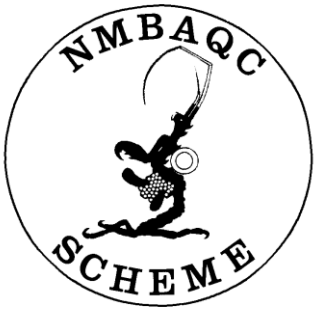

BEQUALM
NATIONAL MARINE BIOLOGICAL ANALYTICAL QUALITY CONTROL SCHEME
Annual Report - Year 18 - 2011/2012

A report prepared by the NMBAQC Coordinating Committee April 2014

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This Year 18 Annual Report provides synopsis of the scheme year's activities over 2011/2012. 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 three meetings during the scheme Year 18 on 11th May 2011, 23 August 2011, 24th November 2011 and 8th March 2012. Committee Membership for Year 18 is shown in Appendix 1.

1. Scheme Review

The scope of the NMBAQC scheme continued to develop in Year 18 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). In Yr 17 the NMBAQC agreed their Terms of Reference with HBDSEG.

Year 18 of the scheme followed a similar format to the previous year and involved training and testing exercises for the Invertebrate, Particle Size, Fish, Phytoplankton and Macroalgae components. The Year 18 participation level in the NMBAQC was very similar to the previous year with a total of 47 (Plus Macroalgae) organisations involved in its training and testing exercises (see Appendix 2).

Summaries of all the component activities are provided below.

2. Invertebrate component

Contract Manager: Myles O' Reilly, SEPA
Component Administrator: David Hall, Unicomarine

2.1 Summary of activities

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

- Identification of two sets of twenty-five invertebrate specimens (RT, Invertebrate Ring Test module).
- Analysis of a single fully marine macrobenthic sample (MB, Macrobenthic Sample module).
- Re-identification of a set of twenty-five specimens supplied by each of the participating laboratories (LR, Laboratory Reference module).
- Re-analysis by Thomson Unicomarine of three own samples supplied by each of the participating laboratories (OS, Own Sample module).

The analytical procedures of the various modules were the same as for Year 17 of the Scheme, which includes the specification that the Macrobenthic Sample module and CSEMP samples within the Own Sample module should be conducted using the NMBAQC guidance for macrobenthic invertebrate sample analysis (Worsfold, Hall & O'Reilly (Ed.) 2010).

The Invertebrate component held a five-day Beginners' Taxonomic Workshop in March 2012 at the Thomson Unicomarine Laboratory in Letchworth. The committee commissioned a 'Summary of CSEMP Own Sample Audits 1999 to 2008' report which identifies the ownership by Competent Monitoring Authorities (CMAs) of all the UK CSEMP invertebrate sampling sites and their audit history over a ten year period. The report provides a traceable link between the samples submitted to the scheme by UK CMAs and the invertebrate datasets held on the Merman database.

2.2 Summary of exercise results

Forty laboratories participated in the benthic invertebrate component of the NMBAQC Scheme in Year 18. Fifteen participants were CMAs; twenty-five were private consultancies. One of the participants was a consortium of sole traders. Four of the CMA participants were responsible for CSEMP sample analysis. A summary of the overall NMBAQC participation levels is shown in Appendix 2.

Two **Ring Tests (RT)** of 25 specimens were distributed. One set contained 25 general invertebrate specimens **(RT41)** and a second set consisted of 'targeted' specimens from taxa that occur in Scottish waters **(RT42)**. For the general set of fauna (RT41) there was fairly good agreement between the identifications made by the participating laboratories and those made by Thomson Unicomarine On average each participating laboratory recorded 2.1 generic differences and 6.3 specific differences. Eight taxa (two molluscs, four polychaetes and two crustaceans) were responsible for two thirds of the specific differences. The 'targeted' ring test (RT42, taxa from Scottish waters) produced much better results than the standard exercise. On average each participating laboratory recorded 2.9 generic differences and 3.6 specific differences. Four taxa (three molluscs and one polychaete) were responsible for more than half of the differences.

Analysis of the **Macrobenthic Sample (MB19)** by the participating laboratories and subsequent re-analysis by Thomson Unicomarine provided information on the efficiency of extraction of the fauna, accuracy of enumeration and identification and the reproducibility of biomass estimations. For MB19 fully marine samples from the southern North Sea were distributed. This was the second MB exercise with strict extraction and processing instructions and, in contrast to the previous year, results for MB19 showed a high degree of agreement to the re-analysis by Thomson Unicomarine. Extraction efficiency (of individuals) was on average 95.84% with only one laboratory extracting less than 90 % of the individuals. Comparison of the results from the laboratories with those from analysis by Thomson Unicomarine (following the NMBAQC macrobenthic analysis guidelines) was made using the Bray-Curtis similarity index (untransformed). The value of the index varied between 76.02% and 99.06%. It was better than 90% in 71% of the comparisons and less than 85% in only one laboratory.

Laboratory Reference (LR16): Eleven laboratories submitted their specimens for confirmation. Six of these 11 laboratories presented one third or more differences to those made by Thomson Unicomarine. The taxa responsible for these differences were mainly bivalves, amphipods and polychaete families which are either speciose or which lack adequate keys.

The revised protocols of Scheme Year 10 for 'blind' **Own Sample (OS47, OS48, & OS49)** audits were continued in this Scheme year. Laboratories were to submit full completed data matrices from their previous year's Clean Seas Environment Monitoring Programme (CSEMP 2010; formerly NMMP) samples or similar alternative sampling programmes (if not responsible for CSEMP 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). The results for the Own Sample Module were slightly better than those from the Macrobenthic Sample. Agreement between the laboratories and Thomson Unicomarine was generally very good. Extraction efficiency was better than 90% in 96% of the comparisons and better than 95% in 87% of all comparisons. All countable faunal specimens were extracted from the sample residues in 58% of the samples. The Bray-Curtis similarity index ranged from 86% to 100% with an average figure of 97%. The Bray-Curtis similarity index was greater than 95% in 82% of comparisons and in most cases (96%) the value of the index was greater than 90%. These samples all achieved 'Pass' flags. Twenty-seven samples (27%) achieved 'Pass-Excellent' flags with Bray-Curtis similarity scores of 100%.

2.3 Issues and recommendations

1. Late submission of data or samples by participants continues to result in significant reporting delays.
2. Labs submitting samples for audit must ensure they submit all sorted residue and all faunal fragments.
3. It is the responsibility of participating labs to ensure they return data or submit samples. Labs who have signed up to exercises but do not complete them will still be charged. CSEMP labs who fail to submit samples for audit will receive a "deemed fail" flag.
4. Labs need to ensure the follow the standardised protocol fo biomass assessment.
5. Labs should make use to the Lab Reference exercise to develop and verify their reference collections.
6. Ring Test participants should complete the "Confidence Level" column to allow the test administrators to gauge the level of difficulty on each taxon.
7. Participants should ensure they are familiar with taxonomic literature produced by, or highlighted by, the scheme.
8. Own Sample submission sheets should be completed in full and sample processing should follow the NMBAQC guidelines.
9. Own Sample participants should address all taxonomic errors, including those in samples that have receieved a Pass flag.
10. Own Sample participants should investigate and address issues raised with samples that fail to achieve targets for sorting efficiency.
11. There is a need for the scheme to develop a Taxonomic Discrimination Policy (TDP) to standardise acceptable identification levels within different taxonomic groups.
12. Participants should be actively encouraged to provide more feedback on exercises (whether positive or negative) to ensure they receive the most benefit from their participation.

Year 18 Invertebrate Component Annual Report:

Year 18 Annual report, 2013

Barnich, R, 2013. National Marine Biological Analytical Quality Control Scheme. Benthic Invertebrate Component Report, Scheme Operation Year 18 - 2011/2012. 27 pp, November 2013.

Year 18 Own Sample Report:

Own Sample Module Summary Report OS44, 45 & 46 - September 2012

Hall, D.J., 2012. National Marine Biological Analytical Quality Control Scheme. Own Sample Module Interim Summary Report OS44, 45 & 46. Report to the NMBAQC Scheme participants. 25pp, September 2012.

Year 18 Ring Test Bulletins:

RTB 40 - October 2011

Hall, D.J., Taylor, J.G. and Worsfold, T.M., 2011. National Marine Biological Analytical Quality Control Scheme. Ring Test Bulletin: RTB#40. Report to the NMBAQC Scheme participants. Unicomarine Report NMBAQCrtb#40, 33pp, October 2011

RTB 41 - July 2012

Hall, D.J., Worsfold, T.M. and Neilson, D., 2012. National Marine Biological Analytical Quality Control Scheme. Ring Test Bulletin: RTB#41. Report to the NMBAQC Scheme participants. Unicomarine Report NMBAQCrtb#41, 36pp, July 2012.

Year 18 Macrobenthic Exercise Report:

MB 19 - June 2012

Taylor, J.G. and Hall, D.J., 2012. National Marine Biological Analytical Quality Control Scheme. Macrobenthic Exercise Results - MB19. Report to the NMBAQC Scheme participants. 19pp, June 2012.

3. Particle Size Analysis Component

Contract Manager: Myles O' Reilly, SEPA
Component Administrator: David Hall, Unicomarine

3.1 Summary of activities

In the Year 18 NMBAQC Scheme eleven laboratories participated in the particle size analysis exercises PS40, PS41, PS42 and PS43; five were government laboratories; six were private consultancies. Five of the participants were responsible for CSEMP (Clean Seas Environment Monitoring Programme) sample analysis.
This PSA component consisted of one module with four exercises: Analysis of four sediment samples for physical description (Particle Size module):

PS40 - Sandy Mud (natural sample)
PS41 - Sand (natural sample)
PS42 - Gravel (artificially created sample)
PS43 - Gravelly Muddy Sand (artificially created sample)

Following on from the particle size analysis workshop, held at the Cefas laboratory in February 2009, the NMBAQC has now produced a Best Practice Guidance for Particle

Size Analysis (PSA) for Supporting Biological Analysis. This describes standard procedures for collecting and analysing sediment samples including sampling, analysis, data recording and quality assurance. The UK CMAs undertaking PSA in support of biological analysis for CSEMP and WFD monitoring programmes are required to adopt these procedures and recommendations.

3.2 Summary of results

The analytical procedures of this module were the same as for the seventeenth year of the Scheme. 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. Scheme Year 17 trialled the use of z-scores calculated for each half-phi interval, Scheme Year 18 trialed the use of multivariate analysis using Euclidean distance matrices (dendrograms and nonmetric MDS plots).

The variation within the ten replicate results produced by the benchmark laboratories using the NMBAQC PSA SOP was minimal for PS40-43; 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.
There was generally good agreement for **PS40** between the results from the analysis of replicates and those from the majority of participating laboratories. One lab (LB1830) had missing data values for some of the half-phi intervals towards the end of the data set. All of the participants used the laser diffraction technique to analyse the sample. The derived statistic for %silt for those laboratories following the NMBAQC methods ranged from 78.99% to 92.00%. The two laboratories following alternate methods recorded a %silt of 90.38% and 86.32% .

There was generally good agreement for **PS41** between the results from the analysis of replicates and those from the participating laboratories. Of the laboratories following the NMBAQC methods six stated that they used laser diffraction only to analyse the sample and three used sieves and laser diffraction. Of the laboratories not following NMBAQC methods one used only laser diffraction and one used sieves and laser diffraction. The derived statistic for laboratories following the NMBAQC methods for %silt ranged from 6.39% to 18.94%. The two laboratories following alternate methods recorded a %silt of 12.27% and 15.07%.

There was generally good agreement for **PS42** between the results from the analysis of replicates and those from the participating laboratories. Seven out of the nine laboratories following the NMBAQC methodology used dry sieving only to analyse the sample. The remaining two laboratories attempted laser diffraction as well as dry sieving but found there was insufficient sediment to do more than one run through the laser.

Two participating labs used alternate methods; one used dry sieves from -6.5 to 4.0 phi and the other dry sieves from -6.5 to 0 phi. Two laboratories did not provide the data in half phi intervals. The derived statistic for the % silt was 0% for all laboratories except for those who attempted laser diffraction. The %silt for these two laboratories was 0.07% and 0.13%.

There was a fair amount of variation in **PS43** between the results from analysis of replicates and those from the participating laboratories. Ten laboratories used sieve and laser analysis to analyse the sample; one lab only used laser analysis. One lab only recorded above -2.5, displacing their cumulative curve by 2 phi at the beginning. The stone that the majority of laboratories recorded at -4.5 to -4.0 phi was recorded half a phi out by one lab and one phi out by another. For participating laboratories using the NMBAQC method the derived statistic for the % silt/clay ranged from 1.4% to 89.4%. The variability in this result clearly demonstrates some of the laboratories were not following methodology (not sieving at 0.5phi intervals, completing laser analysis only) but also that there are some laboratories who would benefit from further training. This sample (a diamicton) was the hardest sample to analyse and most useful for identifying non-conformities. Inclusion of such samples in future rounds is strongly recommended.

Z-scores and cluster dendrogram figures were presented in each of the PS exercise reports; however these were only for illustration purposes. The investigations into new pass/fail standards are still underway. Pass/fail criteria will be introduced when sufficient data are collected using the new analysis guidance method.

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. A modified workbook has been designed for use in Scheme Year 18 to enable laboratories to provide data in a comparable format. This has been modified slightly for Year 19 to resolve any issues that have arisen. 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 past sixteen 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 after the exercise deadlines as practicable. Participants that submit significantly incorrect data are contacted immediately to ensure that in-house checks can be implemented to ensure future quality assurance. The PS40, PS41, PS42 and PS43 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. The z-score method also assumes that the majority of respondents are correct and raised genuine concerns regarding technique and method bias. In Scheme Year 18 (2011/12) z-score analysis was run alongside cluster analysis using Euclidean distance matrices. PS40 and PS41 tentatively examined using confidence intervals, this approach will be examined in more depth in Scheme Year 19

PSA Component Annual Report, Year 18 (2011/12)
Finbow, L.A. and Hall, D.J., 2012. Particle Size component - Report from the contractor. Scheme Operation - Year 18 - 2011/12. A report to the NMBAQC Scheme participants. 15pp, August 2012.

PS39 July 2011
Finbow, L.A. and Hall, D.J., 2011. National Marine Biological Analytical Quality Control Scheme. Particle Size Results: PS39. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQCps39, 31pp, July 2011.

PS40 December 2011
Finbow, L.A. and Hall, D.J., 2011. National Marine Biological Analytical Quality Control Scheme. Particle Size Results: PS40. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQCps40, 38pp, December 2011.

PS41 December 2011
Finbow, L.A. and Hall, D.J., 2011. National Marine Biological Analytical Quality Control Scheme. Particle Size Results: PS41. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQCps41, 38pp, December 2011.

PS42 June 2012
Finbow, L.A. and Hall, D.J., 2012. National Marine Biological Analytical Quality Control Scheme. Particle Size Results: PS42. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQCps42, 26pp, June 2012.

4. Fish component

Contract Manager: Steve Coates, Environment Agency
Component Administrator: David Hall, Unicomarine

4.1 Summary of activities

A fish identification workshop was held in April 2011 at the Dove Marine Lab (See Appendix 3). This provided an opportunity for CMAs and consultants to improve fish ID and monitoring skills as part of a UK-wide initiative. The Fish Component contract manager, Steve Coates, left the Environment Agency (and effectively resigned from the committee) around November 2011. As no new fish lead was available, then interim management of the Fish Component contract was undertaken in conjunction with the Invertebrate and Particle Size Components.

There were two modules in the fish component for Scheme year eighteen; Fish Reverse Ring Test identification (F_RRT) module and Fish Ring Test identification (F_RT) module. The F_RRT Module enables the identification of fish specimens to be externally verified and encourages laboratories / fish teams to build extensive, verified reference collections to improve identification consistency. The F_RT Module examined inter-laboratory variation in the participants ability to identify fish taxa and attempted to determine whether any errors were the result of inadequate keys, lack of reference material (e.g. growth series), or the incorrect use of satisfactory keys.

4.2 Summary of results

In total twenty-five laboratories / fish teams subscribed to F_RRT03, with all laboratories returning specimens for verification. Four laboratories submitted data and specimens after the submission deadline. Six laboratories submitted less than the specified number of taxa. In total three hundred and forty-nine fish taxon bags were submitted for verification.

In the majority of instances identifications made by Thomson Unicomarine Ltd. were in agreement with those made by the participating laboratories, just twenty-nine errors (from a potential three hundred and forty-nine). In view of the different species that were sent by laboratories for identification it is difficult to make detailed inter-lab comparisons with such a small data set and the potentially differing approaches taken to this exercise. However over a third of the sixteen specimens of grey Mullets sent by participating laboratories were identified incorrectly. Another recurring error was noted for Gobies (*Pomatoschistus minutus, P. pictus* and *P. microps*). Similar errors were noted in F_RRT02. Such trends will be monitored in future reverse fish ring tests and potentially difficult taxa could be specifically targeted in future fish ring tests (F_RT exercises) to quantify and resolve problems via the circulation of standardised specimens.

For F_RT05 fifteen fish specimens were circulated to eleven participating laboratories. As with previous Scheme years, participating laboratories were permitted to supply multiple data entries for each exercise to maximise results and enhance the training aspect of this module. Other aspects of the circulation, in particular the method of scoring results, were the same as for previous circulations. Participating laboratories were permitted to retain F_RT05 fish specimens as part of their in-house reference

collections. All eleven laboratories returned data for this exercise; seventeen individual data sets in total via multiple data submissions.

This is the fifth fish ring test circulated through the NMBAQC Scheme and the results were comparable with those from the four previous exercises RT28 (F_RT01), RT31 (F_RT02), RT33 (F_RT03) and F_RT04, 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 problem 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_RT05 indicated that the majority of laboratories are using the same literature to identify most specimens; Wheeler 1969 and Maitland & Herdson 2009. However, several of the participating laboratories did not provide information as to the literature used for identification. None of the participants identified all of the specimens correctly. Several participants mis-identified species that are perceived to be common and readily identifiable (*Pleuronectes platessa, Trispoterus minutus* and *Microchirus variegatus*). The most common error was for the lesser sandeel (*Ammodytes tobianus*). Deterioration of ring test material may also have contributed to some mis-identifications, for example fin damage due to repeated examination could produce inaccurate fin ray counts. It must be noted that the vast majority of participants in this exercise would not routinely encounter fixed and preserved fish specimens and these results do not necessarily compromise identifications in routine fish monitoring surveys. Further details and analysis of results can be found in the fish ring test bulletin (Fish Ring Test Bulletin – F_RT05) which was circulated to all participants and is available on the Scheme's website.

Fish Component Annual Report, Year 18 (2011/12)
Taylor, J.G. and Hall, D.J, 2012. Fish component - Report from the contractor. Scheme Operation - Year 18 - 2011/12. A report to the NMBAQC Scheme co-ordinating committee. 13pp, August 2012.

RRT03 February 2012
Taylor, J.G and Hall, D.J, 2012. National Marine Biological Analytical Quality Control Scheme. Fish Reverse Ring Test Bulletin: F-RRT03. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQCf-rrt03, 29pp, February 2012.

FRT05 July 2012
Taylor, J.G and Hall, D.J, 2012. National Marine Biological Analytical Quality Control Scheme. Fish Ring Test Bulletin: FRT#05. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQCfrtb#05, 15pp, June 2012.

5. Phytoplankton component

Scheme Administrator: Joe Silke, Marine Institute, Galway, Ireland. Registration and fee collecting arranged through BEQUALM Website (based at CEFAS Lab, Lowestoft).

5.1 Summary of activities

Collaboration between the Marine Institute in Ireland and the IOC UNESCO Centre for Science and Communication of Harmful algae in Denmark on the Bequalm intercomparison exercise commenced in 2011. This collaboration involved the use of algal cultures from the Scandinavian Culture Collection of Algae and Protozoa in Copenhagen and also included the elaboration of a marine phytoplankton taxonomy quiz using an online platform called 'Ocean Teacher'. This HAB quiz was designed by Jacob Larsen (IOC).

This year, 34 analysts from 20 laboratories across Europe took part in this exercise. It is the first year we had participants from Greece. There are now three countries in the Mediterranean area taking part in this intercomparison; this includes laboratories from Spain (3), Croatia (1) and Greece (1). In the Atlantic area of influence, there are 9 laboratories across the UK, 2 in the Netherlands, 2 in Spain and 2 in Ireland.

This intercomparison 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-11-MI1. PHY standing for phytoplankton, ICN for intercomparison, 11 refers to the year 2011, MI refers to the Marine Institute and 1 is a sequential number of intercomparisons for the year. So, 1 indicates the first intercomparison for the year 2011.

The Phytoplankton Component workshop was held on the 8th of November 2011 at the CEFAS laboratory in Weymouth. There were 15 participants from 10 laboratories including representatives from Croatia, Denmark and Holland. Presentations were provided by Katerina Aligizaki (Greece) on benthic dinoflagellates and Sarah Swan (Aberdeen) on toxic dinoflagellates.

5.2 Summary of results

- 30 analysts from 20 laboratories across Europe returned results. There is a lack of reproducibility between laboratories and reference values: If the reference values were validated, most laboratories would be outside the accepted variance of 2 standard deviations of the mean.
- The descriptive statistics suggests, the data don't follow a normal distribution for most counts, despite this, most Individual charts and Z-scores suggest most analysts perform within the 2 standard deviations of the mean of the other analysts' results.
- Six organisms were preserved and spiked in the samples. Three toxic species and three non-toxic species. Analysts were better overall at identifying the toxic from the non-toxic species.
- Three of the species were large in size (*A.sanguinea, G.pacificus, P.lima*) compared to the other three which were smaller in size (*A.minutum, S.trochoidea,*

H.minima). Analysts performed better at identifying the larger species from the smaller ones.

- *Heterocapsa minima* were the most difficult organism to identify: 13 analysts did not find this species in the sample. 4 analysts misidentified it, three of which named it as *Azadinium*.
- *Akashiwo sanguinea* and *Prorocentrum lima* were the easiest organisms to identify. All analysts recorded these species correctly. *Gambierdiscus pacificus* was easy to identify to genus level but most analysts (15 in total) thought it was the species *G.toxicus*.
- *G.pacificus* was not identified by four analysts. These analysts came from laboratories which don't find these species in their waters.
- A reliability qualitative measure calculated for the method indicates that the method is more sensitive (91%) than specific (76%) and its efficiency based on the data is 83%. The false positive rate is higher (29%) than the false negative rate (8%) indicating that we are more likely to mis-identify a non-toxic species than the other way around.
- Most analysts performed above the 90% mark for the 'Ocean Teacher' Bequalm HAB quiz exercise. Questions 4,7,8,9 and 10 were perfectly answered by all analysts. Q12 was the worst answered question. This was the question on the diatom taxonomy of the genus *Pseudo-nitzschia spp.*

Phytoplankton Enumeration And Identification Ring Test, 2011

Salas, R.G., Larsen, J., 2011. BEQUALM Phytoplankton proficiency test in the abundance and composition of marine microalgae 2011 report. PHY-ICN-11-MI1 VR 2.0

6. Macroalgae component

Contract Manager: Clare Scanlan, SEPA
Component Administrator: Emma Wells, Wells Marine

6.1 Summary of activities

This component has been ongoing since its development in year 13 (2006/2007), but has not been reported upon since the Annual report in year 15 (2008/2009). In the interim two new modules were introduced in year 16 (2009/2010), the Opportunistic Macroalgae and Seagrass % cover module (OMC) and the Opportunistic Macroalgae Biomass module (OMB). The new OMC and OMB modules were repeated in year 17 (2010/2011) and now, in year 18 (2011/2012), are therefore in their 3rd year (RT03), whereas the macroalgae identification module is running in its 6th year (RT06). The results for years 16 & 17 are available on the scheme website, while those for year 18 are outlined and discussed below.

6.1.1 The Macroalgae and Seagrass % Cover Module (OMC RT03)

This module consisted of one macroalgae and one seagrass exercise, which was subsequently split into three alternative means of assessment which could be considered as separate modules from which laboratories could complete one or more module.
There were a total of 12 participating laboratories and 36 individuals. Most labs were CMAs, the rest consultancies. Some laboratories submitted results late, which meant

bulletins and reports were later than planned. In subsequent years reminders will be distributed prior to the deadline.
Two sets of fifteen quadrat photographs consisting of various % covers one for opportunist macroalgae and one for seagrass were used for the exercise. These could be assessed by three types of overlaid quadrat: open, 10 x 10 square grid, 5 x 5 square grid. Each photo represented natural levels of opportunist macroalgae and seagrass cover.

6.1.2 Macroalgae Biomass Module (OMB RT03)

This module consisted of a single exercise producing a single set of results from each laboratory. The analytical procedures of the exercise remained consistent with earlier rounds one and two of the scheme (OMB RT01 & RT02). A total of nine laboratories (all CMAs) took part. The deadline was extended slightly for one laboratory. In future a reminder will be sent out to participants one week before the results return deadline, as delayed submissions mean delayed reports.

6.1.3 Macroalgae Identification Module (RT 06)

This component consisted of a single macroalgae exercise the analytical procedures of which remained consistent with rounds two and three of the scheme.

1.5.1.4 The need for certificates was discussed, and these will be issued from the next round of tests.

6.2 Summary of results

6.2.1 The Macroalgae and Seagrass % Cover Module (OMC RT03)

1. There is evidently still a high degree of error between assessment methods as well as between participants and this may prompt the need for a specific workshop whereby methods may be discussed and possibly % cover estimations compared in the field. It is not possible from the current ring test to conclude which % cover estimation method provides the most accurate results. However it is evident through the number of participants that Test B is the most favoured method albeit just for macroalgae.
2. The image analysis method used during RT03 is considered more objective than skilled eye estimation and likely to produce more accurate results. RT03 also incorporated ground-truthing to pick up subtleties of variations in cover within the defined affected area. However, this method is still under development and will continue to undergo improvements prior to the next round of tests. Despite this round incorporating a classified and ground-truthed image analysis method with more accurate results, it is suggested at this time that participants should use the Z-scores derived from comparisons with the mean if they are required for internal quality reports.
3. There are still some issues over the timing of the test, and there are suggestions that the time allowed for completion of the test should be extended to accommodate increased workloads. Although this is still the most appropriate time of year to complete the tests, a longer time scale within which to complete the exercises would allow more laboratories to complete all three methodologies for both the seagrass and macroalgae. This will be implemented.
4. It is accepted that the nature of photographs can produce difficulties when assessing the density of algae or seagrass, and the presence of some shadows can hinder this further. However, it should be noted that many seagrass beds remain

waterlogged regardless of tidal height, so are difficult to photograph. It is equally accepted that sometimes it is difficult to count algal cover accurately when obscured under cross hairs, which would not be an issue in the field, but cannot be prevented within the test; therefore it remains important to include the open quadrat test method for a full view of the quadrat. It was also considered that the higher % cover band was not sufficiently considered within the ring test and that subsequent tests should include a wider range of cover bands.

5. Feedback on quadrat type was received and options will be discussed for future ring tests.
6. Due to the unfamiliarity of some methods of estimating % cover, it is suggested that such methods be clarified to ensure the tests are carried out accurately and with a level of consistency between laboratories. The methods that are currently included within the ring test were those considered to be most frequently used. It is agreed that where laboratories use alternative methods such as subtidal quadrat % cover estimations these methods may not accurately represent their commonly used procedures. The exercise is for intertidal, not subtidal, beds. However, by completing all three methods for both seagrass and macroalgae it is still possible to compare results with other laboratories in order gauge the level of accuracy.
7. 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 analysis. This will be discussed for inclusion in future ring tests.

6.2.2 Macroalgae Biomass Module (OMB RT03)

A number of observations may be made from the results of the exercise which have been summarised below:

1. Despite the artificial nature of the sample material, the test has been generally well accepted by all laboratories with positive comments on points of possible improvements. All samples arrived in good condition and apart from some extensive drying times the tests were considered quick and easy.
2. Materials used in the samples are acceptable but will be improved where possible.
3. This year all laboratories managed to complete both wet and dry weights for all samples, however there is still a question over the necessity to incorporate dry weights within the ring test. Although many in house field procedures do not incorporate dry weight of algal samples these values are included within NMBAQC scheme to enable analysis of laboratory procedures. The values provide evidence of insufficient rinsing of samples, whereby the dry weight would be considerably higher than the actual dry weight. Also there is no definite wet weight from which to compare the individual laboratories submissions so it is difficult to conclude which results are the most representative. The dry weight however can be compared directly with the original weight of the samples which was measured very accurately prior to addition of debris. Most laboratories submitted dry weight values that were considered well within an acceptable limit of the actual biomass; however wet weight still remains highly variable. Therefore the level of squeezing still remains an issue within the overall procedure and should be addressed. During subsequent ring tests, all laboratories

should continue to complete the full exercise even if it is not part of their routine monitoring.

4. Two laboratories contributed much of the variation by having large outliers. The differences in sample processing have become evident through the degree of variation in the results submitted. There needs to be a greater level of consistency in the methodology utilised for both rinsing and squeezing of samples and documented in a Standard Operating Procedure to be distributed to all laboratories involved in such practices.
5. In total six results were flagged as "Fail" when using Z-scores based on sample means; these were split between two labs only, all others being within +/- 1.0 Z-score.
6. There may be future requirements to include biomass analysis within a workshop to further discuss processing procedures and levels of intensity for manual removal of debris and water.
7. A number of laboratories submitted results to a lesser degree of accuracy than others. It is stipulated that both wet and dry weights be provided to 2 decimal places where possible. This will highlight smaller variations in weight as the samples are relatively small compared with some field samples. An agreement needs to be made on the most applicable number of decimal places, prior to the next exercise, to ensure all laboratories are content with, and follow, the methodology.

6.2.3 Macroalgae Identification Module (RT06)

1. Seven laboratories subscribed, but only six returned results, with a total of 11 individuals taking part. The majority of participants submitted results within the designated timescale, but not all. In subsequent years reminders will be sent close to the submission deadline.
2. There were 13 errors at genus level, 6 of these for the same taxon. There were 21 errors in total at species level. 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 may be targetted in future exercises. This represented a good performance by participants.
3. There were still quite a few incorrect spellings, showing participants were not showing sufficient care.
4. There was some disagreement as to the correct identification of *Epicladia flustrae*, with several participants calling it *Pseudendoclonium dynamenae*. After some discussion and consideration of the keys and descriptions, it was decided to accept either identification as correct.
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 and up to date nomenclature.
6. Although there was generally 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. In some instances the specimen photographs would have benefited further from a scale and some

details of habitat, general location, exposure of shore, height present on shore etc. This additional information will be suggested for inclusion on subsequent tests to allow accurate identification and reduce error or confusion.

RM RT06 Preliminary Results Bulletin March 2012
Wells, E., 2012. National Marine Biological Analytical Quality Control Scheme- Ring Test Bulletin- RM RT06. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

OMB RT03 Preliminary Results Bulletin March 2012
Wells, E., 2012. National Marine Biological Analytical Quality Control Scheme- Ring Test Bulletin - OMB RT03. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

OMC Seagrass RT03 Preliminary Results Bulletin year April 2012
Wells, E., 2012. National Marine Biological Analytical Quality Control Scheme- Ring Test Bulletin - Seagrass OMC RT03. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

RM RT06 Final report April 2012
Wells, E., 2012. National Marine Biological Analytical Quality Control Scheme- Macroalgae Identification Component RM RT06. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

OMB RT03 Final Report April 2012
Wells, E., 2012. National Marine Biological Analytical Quality Control Scheme- Macroalgae Biomass Component Report - OMB RT03 2012. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

OMC RT03 Seagrass Final report April 2012
Wells, E., 2012. National Marine Biological Analytical Quality Control Scheme- Macroalgae and Seagrass % Cover Component Report - OMC RT03 2012. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

7. Epibiota component

Contract Manager: Matt Service, AFBI
Component Administrator: Ian Sotheran, Envision

In year 18 the NMBAQC looked into the development of some training tools, e.g. a video or image library for everyone to use. It has been recognised from feedback at previous workshops that such a resource would be beneficial as a QA procedural tool with more longevity and broader availability than occasional workshops. There was potentially a PhD student who was going to investigate this matter, but his project was too different to combine with this. HBDSEG regarded development of Epibiota Video QA and automated Biotope Classification as priority areas but unfortunately due to constrained funding and time no further progress was made. It is clear that both additional funds and dedicated personnel are required to take on these epibiota proposals and help develop the component.

Appendix 1: NMBAQC Co-ordinating Committee – Year 18 - 2011/2012

Name	Organisation	Position
Tim Mackie	Environment & Heritage Service, NI	Chair
Amanda Prior	Environment Agency	Finance Manager
Myles O'Reilly	Scottish Environment Protection Agency	Invertebrate Contract Manager
Steve Coates	Environment Agency	Fish Contract Manager
Joe Silke/ Rafael Salas	Marine Institute, Ireland	Phytoplankton Contract Manager
Clare Scanlan	Scottish Environment Protection Agency	Macroalgae Contract Manager
Carol Milner	APEM Ltd	Contractors Representative
Gavin McNeill/ James Strong	Agri-Food and Biosciences Institute	Epibiota Contract manager
David Hall	Unicomarine	Invertebrate, Particle Size and Fish Components Administrator
Keith Cooper/ Claire Mason	Centre for Environment, Fisheries & Aquaculture Science	CMA Representative
Mark Charlesworth	British Oceanographic Data Centre	CMA Representative
Lucie Skates/ Rob Cooke	Countryside Council for Wales	CMA Representative
Jessika Haapkylä/ Milly Hatton-Brown	Sir Alister Hardy Foundation for Ocean Science	Technical Secretary

Appendix 2: NMBAQC scheme participation for Year 18

ORGANISATION	Fish	PSA	Invertebrate	Phytoplankton	Macroalgae
Agri Food Biosciences Institute	✓	✓	✓		
APEM Ltd	✓		✓	✓	
Aristotle University of Thessaloniki, School of Biology, Dept of Botany, Greece				✓	
Benthic Solutions Limited		✓	✓		
Biotikos Limited			✓		
CEFAS - Lowestoft	✓	✓	✓	✓	
Centro Balear de Biologia Aplicada, Spain				✓	
CMACS Ltd		✓	✓		
CountrySide Council for Wales			✓		✓
EA Marine Monitoring Service	✓				
Ecospan Environmental Ltd			✓		
EMU Ltd.	✓	✓	✓		✓
Environment Agency	✓		✓		✓
Fisheries and Aquatic Ecosystems Branch				✓	
Fish Vet Group (Environment Dept)			✓		
Fugro Environmental Taxonomy Lab			✓		✓
Fugro ERT	✓	✓	✓		
Gardline Environmental Limited		✓	✓		
Grontmij Nederland B.V., Team Ecologie, the Netherlands			✓		
Hebog Environmental Ltd			✓		
Hunter Biological			✓		
ILVO (Institute for Agricultural and fisheries Research)			✓		
IMARES B.V., the Netherlands			✓	✓	
Institute of Estuarine & Coastal Studies	✓	✓	✓		
INTECMAR, Spain				✓	
IRTA, Spain				✓	
Isle of Man Government Laboratory				✓	
IZOR, Croatia				✓	
Jacobs UK Ltd			✓		
Koeman en Bijkerk B.V., the Netherlands				✓	

ORGANISATION	Fish	PSA	Invertebrate	Phytoplankton	Macroalgae
Laboratorio de Control de Calidad de los recursos pesquoros, Spain				✓	
LexEcology	✓				
LVCC Palmones, Spain				✓	
MARILIM GmbH, Germany					✓
Marine Ecological Surveys Ltd			✓		✓
Marine Farm Services, Shetland Seafood Quality Control Ltd (SSQC Ltd)			✓		
Marine Institute, Ireland				✓	
Marine Invertebrate Ecological Services			✓		
Marine Scotland - Science		✓	✓	✓	
Monitor Taskforce, Netherlands Institute of Ecology, the Netherlands			✓		
Myriad Taxonomy			✓		
National Laboratory Service		✓			
Northern Ireland Environment Agency (NIEA)	✓	✓	✓	✓	✓
OBIONE			✓		
Precision Marine Survey Ltd			✓		
Scottish Association for Marine Science (SAMS)				✓	
SEPA (South)	✓	✓	✓	✓	✓
SOI Ltd (previously SERG:ES)			✓		

Appendix 3: Marine Fish ID course, Dove Marine Laboratory: 5-7 April 2011

Tuesday 5th April

0900hrs Arrival & delegate packs etc.
0930hrs Introductions, H&S, structure of the course - Steve Coates (Environment Agency).
1000hrs NMBAQC fish ring-tests - Jessica Taylor (Thomson Unicomarine)
1030hrs Introduction to UK Marine Fish fauna – Peter Henderson (PISCES Conservation Ltd).
1130hrs Fyke net monitoring, followed by deployment in Cullercoats Bay - Steve Coates.

1245hrs lunch.

1330hrs Common inshore/estuarine fish species encountered – Peter Henderson.
1630hrs Beam trawl monitoring, followed by practical demonstration in Cullercoats bay - Steve Coates.
Finish @ 1730hrs

Wednesday 6th April

0900hrs Coffee
0930hrs Small juvenile fish – Peter Henderson.
1200hrs Fyke net recovery, followed by fish ID.

1245hrs lunch.

1330hrs Introduction to British Gobies, – Peter Miller (Snr. Research Fellow, Bristol University).
1500hrs How to identify gobies – Peter Miller
1630hrs WFD Fish monitoring.- Steve Coates
Finish @ 1730hrs

2000hrs – Workshop Dinner, Hanahana, 45, Bath Lane, Newcastle, NE4 5SP :-
http://www.hanahananewcastle.com/

Thursday 7th April.

0900hrs Coffee
0930hrs Seine net monitoring practical demonstration in Cullercoats bay - Steve Coates
1100hrs Flatfish – Peter Miller..

1245hrs lunch.

1330hrs Pipefish – Peter Miller
1600hrs Finish & depart