Marine Research, Belgium	✓				
Ecospan Environmental Ltd, UK	✓				
Eidikos Logariasmos Kondilion Erevnas, Greece					✓
Environment Agency, UK		✓	✓	✓	
Environmental Protection Agency (EPA), Ireland				✓	
Fish Vet Group, UK	✓	✓			
Fugro EMU Limited, UK	✓	✓	✓	✓	
Gardline Ltd., UK		✓			
Grontmij Nederland B.V., Team Ecologie, the Netherlands	✓				
Hebog Environmental Ltd, UK	✓				
Hunter Biological, UK	✓				
IFREMER, France					✓

ORGANISATION	BENTHIC INVERTS	PARTICLE SIZE	FISH	MACROALGAE/ SEAGRASS	PHYTO
ILVO (Institute for Agricultural and Fisheries Research)- ANIMILAB, Belgium	✓				
IMARES, the Netherlands	✓				✓
Institute of Estuarine & Coastal Studies (IECS), UK	✓	✓	✓		
Institute of Oceanography and Fisheries, Croatia					✓
Inter University Center of Marine Biology and Applied Ecology (CIBM), Livorno, Italy	✓				
IRTA, Spain					✓
Isle of Man Government Laboratory, Isle of Man					✓
IVL Swedish Environmental Institute, Sweden					✓
Jacobs Ltd., UK	✓				✓
Kenneth Pye Associates, UK		✓			
Koeman en Bijkerk BV, the Netherlands	✓				✓
Laboratoire des sciences de l'environment Marin (LEMAR), France					✓
Laboratorio de Control de calidad de los recursos pesqueros, Spain					✓
Laboratorio de Medio Ambiente de Galicia (LMAG), Spain					✓
Marine Ecological Solutions Ltd,UK				✓	
Marine Ecological Surveys Ltd, UK	✓				
Marine Farm Services, Shetland Seafood Quality Control (SSQC), UK	✓				
Marine Institute, Ireland					✓
Marine Invertebrate Ecological Services, UK	✓				

ORGANISATION	BENTHIC INVERTS	PARTICLE SIZE	FISH	MACROALGAE/ SEAGRASS	PHYTO
Marine Scotland (Marine Laboratory), UK	✓	✓			✓
Microalgal Services, Australia					✓
Monitor Taskforce, Royal Netherlands Institute for Sea Research, the Netherlands	✓				
Myriad Taxonomy, UK	✓				
Natural England, UK	✓			✓	
NIEA, (DOE(NI)),UK	✓	✓	✓	✓	
Nostoca Algae Laboratory, USA					✓
Phytoplankton Monitoring Program National Direction of Aquatic Resources, Uruguay					✓
Precision Marine Survey Ltd (PMSL), UK	✓	✓	✓		
SAMS Research Services Limited, UK		✓			✓
SEPA, UK	✓	✓	✓	✓	✓
Stazione Zoologica Anton Dohrn Villa Comunale, Italy					✓
Sue Hamilton, UK	✓				

Appendix 3 – Beginners Invertebrate Taxonomic Workshop Programme – Thomson Unicomarine, Letchworth – Nov. 2013

Day	Session	Discussion / Demonstration / Practical	Aims	Session Leader
Monday 11th Nov. 2013	1:00pm	Arrival. Laboratory set-up.	Prepare laboratory equipment for practical sessions.	Ruth Throssell & Laura Hearnden
	1:30pm	Introduction. General information. Lab. rules (H&S issues). Q&A session.	Welcome participants. Outline folder / timetable / daily structure. Give history of Thomson Unicomarine and facilities. Present pub & food guide.	Ruth Barnich, Janie Cloote & Laura Hearnden
	2:00pm	Demonstration / Discussion - Sample Processing.	Requirements, SOP's and best practice for sample analysis.	Janie Cloote & Laura Hearnden
	2:20pm	Practical - Phyla recognition.	Review starting position of knowledge.	
	pm	Demonstration - Porifera, Cnidaria, Platyhelminthes, Nematoda, Nemertea, Priapulida, Sipuncula & Echiura.	Introduce the major features / terminology used for these Phyla. Show major literature required for identification.	
	pm	Practical – Examination of reference material.	Obtain familiarity with the major identification features. Gain experience of identification.	
Tuesday 12th Nov. 2013	9:15am	Demonstration - Annelida.	Introduce the major features / terminology used for this Phylum. Show major literature required for identification.	Ruth Barnich & Laura Hearnden
	pm	Practical – Examination of reference material.	Obtain familiarity with the major identification features. Gain experience of identification.	
	4:30pm	Practical – test specimens.	Allow identification of unnamed material.	
Wednesday 13th Nov. 2013	9:15am	Demonstration - Mollusca.	Introduce the major features / terminology used for this Phylum. Show major literature required for identification.	Anita Gajda, Alice Lodola & Laura Hearnden
	pm	Practical – Examination of reference material.	Obtain familiarity with the major identification features. Gain experience of identification.	
	4:30pm	Practical – test specimens.	Allow identification of unnamed material.	
Thursday 14th Nov. 2013	9:15am	Demonstration - Crustacea.	Introduce the major features / terminology used for this Phylum. Show major literature required for identification.	Lauren Hughes & Laura Hearnden
	pm	Practical – Examination of reference material.	Obtain familiarity with the major identification features. Gain experience of identification.	
	4:30pm	Practical – test specimens.	Allow identification of unnamed material.	
Friday 15th Nov. 2013	9:15am	Demonstration – Echinodermata.	Introduce the major features / terminology used for this Phylum. Show major literature required for identification.	Janie Cloote & Laura Hearnden
	am	Practical – Examination of reference material.	Obtain familiarity with the major identification features. Gain experience of identification.	
	pm	Discussion - Summary of week. Q&A session. Departure.	Distribute / collect workshop feedback forms.	

Appendix 4 - BEQUALM/NMBAQC Scheme Phytoplankton Workshop

Agenda BEQUALM Phytoplankton Inter-comparison Workshop

Marine Institute, Rinville, Oranmore, County Galway, Ireland 7-9 Oct 2013.

	Morning 9.30am-13.00pm	Afternoon 14.00pm-17.30pm
Monday, 7 Oct	Intercomparison exercise results Enumeration and identification exercise results. Ocean teacher HABs quiz exercise results. (R.Salas) ISO13528 statistical methods (R.Salas) Discussion of exercise and ideas for 2014 (All)	Community analyses of North Sea phytoplankton (R. van Wezel) Calculating Phytoplankton Biovolume, Biomass and Carbon - How and Why! (Lars Edler) Field samples from participants (microscopy and identification) All
Tuesday, 8 Oct	Lecture and microscope demonstration of the Raphidophytes group (J.Larsen)	Lecture and microscope demonstration of the nanoflagellates group (J.Larsen)
Wednesday, 9 Oct	Lecture and microscope demonstration of naked dinoflagellates with emphasis on *Gyrodinium* and *Gymndinium* genera (J.Larsen)	Departure