NE ATLANTIC MARINE BIOLOGICAL ANALYTICAL QUALITY CONTROL SCHEME

Annual Report 2016/2017

A report prepared by the NMBAQC Scheme Coordinating Committee – Nov. 2017

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This Annual Report provides synopsis of the scheme year's activities over 2016/2017, the 23rd year of the NMBAQC scheme. 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 2016-2017 on 18th April 2016, 8th November 2016, and 6th February 2017. The minutes of the meetings are on the NMBAQC web site **http://www.nmbaqcs.org/reports/.**

Committee Membership for 2016/2017 is shown in Appendix 1.

1 Scheme Review

The scope of the NMBAQC scheme continued to develop in 2016/2017 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 the UK CSEMP (Clean Seas Environment Monitoring Programme). Under the UK Marine Monitoring and Assessment Strategy (UKMMAS) the NMBAQC scheme coordinating committee reports to the Healthy and Biologically Diverse Seas Evidence Group (HBDSEG).

All components followed a similar format to the previous year and involved training and testing exercises for the Invertebrate, Particle Size, Fish, Phytoplankton and Macroalgae components and the first official ring test for the Zooplankton component. Administration of the macroalgae component went out for new tender and this was awarded to Wells Marine.

The 2016-2017 participation level in the NMBAQC scheme 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: David Hall, Apem Ltd.

2.1 Summary of activities

Forty-three laboratories participated in the Benthic Invertebrate Component of the NMBAQC Scheme in 2016 / 2017 (year 23). Sixteen of the participants were Competent Monitoring Authorities (CMAs) and twenty-seven were private consultancies, one of which 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.

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

- 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.
- Own Sample module (OS) - re-analysis by APEM Ltd. of three samples supplied by each of the participating laboratories;

The format and analytical procedures of the various modules were the same as for 2015 / 16 (year 22) of the Scheme. Protocols for the 2016/17 RT, LR, and OS modules were placed in the scheme website in June 2016. A new summary report for the Laboratory Reference module was introduced for the first time, in agreement with participating labs, which allows access to all the results of the module.

Laboratory Reference (LR): Ten laboratories signed up for the LR21 module but only six laboratories submitted specimens for confirmation. Previously the LR results for each individual lab were returned to that lab only. This year a new LR Module Summary report was introduced which enabled participants to view the taxa sets submitted by other labs and the taxonomic edits and comments provided by the component administrator. It is anticipated that this will prove of some value, especially in relation to the submission and review of unknown or problem taxa.

A benthic invertebrate Taxonomic Workshop was held in October 2016 at the Field Studies Council, Millport, Isle of Cumbrae. The workshop programme focussed on the polychaete families Paraonidae and Spionidae (see Appendix 3).

2.2 Summary of results

Two Ring Tests (RT), each of 25 specimens, were distributed (RT51 and RT52). The second (RT52) was targeted on bivalves.

The results for the ring tests were in general comparable with those from previous exercises, with an average of 4% generic and 8.4% specific differences across the participating laboratories in RT51 and 4% generic and 5.9% specific differences across the participants in RT52.

For RT51, the average numbers of differences per participating laboratory (for a total of 20 laboratories with 22 submissions) were 4 generic differences and 8.4 specific differences. Eight taxa (three annelids, three molluscs, one crustacean and one cnidarian) were responsible for almost two thirds (65%) of the specific differences.

For RT52, the average numbers of differences per participating laboratory (for a total of 21 participants) were 4.0 generic differences and 5.9 specific differences. Four taxa (*Scrobicularia plana, Cerastoderma edule* – 2 circulations at different sizes – and *Nucula nucleus*), all circulated as small sizes, were responsible for almost half (46%) of the specific differences.

In RT51, several of the most significant differences (e.g. for *Tanaissus danica, Terebellides shetlandica* and *Vitreolina antiflexa*) were the result of lack of knowledge of literature and recent taxonomic work (citations were provided in the bulletin). Others (e.g. for *Paramphitrite birulai, Ecrobia ventrosa* and *Nematostella vectensis*) were due to inherent difficulties in recognition of identification features for the species. The high error rate for *N. vectensis* is notable as this is a protected and non-native species. The majority of RT52 (and some RT51) differences were due to the inadequacy of identification keys for small bivalves (circulated sizes were included in RTB52). Growth series are often required for these and some were provided in the bulletins but it is ultimately the responsibility of participants to maintain their own reference collections.

Only six labs submitted material for the LR exercise. Most misidentifications were for Annelida (56%), followed by Mollusca (30%) and Crustacea (14%); many belonged to genera which are either speciose, or for which the taxonomy has yet to be finalized. In addition, changes were made to taxonomic resolution, recording notation and spelling for many specimens. A summary of results from this module is presented in the Laboratory Reference Module Summary Report – LR21. Some of the differences resulted from policy changes and recent literature and workshop outcomes (e.g. *Syllis parapari, Dipolydora saintjosephi, Owenia borealis, Vitreolina antiflexa*). The submitted specimens also included several species that cannot yet be named and may be undescribed (e.g. *Sphaerosyllis cf. taylori, Scolelepis squamata* type 1, *Melinna sp., Cochliopidae* species A). The taxonomic resolution and recording policy differences were used to revise and standardize the notes made on such differences in future exercises (see report), with a view to the later development of a taxonomic discrimination protocol.

There were 84 samples submitted for the Own Sample module, including the seven processed by the Scheme's external auditor. Of the 84 samples, 72 (86%) exceeded the 90% Bray-Curtis Pass mark and 62 (74%) of the samples exceeded 95% BCSI. Since the beginning of this module in Year 02 of the Scheme, 79% of the samples received have exceeded the 90% Bray-Curtis Pass mark. Twenty of the 32 participating laboratories achieved a Bray Curtis of >90% ('pass' flag) for all three of their Own Samples this year. Overall, 73% of the comparisons were considered to have passed the enumeration of taxa standard, 83% exceeded the enumeration of individuals standard and 86% passed the Bray-Curtis comparison standard (>90%). All the laboratories with 'Poor' or 'Bad' sample flags have been provided with specific recommendations of remedial actions to quality assure their Own Sample data sets. Performance with respect to the biomass standard was generally good with 80% of the samples with submitted biomass values meeting the required standard.

2.3 Issues and recommendations

The majority of participating laboratories submit data / samples in accordance with the Scheme's timetable. Late submissions, however, are still the major contributing factor for delaying the production of exercise bulletins / reports. Also, the number of samples in data sets provided for selection of Own Samples varied considerably with several laboratories offering relatively few samples for audit selection, plus residues were not

always retained for re-analysis. Duplicate date from contractors/CMAs has been submitted. Where contractors submit data and samples belonging to CMAs the contractor should declare this so that audit results can be shared accordingly and CMA data audit can be tracked and co-ordinated. Data submission forms will be revised to show this.

There were continued problems associated with the measurement of biomass for individual species in the Own Sample module. In this and previous Scheme years, several laboratories, despite using blotted wet weight biomass techniques, rendered some of their specimens too damaged to be re-identified. Additionally, some laboratories had erroneous results where it appeared that biomass had been estimated or mis-transcribed. There were some instances (OS & LR modules) of specimens being provided in vials / containers that were not airtight and, as a consequence, specimens were dry and in some case identification was impossible. Participants are reminded that specimens should be stored in suitable air-tight containers so that viability is maintained for the audit process

The maintenance of a comprehensive reference collection has numerous benefits for improving identification ability, maintaining consistency of identification between surveys and access to growth series material. The LR exercise can be used as a means of verifying reference specimens. Participants submitting data for laboratory reference exercises should add a note on habitat / location of sample to aid identification

Participants submitting data for the ring test exercises should attempt to identify the specimen / specimens to species and complete the 'confidence level' section of their ring test datasheets to enable additional information to be gathered regarding the difficulty of ring test specimens.

The Own Sample module has shown repeated taxonomic errors for some laboratories over several years. Participating laboratories are encouraged to redress or resolve disagreements for taxonomic errors reported in their Own Samples even if their samples achieve an overall 'Pass' flag. There are still some problems of individuals and taxa missed at the sorting stage of Own Sample analysis. This is an area that is often the major contributing factor in samples with 'Fail' flags or low Bray-Curtis similarity indices. When taxa and individuals are missed during the extraction of fauna from the sediment, laboratories should determine why certain taxa have not been extracted. This could be due to the taxon not being recognised as countable, or due to problems with the effect of stains upon the specimens. Additional training may be required and a review of existing extraction techniques and internal quality control measures may be beneficial. Remedial action should concentrate on the specific causes of the failure and should be targeted accordingly e.g. analyst or method related discrepancies.

A detailed taxonomic discrimination policy (TDP) needs to be developed and added to the processing requirement protocol (PRP) to ensure that macrobenthic data from multiple analysts are as consistent and inter-comparable as possible. It has been noted that some laboratories are producing data with an atypical number of over-cautious identifications and multiple taxa recorded for a single species, which will lead to data

comparison issues for spatial and temporal studies. The Own Sample pass / fail criteria will be reviewed to ensure that they are fit for purpose and uphold data consistency between the Scheme participants.

2.4 Reports

Ring Test Protocol 2016-2017

Milner, C. and Hall, D.H., 2016. Benthic Invertebrate component – Ring Test Protocol. Report to the NMBAQC Scheme participants. 5pp, June 2016

Laboratory Reference Protocol 2016-2017

Milner, C. and Hall, D.H., 2016. Benthic Invertebrate component – Laboratory Reference Protocol. Report to the NMBAQC Scheme participants. 5pp, June 2016

Own Samples Protocol 2016-2017

Milner, C. and Hall, D.H., 2016. Benthic Invertebrate component – Own Sample Protocol. Report to the NMBAQC Scheme participants. 5pp, June 2016

Own Sample Interim Report Review and Remedial Action Processes

Hall, D.J., 2016. Benthic Invertebrate component – Own Sample Interim Report Review and Remedial Action Processes. Report to the NMBAQC Scheme committee and participants. 5pp, June 2016

RTB51 – Oct 2016 (General/Mixed taxa)

Milner, C., Worsfold, T., Hall, D. & Pears, S., 2016. NE Atlantic Marine Biological Analytical Quality Control Scheme. Ring Test Bulletin: RTB#51. Report to the NMBAQC Scheme participants. APEM Report NMBAQC RTB#51, 39pp, Oct, 2016.

RTB52 – Targeted, Bivalvia - Mar 2017

Worsfold, T., Hall, D. & Pears, S., 2017. NE Atlantic Marine Biological Analytical Quality Control Scheme. Ring Test Bulletin: RTB#52. Report to the NMBAQC Scheme participants. APEM Report NMBAQC RTB#52, 33pp, Mar, 2017.

Own Sample Module Summary Report OS62, 63 & 64 – May 2017

Hall, D. 2017. NE Atlantic Marine Biological Analytical Quality Control Scheme. Own Sample Module Summary Report OS62, 63 & 64. Report to the NMBAQC Scheme participants. 16pp, May 2017.

Review of recording and identification policy differences

Worsfold, T.M., Hall, D.J., 2017. Review of recording and identification policy differences in Benthic Invertebrate Component exercises (OS, LR, MB) for Scheme Operation 2014 - 2016 (Years 21, 22, 23). Report to the NMBAQC Scheme committee and participants. 18pp, July 2017

Benthic Invertebrate Component Annual Report, 2016/2017 (Year 23)
Hall, D.J., Worsfold, T.M., and O'Reilly, M. (Ed.), 2017. Benthic Invertebrate Component Annual Report. Scheme Operation 2016/2017 (Year 23). A report from the contractor to the NMBAQC Scheme co-ordinating committee. 26pp, August 2017

3 Particle Size Analysis component

Contract Manager: Claire Mason, Cefas.
Component Administrator: Lydia Finbow and David Hall, Apem Ltd.

3.1 Summary of activities

The Particle Size (PS) module followed the format of 2015/16. A series of exercises involved the distribution of test materials to participating laboratories and the centralised examination of returned data and samples.

The Particle Size Own Sample (PS-OS) module, introduced in the 2014/15 Scheme year, followed the same logistical format as the previous year. The changes made to the reporting format for 2015/16 (Scheme year 22) were maintained for Year 23. The report compared primary and AQC sieve and laser data separately along with data merging accuracy and assessed whether a representative sample was supplied for reprocessing. The purpose of this module was to examine the accuracy of particle size analysis for participants' in-house samples. The Particle Size Own Sample module is a training / audit module. Participants' samples are re-analysed by the NMBAQC Scheme PSA contractor and the results are compared. PS-OS exercises receive a "Good" or "Review" flag for each element; a "Review" flag is provided with additional comments highlighting errors and areas for improvement.

Fourteen laboratories signed up to participate in the 2016/17 PS module exercises (PS60, PS61, PS62 and PS63); five were government laboratories and nine were private consultancies. Thirteen laboratories signed up to participate in the PS-OS module exercises (PS-OS07, PS-OS08 and PS-OS09); nine were government laboratories and four were private consultancies. One government laboratory had two Lab Codes to submit six PS-OS samples for AQC analysis.

3.2 Summary of Results

Fourteen laboratories subscribed to the PS exercises in 2016/17. For the first (PS60 and PS61) and second (PS62 and PS63) circulation all subscribing participants provided results.

The exercise reports show that the majority of participants follow the NMBAQC methodology for these exercises. Participant PSA_2305 used different methodologies as they do not have access to a laser, PSA_2304 followed an alternate method of sieving to 63 microns for exercise PS62 and PSA_2310 attempted laser analysis on exercise PS63 which consisted of gravel. All four exercises show that the sieve analysis (>1mm) undertaken by participants was generally in agreement even for those using alternative methods. The main causes for concern were found in the laser analysis. One participant (PSA_2309) did not re-scale laser data to 100% before merging with sieve

data for exercises PS61 or PS62. It was apparent in all exercises that required laser analysis (PS60, PS61 and PS62) that there were differences in results depending on which laser instrument was being used. The Coulter instruments had a greater measurement of sensitivity and were the only instruments capable of detecting particles below 11 phi. The results of the Coulter instruments also showed a much greater degree of similarity to each other than those using the Malvern machines. There were still slight differences detected between the participants using Coulter instruments. However these could be due to differences in the samples supplied to each lab, different sub-sampling, sample dispersion and/or sample presentation procedures being used.

Fourteen laboratories subscribed to the PS-OS module in 2016/17. Two of the fourteen lab codes (PSA-2316 and PSA_2317) belonged to the same participant to facilitate multiple PS-OS submissions due to the sub contraction of samples. One potential participant (PSA_2318) did not submit any own samples for reanalysis, but sent an email confirmation of their non-participation. Three participants (PSA_2315, PSA_2316 and PSA_2317) opted to use their PS-OS subscription for bespoke AQC of a project's data outside of the official Scheme as their data would not be ready in time to be reported within the routine timescales of the PS-OS module.

Laboratories generally provided workbooks with all the correct information. Seven participants (PSA_2302, PSA_2303, PSA_2306, PSA_2309, PSA_2312, PSA_2313 and PSA_2319) provided all necessary fractions of their sample for re-analysis, however the samples for PSA_2303 were considered by the AQC laboratory to be too small to be representative of sediment in the field. Participant PSA_2320 did not provide any laser sub-sample, therefore the dried < 1mm fractions were used for laser analysis but this required soaking for 48 hours to soften, before thoroughly mixing and subsampling for laser analysis. Participant PSA_2314 provided freeze dried bulk samples, but they did not supply any >1mm or <1mm fractions, even though gravel and whole shells were present in two of the samples. For the re-analysis the AQC lab wet-separated the bulk sample provided over a 1mm sieve and carried out the usual NMBAQC methodology. Participant PSA_2214 reported that they were only interested in the < 1mm fraction; therefore, although there was > 1mm sediment present in the samples it had not been analysed. Participant PSA_2314 were also not following the NMBAQC methodology, samples were instead freeze dried and screened over a 2mm sieve before being presented to the laser analyser. Participant PSA_2311 also used an alternate method, comments from the AQC lab were that the laser subsamples had been supplied in large bags which appeared to have been the original sample bags. It is possible therefore that the majority of the sediment had been removed for wet separation and sieving, leaving a small amount in the bag for laser analysis which might not be representative of the original bulk sample. It does not appear a separate laser subsample was taken from the bulk sample, after thorough mixing, as required under NMBAQC guidelines.

There was generally good agreement between the participants and the AQC results, particularly in terms of basic sediment textural classification. There were a few discrepancies in the sieve data but these are to be expected due to factors such as breakage of particles during repeat analysis and variations in sieving time and vibration

amplitude. The AQC analysis of a few samples found small amounts of material greater than 1mm in samples where participants had undertaken laser analysis only, therefore sieve and laser analysis should have originally been carried out, however these small amounts of greater than 1mm particles had minimal effect on the overall distribution of the sample and were usually deemed not materially significant. The majority of participants merged data correctly with only one participant not re-proportioning laser data to 100%; this had a knock-on effect on the final merged data. In some of the results there was a fair amount of variability in the laser data; some of this variability can be explained by differing laser instruments used by the AQC lab and participants. The Malvern Mastersizer 2000 and 3000 instruments do not have the same resolution as the Coulter LS13320, especially at the finer end; the Coulter uses a PIDS (Polarization Intensity Differential Scattering) system at the bottom end, rather than diffraction, so provides better sensitivity than the Malvern system which employs diffraction of two different wavelengths of light (red and blue). Often the Coulter system reports higher mud content than the Malvern machines and the distributions produced by the Malvern tend to be more smoothed, and less able to identify discrete size modes. The output size distribution from the Malvern instruments machines is very dependent on the diffraction pattern interpretation model used; this can be selected by the operator as "General Purpose, Unimodal, and Multimodal etc." and can give rise to uncertainty. There is no such specification requirement with the Coulter instruments.

3.3 Issues and recommendations

Additional analysis undertaken on the laser replicates and metadata provided revealed there was a great deal of variation and some major problems for the PS exercises. Using PS62 as an example, there was no consistency in whether red light alone or red and blue light were used by operators of the Malvern instruments and it was not clear which diffraction pattern interpretation model had been applied. Different laboratories apparently have used the multipurpose model, the uni-modal model, the bi-modal model and the poly-modal model, although in most cases this had not been specified. One participant has used the Fraunhofer optical model while others have apparently used the Mie model, but in the latter case most labs do not state the optical property values chosen. These factors are probably mostly responsible for the deviant laser distributions demonstrated by a number of participants. A few participants queried results and asked for additional replicates to re-analyse. It is not always obvious why a result appears to be different without detailed laser metadata. This is an issue that needs to be addressed before the next scheme year.

As in previous years, the PS-OS module raised issues over the interpretation of the methodology set out in the NMBAQC Best Practice Guidelines (Mason, 2016), in particular how the laser analysis is undertaken. These guidelines, originally written in 2011, were based on the widespread use at that time amongst participants of Malvern Instruments laser diffraction instruments that have 15 – 25 second standard run times and generally are restricted to the analysis of material < 1mm in size. The original methodology suggested that:

1. A homogenised sub-sample of approximately 100ml is taken from the bulk sample for laser analysis (Laser Pot).

2. A small representative sub-sample is taken from the Laser Pot and passed over a 1mm sieve using as little water as possible (Replicate 1).
3. Replicate 1 is then run through the laser at the desired obscuration, producing three run results.

Steps 2 and 3 are then repeated to create Replicates 2 and 3, giving a final result of 9 runs to create the final laser data, the average of these 9 runs. The completion of nine analyses, and subsequent merging of results is necessarily a time consuming process, especially if standard run times longer than 15 to 25 seconds are used (e.g. 60 seconds is standard with Beckman Coulter instruments (if the PIDS system is activated), which are used by some NMBAQC Scheme participants).

It has been demonstrated by KPAL that, for the vast majority of samples, there is little practical benefit in routinely carrying out analysis of three replicate sub-samples if samples are homogenised properly both before the laser sub-sample is taken from the bulk sample and when the test sample is taken from the laser sub-sample, and the sample is adequately dispersed prior to presentation to the instrument. In relatively rare instances where samples consist very largely of > 1mm size material and it is impractical to obtain a representative laser sub-sample from the bulk sample, more consistent laser results can be obtained by taking a laser sub-sample from the wet separated < 1mm fraction of the sediment, rather than from the bulk sample.

3.4 Reports

PS60 August 2016

Finbow, L. & Hall, D., 2016. National Marine Biological Analytical Quality Control Scheme. Particle Size Results: PS60. Report to the NMBAQC Scheme participants. Apem Report NMBAQCps60, 36pp, August 2016.

PS61 August 2016

Finbow, L. & Hall, D., 2016. National Marine Biological Analytical Quality Control Scheme. Particle Size Results: PS61. Report to the NMBAQC Scheme participants. Apem Report NMBAQCps61, 37pp, August 2016.

PS62 January 2017

Finbow, L. & Hall, D., 2017. National Marine Biological Analytical Quality Control Scheme. Particle Size Results: PS62. Report to the NMBAQC Scheme participants. Apem Report NMBAQCps62, 36pp, January 2017.

PS63 January 2017

Finbow, L. & Hall, D., 2017. National Marine Biological Analytical Quality Control Scheme. Particle Size Results: PS63. Report to the NMBAQC Scheme participants. Apem Report NMBAQCps63, 36pp, January 2017.

PSA Component Annual Report Year 23 (2016/17)

Finbow, L, Pye, K. and Hall, D. Particle Size component - Report from the contractor. Scheme Operation - Year 2016/2017. A report to the NMBAQC Scheme co-ordinating committee. 25pp, May 2017.

4 Fish component

Contract Manager: Jim Ellis, CEFAS.

Component Administrator: Sarah Hussey (until July 2016), Karina Jakobsen and Ruth Barnich (after July 2016), Thomson Unicomarine.

4.1 Summary of activities

This component consisted of two official 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 twenty-second year of the Scheme.

Fish Reverse Ring Test (F_RRT):

The identification of a set of fifteen fish species selected and supplied by the participating laboratories was relatively accurate (F_RRT08) (13 differences for 245 specimens submitted). The majority of specimens were collected by fish teams during their 2016 autumn monitoring surveys. There was a range of families where differences in identification occurred, including the Clupeidae (herrings), Mugilidae (grey mullets) and Gobiidae (gobies). The grey mullet and gobies were the main families where differences occurred. Each had three individuals incorrectly identified and one uncertain or unknown specimen.

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

Fish Ring Test (F_RT):

Fifteen fish specimens were distributed by Thomson Unicomarine Ltd. This Fish Ring Test (F_RT10) produced good agreement between the identifications made by the participating laboratories and those made by Thomson Unicomarine Ltd. On average, each laboratory recorded 0.4 generic differences and 0.6 specific differences, which is an improvement on last year's results.

4.2 Summary of results

Fish Reverse Ring Test (F_RRT):

In the majority of instances, identifications made by Thomson Unicomarine Ltd. were in agreement with those made by the participating laboratories with thirteen differences occurring from two hundred and forty-five identification submissions. Most identification issues were associated with grey mullets and gobies, with misidentifications between *Chelon labrosus*, *Liza aurata* and *Liza ramada*, and between

Pomatoschistus microps, *Pomatoschistus minutus* and *Pomatoschistus pictus*. Three out of the forty-two goby specimens submitted by participating laboratories were incorrectly identified. Identification issues with these taxa have been observed in previous years. There were also discrepancies for species such as bull rout, topknot, scad, reticulated dragonet, tub gurnard and herring. Potentially difficult taxa such as the gobies and grey mullets could be specifically targeted in future fish ring tests (F_RT exercises) to quantify and resolve problems via the circulation of standardised specimens.

Fish Ring Test (F_RT):
This is the tenth fish ring test circulated through the NMBAQC Scheme and the results were comparable with those from the nine previous exercises 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 to 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_RT10 indicated that most laboratories are using the same literature to identify fish specimens (Wheeler 1969, 1978; Maitland & Herdson 2009; Henderson 2015). Ring test specimens were sent to participating laboratories frozen. Frozen specimens tend to maintain their integrity and preserve colour better than those preserved in alcohol.

4.3 Issues and recommendations

Two samples identified as tub gurnard by the participants were submitted as part of the reverse ring test (F_RRT08). One of which was identified as a Piper (*Trigla lyra)* by Thomson Unicomarine Ltd, the other confirmed as Tub Gurnard (*Chelidonichthys lucerna)*. Piper is similar in appearance to the tub gurnard but tends to have a more offshore distribution (Figure 1).

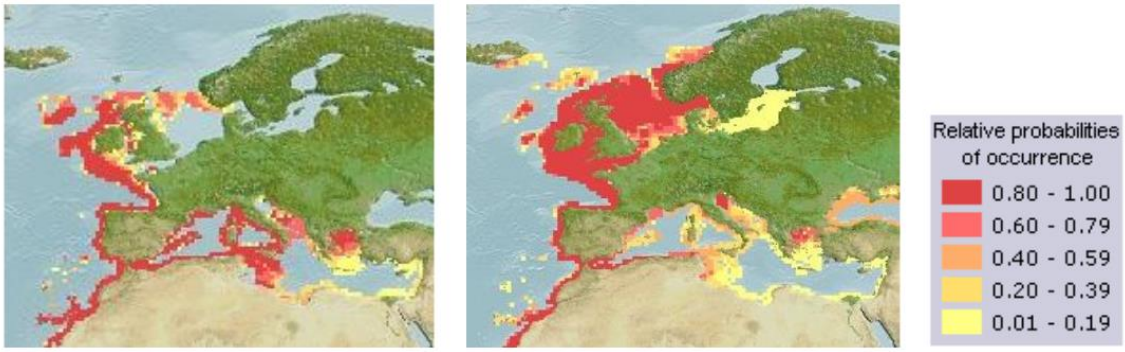

Figure 1: Potential distribution of piper (left) and tub gurnard (right). From www.aquamaps.org, version of Aug. 2016. Accessed 29/03/2017.

Piper are found typically in deeper waters, but can be caught closer to shore in areas close to deeper water. Some of the key factors in determining between the two species are the head profile and the length of the cleithral spine, above the pectoral fin. The cleithral spine is long in piper (extending backwards to the middle of the pectoral fin), and the snout of piper is produced into two flattened and spiny lobes (Wheeler, 1969; Whitehead et al., 1986). The dorsal colouration of piper is often bright red.

Whilst not an issue this year, previous Fish Ring Tests have highlighted instances of differences due to the incorrect translation of a common name. Fish teams are to incorporate scientific names in field data records and/or ensure that common to scientific name translations are correct prior to database submission.

Recurring differences have been highlighted in the identification of grey mullets(*Liza aurata*; *Chelon labrosus* and *Liza ramada*) and gobies(*Pomatoschistus microps*; *Pomatoschistus minutus* and *Pomatoschistus pictus*) in all reverse ring test exercises. These groups could usefully be targeted at workshops or in future ring test exercises. Future Fish Ring Test (F_RT) circulations will target taxa identified in the Fish Reverse Ring Tests (F_RRT) as potentially problematic.

4.4 Reports

FRT 10 March 2017

Jakobsen, K., 2016. NE Atlantic Marine Biological Analytical Quality Control Scheme. Fish Ring Test Bulletin: FRT#10. Report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQCfrtb#10, 20pp, March 2017.

FRRT 08 - March 2017

Jacobsen, K., 2017. National Marine Biological Analytical Quality Control Scheme. Fish Reverse Ring Test: FRRT08. Final report to the NMBAQC Scheme participants. Thomson Unicomarine Report NMBAQC FRRT08, 11pp, March 2017.

Fish Component Annual Report, Year 2016/2017

Jakobsen, K., 2017. Fish component - Report from the contractor. Scheme Operation - 2016/2017. A report to the NMBAQC Scheme co-ordinating committee. 16pp, March 2017.

5 Phytoplankton component

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

5.1 Summary of activities

The phytoplankton component is undertaken by the Marine Institute (Ireland) in collaboration with the IOC Science and Communication Centre on Harmful Algae Denmark (and in association with the NMBAQC, UK). Previously this component undertook intercomparison exercises under the BEQUALM banner. However, as the BEQUALM programme closed in 2014, these exercises were renamed in 2016 as IPI (International Phytoplankton Intercomparison).

Participants undertake Identification and Enumeration exercises on three preserved 50ml marine water samples which have been spiked with cultured material. They also take part in an online Harmful Algal Bloom (HAB) quiz where they are required to identify planktonic algae from photos or diagramms. Each year the exercises are followed by workshop with discussion of the exercise results and additional presentations on phytoplankton issues (see Appendix 4).

82 analysts from 43 laboratories took part in the 2016 intercomparison exercise. 81 analysts returned sample results and 79 completed the online HAB quiz. There were 69 participants from laboratories across Europe, 5 from South America, 2 in Australia, 1 in New Zealand and 5 in Africa.

Ten species were used in the Identification and Enumeration test. These were the dinoflagellates *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen, *Prorocentrum triestinum* J.Schiller, *Karenia selliformis* A.J.Haywood, K.A.Steidinger & L.MacKenzie, *Karlodinium veneficum* (D.Ballantine) J.Larsen, *Dinophysis acuta* Ehrenberg and the diatoms *Pseudo-nitzschia australis* Frenguelli, *Guinardia delicatula* (Cleve) Hasle, *Chaetoceros didymus* Ehrenberg, *Coscinodiscus wailesii* Gran & Angst and *Thalassiosira gravida* Cleve.

The cell counts of the species *Karlodinium veneficum* which did not past the minimum requirements for homogenization and stability were discounted for statistical purposes and also *Karenia selliformis* which did not preserve well in the samples was not used here. All the other species counts were used.

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 and stability test. ISO 13528 is only valid for quantitative data. The consensus values from the participants was used.

This year there was a mixture of dinoflagellates and diatoms in the samples and also a mixture of toxic and non-toxic species. The samples had 5 dinoflagellates (if we count *K.selliformis*) and 5 diatom species, although at the end only 8 species had to be identified. There was also 4 toxic species in the samples. However as mentioned before lugol's preservation caused problems with *K.selliformis* and *K.veneficum* did not homogenise properly in the samples giving poor repeatability between analysts. These 2 species were disregarded for statistical analysis.

5.2 Summary of results

All measurands passed the F-test except for *K.veneficum*. Only *A.ostenfeldii* passed the homogeneity test according to ISO13528 but they all passed the expanded criterion except for *K.veneficum*. The stability test was passed by 5 out of the 9 measurands but failed *K.veneficum, D.acuta, T.gravida* and *P.australis*. All measurands passed the stability test according to the expanded 13528:2015 except for *K.veneficum*.
The consensus values new Standard deviation (STD) was used for all measurands regardless of the Pass/Fail flags from the homogeneity test. There were a small number of action signals across all measurands. 9 Red flags in total (1.4% of results), 22 (3.4%) yellow flags and 6 (0.93%) orange flags (Non-Ids) from 648 scores is evidence of good performance overall. Eight analysts did not pass the full test with a below 80% score. There is evidence of method bias on low cell density measurands due to the volume analysed.

The Ocean teacher online HAB quiz results suggests a high rate of proficiency. 68% of analysts achieved a score over 90% (Proficient). Another 21.5% of analysts above 80%, 8% between 70 and 80% and 2.5% needs improvement.

There was good consensus on the various identifications of diatom species from images in questions 1 to 3. Although the images of *T.mobiliensis* and *C.densus* were the most difficult organisms to identify from these images, results suggest a good performance overall. In Questions 4 to 6, there were good overall marks on flagellate identification based on depictions. Q7-9 Good scores on Peridinioid terminology but difficulties with the lesser known Suessiaceae group. Q10-12 Problems identifying *T.macroceros* group (Q10) worst score (68.8% correct). Q12-15 Theory based on 1' and 2a plate for identification of *Protoperidinium* is understood but difficult to execute using images.

5.3 Reports

Phytoplankton Identification and Enumeration Ring Test, 2016

Salas, R.G., Larsen, J., 2016. International Phytoplankton Intercomparison profiency test in the abundance and composition of marine microalgae 2016 report. PHY-ICN-16_MI1 VR 1.0. 126 pp.

6 Macroalgae component

Contract Manager: Clare Scanlan (until 1st Feb 2017), Scottish Environment Protection Agency. Claire Young (after 1st Feb 2017), DAERA-NI.
Component Administrator: Emma Wells, Wells Marine.

6.1 Summary of activities

The format for 2016 -17followed that of the previous year.

The component consisted of three modules:

- **Rocky Shore Macroalgae Ring Test (RM - RT)**: - Identification of twenty macroalgae species based on a series of images.
- **Opportunistic Macroalgae Biomass Ring Test (OMB - RT)**: - synthetic samples of different weights for washing and drying to both wet and dry weights.
- **Opportunistic Macroalgae/Seagrass Cover Ring Test (OMC-RT)**:- estimation of percentage cover of opportunistic macroalgae and seagrass based on photographs of field quadrats.

The analytical procedures of all modules were the same as for the previous year of the Scheme.

Rocky shore Macroalgae (RM-RT11) Identification of intertidal macroalgae

Six laboratories subscribed to the macroalgae ring test with all six laboratories submitting results with a total of fifteen participants. Four of the subscribing laboratories were government organisations and two were independent consultancies.

This is the eleventh macroalgae identification ring test as circulated through the NMBAQC scheme.

Biomass of macroalgae (OMB-RT08)
This is the eighth year in which biomass of macroalgae has been included as an element of the NMBAQC scheme and was included as a single exercise. The format followed that of previous years. Test material was distributed to participating laboratories from which data forms were completed with algal biomass results and returned for analysis. Ten laboratories were issued with test material. Nine laboratories completed the macroalgae biomass component of the NMBAQC scheme, one laboratory failed to submit any results. All of the participating laboratories were government; no private consultancy took part in this component of the macroalgae exercises.

Cover of macroalgae & seagrass (OMC-RT08)
This is the eighth year in which % cover estimations of macroalgae have been included as an element of the NMBAQC scheme and the sixth year for which seagrass has been assessed as a separate entity. This included a single exercise for macroalgae and one for seagrass both of which were split into three smaller tests (A, B & C) based on methodology. The format followed that of previous years. Test material was distributed to participating laboratories from which data forms were completed with macroalgae and seagrass % cover results and returned for analysis. Fourteen laboratories were issued test material. Twelve laboratories completed the % cover macroalgae/seagrass exercises with a total of 40 participants. Of those laboratories submitting results, all ten were government organisations. There was no limit on the number of participants per lab. Laboratories could complete the % cover test that best represented their own methodology. However, the laboratories were encouraged to complete all three test variations of both the macroalgae and seagrass exercises to facilitate comparisons of the methods.

6.2 Summary of results

Identification of intertidal macroalgae (RM-RT11)
Although the results were broadly comparable with those of previous years there is a noticeable decrease in the level of agreement between participating laboratories and the AQC. As per previous years the test included a number of cryptic and taxonomically challenging species as well as those considered more common. Such genera included *Ulva* sp. and *Porphyra* sp. which are notoriously difficult to identify to species level. *Gelidium* sp. can also been easily misidentified due to confusions with other morphologically similar genera such as *Chondria* sp. and in general it is very difficult to tell these species apart from each other. These genera require an increased depth of knowledge on the cellular attributes, which can be remarkably similar between species, as well as other characteristics, such as overall texture, which can be used to separate such species.

No one participant managed to identify all species and genera correctly and there were only 5 species for which all laboratories were successful in their identification, 4 fewer

than for RT10. The most problematic species were *Ulvella viridis, Gelidium pulchellum* and *Myriotrichia clavaeformis* which may be considered relatively difficult to identify due to the occurrence of morphologically similar species and genera or their microscopic nature, making them less commonly found and identified. With an increased number of misidentifications, it could be concluded that this test was slightly more difficult than previous tests so has little reflection on the level of competency of the participants since the pass rate was lower across all participants.

Biomass of macroalgae (OMB-RT08)

A single test consisting of three biomass samples was distributed. This year each sample consisted of a different synthetic material including j-cloths, wool and synthetic stuffing material. These are currently considered the most representative materials in terms of imitating the overall look and feel of various opportunist macroalgae species. Cloths and wool were cut to different lengths and sizes to represent different foliose and filiform taxa (e.g. *Ulva*).The synthetic stuffing is considered to be more representative of finer opportunist algae such as *Ectocarpus* sp. and *Chaetomorpha* sp. Each sample was contaminated with debris and sediment of a sandy-muddy nature consistent with the substrate type known to support opportunist macroalgal blooms. Results for wet weight of biomass varied between laboratories with some laboratories producing high measures of biomass compared against the average biomass and actual/expected biomass. The dry weights showed a similar level of variability. One laboratory failed to remain within the Z-score limit of +/-2.0 for the average sample dry weight, there was also one 'Fail' for wet weight against the mean despite the high standard deviation caused by the high range of results. Three further laboratories showed significant deviation from the actual sample dry weight with a further five 'Fails' against wet weight, this means of assessment is not as accommodating towards outliers. Sample A had the greatest number of 'Fails' when comparing wet weight against 'expected' wet weight, this may be in part due to the low standard deviation due to the small size of sample material. Four laboratories had dry weights lower than that of the actual dry weight suggesting minor losses of material during the rinsing process, however in most cases this loss was very minimal and had limited effect on the overall results.

Cover of macroalgae & seagrass (OMC-RT08)

a) Macroalgae Exercise Results

Test A (open quadrat) was undertaken by 29 participants and was the most popular of the three methods. The range of results per quadrat varied considerably with the largest range of results produced for quadrats 5, 6 and 14 with ranges of 27%, 26% and 35% respectively. The remaining quadrats had ranges between 10 and 23; these ranges are slightly lower than for the same test last year. Z-scores calculated against the population mean resulted in nine laboratories failing between 1 and 6 quadrats. In total there was a 95% pass rate for test A when using Z-scores derived from the mean which is consistent with previous years' results. Although the number of 'Fails' produced when calculating Z-scores against image analysis participants showed an average % cover deviation from image analysis % cover ranging between 3.25% and 10.28%. The deviation from mean % cover was very similar ranging between 2.21 and 10.52. However, the pass rate was substantially lower for image analysis z-score at only

80% with 24 out of 29 participants failing at least one quadrat. These results were also consistent with those from RT07 with similar pass rates.

Test B (5 x 5 gridded quadrat) had the least number of participants with 12. As with test A there was a greater degree of correlation of % cover against population mean compared with the image analysis. A total of 75% of participants (9 out of the 12) consistently produced Z-scores of less than 2.0, which is regarded as a 'pass'. Two of the remaining labs 'failed' 1 quadrat each and the remaining lab 'failed' a total of 9 quadrats. The largest range of % covers per quadrat was a range of 23% (for quadrats 1 and 14) and 24% (for quadrats 4 and 5). The remaining quadrats had ranges between 6% and 22%. As seen in test A also, these ranges were also slightly lower than previous years. The lowest range of % cover estimates were for quadrat 7 which had a % cover range of 6. Consistent with test A, test B also showed a higher degree of deviation from the image analysis results compared with the population mean, with 11 out of 12 participants failing at least one quadrat and an overall pass rate of only 71% compared with a pass rate of 94% using Z-score from the population mean although this result is consistent with last year(RT07).The greatest number of 'Fails' could be attributed to quadrat 7, with 10 'Fails' followed by quadrat 15 with 8 'Fails'. For 11 out of 12 participants the levels of deviations stayed under 5% when calculated against the mean. The range of deviation was broader with image analysis with more participants resulting in greater than 5% deviation.

Test C (9 x 9 crosshairs quadrat) had a total of 24 participants who opted to use the 100 square method with varying levels of deviation from the population mean. Although not the preferred method this year, Test C had a high number of participants, which was consistent with previous years. The results verified that as with the other two test methods there was a higher degree of deviation when comparing results against the image analysis % cover as opposed to the population mean. The average range of percentage covers per quadrat was 22.4%, lower than for RT07, but higher than for tests A and B. Two quadrats had % cover ranges above 30% (34.3 for quadrat 14 and 32.3 for quadrat 4). Of the remaining quadrats, 8 had ranges between 20% and 30% and 5 between 10% and 20%. The range of results submitted for test C was higher than for tests A and B. Six participants failed at least between 1 and 4 quadrats with an overall pass rate of 96%. There were also more 'Fails' using Z-scores from image analysis with 13 participants failing 1 quadrats and a further 7 'failing' between 2 and 9 quadrats and an overall pass rate of 87%. Quadrat 13 had the greatest number of 'Fails' with 16 out of the 24 participants scoring higher than +/-2.0, the remaining quadrats had between 1 and 5 recorded 'fails' except quadrat 10 which has no 'fails'.

b) Seagrass Exercise Results

Test A (open quadrat) consisted of 35 participants and as with the macroalgae this was the most popular method. The range of results submitted per quadrat also varied considerably but overall were much higher than for the macroalgae test. The largest range was for quadrats 9 and 13 with % and quadrat 12 with 55% and as in previous years the greatest range of results were recorded for those quadrats with the mid range of % cover. The lowest range of results were for quadrats 4 and 10 with 20% cover ranges. Z-scores calculated against the population mean resulted in 9

participants failing between 1 and 5 quadrats. In total there was a 96% pass rate for test A when using Z-scores derived from the mean. When comparing results against % cover as calculated using ImageJ, the number of 'Fails' per laboratory was greater with a total number of 49 'Fails'. However, the overall pass rate was 91% with 15 participants 'passing 'all quadrats. This is a considerably better result than for RT07. Those quadrats with the highest number of 'Fails' were quadrats 2 and 15 with 9 and 8 'fails' respectively. The average deviation of results were similar between image analysis (3.26 to 16.38) and mean % cover (2.04 to 14.67). These results were consistent with previous ring tests.

Test B (5 x 5 gridded quadrat) had the least number of participants with a total of 14 participants opting to complete the 5 x 5 square grid quadrat method, resulting in varying levels of deviation from the population mean. This test followed the same trend as the other tests for both macroalgae and seagrass with comparisons against image analysis resulting in a greater number of failures using the Z-score than when comparing against mean % cover. The range of % cover values were considerably lower than for test A with all quadrats having % cover ranges in the order of between 10% and 40%. Quadrat 15 had the largest range of between 14% and 54%. Quadrat 4 has the smallest range of just 10% between 2% and 12%. Comparing % covers against the mean resulted in just 8 'Fails' distributed between 2 labs and an overall pass rate of 96%. In comparison, the total number of 'Fails' using image analysis was higher at 21and was distributed among all 12 of the 14 participants. The overall pass rates using image analysis % cover was 90%. These results are considerably better than the previous year. The overall deviation from the mean quadrat % cover and that calculated by image analysis was also very similar with a deviation from the mean ranging from 2.66% to 13.49% and deviation from image analysis ranging from 3.87% to 11.35. This was also an improvement from RT07.

Test C (9 x 9 crosshairs quadrat) had a total of 20 participants. The % cover ranges for test c were also consistent with those from Tests A and B with most quadrats having a % cover range between 10% and 40%. However, quadrat 15 had a % cover range between 16% and 67% proving to be consistently the most problematic quadrat within all test methods. Comparison of results against the mean resulted in 19 'Fails' with 5 participants 'failing' between 1 and 6 quadrats and an overall pass rate of 94%. Comparing results against the image analysis resulted in 36 'Fails' with pass rates of 88% with all 7 participants passing all quadrats. Most 'Fails' against image analysis could be attributed to quadrat 2 which had a total of 10 participants failing. Although quadrat 15 had the largest range of % cover estimates this did not result in a high number of 'fails' due to the large standard deviation. Deviation from image analysis % cover was much higher than for the other two test methods with a range of between 3.53 and 22.77.

6.3 Issues and recommendations

Identification of intertidal macroalgae

Certain issues arose with a few species. *Ulvella viridis* was unidentified by a couple of participants while other misidentifications could be attributed to both incorrect genera

and species. It is not commonly recorded in routine monitoring due to its epiphytic nature and may be easily confused with other microscopic epiphytic green algae. Its main distinguishing features include cell size, shape and length as well as cell content. *Gelidium pulchellum* was confused for various species including *Pterocladia capillacea* and *Chondria* sp. as well as with other *Gelidium* species. All the incorrect identifications could be considered incredibly morphologically similar and with such overlapping characteristics it was necessary to look closely at the branching patterns and shape of terminal branches as well as the width of the frond. In the case of *Gelidium pulchellum* one of the most distinguishing features is its association with *Corallina officinalis* on which it is known to be growing epiphytically, this could be seen in the in-situ photos. *Myriotrichia clavaeformis* was misidentified by several laboratories for *Elachista fucicola*, these two species can be distinguished by their multiseriate and uniseriate fronds respectively, but also by the host species on which they grow with *Myriotrichia clavaeformis* characteristically found on *Scytosiphon lomentaria and Elachista fucicola* on *Fucus* sp.

Biomass of macroalgae

Despite the artificial nature of the sample material, the test has been generally well accepted by all laboratories with constructive comments on points of possible improvements. Most samples arrived in good condition and apart from some extensive drying times the tests were considered quick and easy. One sample had become slightly mouldy by the time the participant the started the test, this has been the first instance of this occurring and all attempts will be made to ensure the samples arrive quickly and in good condition. It seems there is now a general agreement that the use of artificial material to mimic algae is an acceptable surrogate for the test albeit less fragile and easier to rinse and squeeze than the real thing. This is the second year in which synthetic stuffing has been used to mimic much finer opportunist algae such as *Pilayella* and *Chaetomorpha*. It is appreciated that the use of synthetic materials do not fully represent the conditions experienced within the field. It may be possible in the future to utilise alternative materials that may be more representative of the texture and general nature of opportunist algae but at this stage alternative materials have not been tested with the same success rate.

This has been the first year in which each sample has consisted of a different artificial material which has enabled a better comparison against actual macroalgae samples. Due to the mixed opinions on which material is the most representative all three materials will continue to be used for future tests or until a more realistic alternative is sourced. However, it was suggested that each sample be a combination of all three materials to ensure they were difficult enough to wash and closer to actual sampling conditions. This year all laboratories submitting results managed to complete both wet and dry weights for all samples, however some participants still question the necessity to incorporate both dry and wet weights within the ring test. Although many in-house field procedures do not incorporate dry weight of algal samples these values are included within the NMBAQC scheme to enable comparison 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. In addition, some laboratories only measure the dry weight therefore, for such an exercise to be appropriate for such laboratories; this measure of biomass needs to remain within the test.

It has been suggested that more mud be added to the samples to enable a more realistic comparison with field procedures. There are further suggestions that more *Hydrobia* could be added to the sample or material to mimic *Hydrobia*. This is definitely something that will be considered and applied for future tests.

It is evident that the larger samples create a greater margin of error with far less consistency between laboratories. However, it has been suggested that these samples are more appropriate in terms of representing natural conditions. This will be taken on board when compiling future tests whereby they will be aimed at including a good range of weights but focusing on some much larger biomass weights.

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. However, if this is not feasible for some laboratories then measurements to the nearest gram are also acceptable but it needs to be recognised by participating laboratories that such measurements will be less accurate particularly with smaller sample sizes. In the instance where the dry weight recorded is less than the actual weight this may be an indication of loss of material but may also be linked to the accuracy of the scales. It is recommended that all laboratories use calibrated scales so as to reduce such minor discrepancies.

The differences in sample processes 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 guidance procedures to be distributed to all laboratories involved in such practices. There are often a number of outliers which significantly skew the results and affect the average weight which is used to compare all other results. If this average is abnormally high or low it will affect the outcome of some laboratories results which might otherwise be considered acceptable. It has also been questioned whether the procedures of the test should be followed or those of the individual laboratory. The two methods may vary in terms of the amount of squeezing pressure applied to the sample. It is important that an individual laboratory has consistent results that are comparable from year to year. However if they are consistently higher of lower than other labs they may be under or overestimating the actual biomass, particularly with regards to wet weight, which may then be reflected in the overall classification of a

water body when applying the WFD blooming tool or any other quality status assessment.

Cover of macroalgae & seagrass

There is evidently still a high degree of difference between tests as well as between participants and this may prompt the need for a specific workshop whereby methods can 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 method A was the most favoured method for macroalgae and seagrass, albeit test B produced the most consistent results. There is still a high level of difference between z-scores calculated from the mean and z-scores calculated from image analysis results and given the varied levels of deviation between the two it is unclear which is the most accurate method from which to compare participants results. The image analysis method used during RT08 is considered more objective than skilled eye estimation and likely to produce a more accurate result; RT08 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 fully classified and ground truthed image analysis method, with more accurate results, it is recommended at this time that participants should use the Z-scores derived from comparisons with the mean if they are required for internal quality reports.

Following consultation with current participants, it has been agreed that the tests are being distributed at the most appropriate time of year for the majority of labs, with a longer time scale within which to complete the exercises. Therefore, tests will continue to be distributed early in the New Year with a time limit of 6 weeks. It will remain the responsibility of the laboratory to ensure all results are submitted within the time provided. It may be considered that during field sampling it may be possible to estimate % cover of opportunist algae with a higher degree of accuracy than when using photos. The nature of the photographs can produce difficulties when assessing the density of the algae and the presence of some shadows and the grids can hinder this further. This point has been highlighted by a couple of labs and in subsequent tests further efforts will be made to ensure this doesn't hinder the ability to accurately estimate the % cover. However, it is to be noted that many seagrass beds remain waterlogged regardless of tidal height and sun reflection may be a problem but all attempts will be made in the future to ensure clear photos are distributed with a broad range of % covers.

It was previously noted that when using the 9 x 9 cross hair method it was difficult to keep orientated when zooming in and out to check cross hair points, therefore it was recommended that a central grid in an alternative colour be place on both axis, thereby dividing the quadrat into four, to assist with the method. This was trialled within RT08 but one laboratory found the central orange cross hairs to be distracting. For the subsequent test, thinner orange lines will be trialled as a slight alternative. Many labs use a slightly alternative method of a 10 x 10 grid and counting the presence within in each square. This is a point worth discussion should a workshop be held. 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. 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. An alternative method has been suggested in which the quadrat is split into 4 equal cells. Although adding an additional test method at this stage may

not be favoured by many labs alternative methods will always be considered for inclusion in subsequent years. At this stage it may be recommended that a review of all laboratories methodologies be undertaken to ensure the most appropriate methods are being included within the ring tests.

It has been suggested that the data collated from the current and previous OMC ring tests be used to produce a set of standard sheets to 'normalise' surveyors results. This proposal will be considered and put forward for investigation. Due to the presence of some anomalies within the results submitted it is recommended that all laboratories review their data prior to submission. Such anomalies can skew the results and fail to recognise any small deviations from the mean; they can also cause the mean to be exceptionally high or low also affecting the outcome of other laboratories, but despite individual failures the overall pass rates are relatively high. In the future, such data may be rejected as outliers. Care should also be taken to ensure the results are in the correct format and page within the spreadsheets provided. It is requested that participants use the spreadsheets provided to submit results using the format provided. Each participants' results should be submitted on a separate sheet and exclude calculations. Where calculations or formulas are included there is greater chance of error when transferring data to a single spreadsheet and during subsequent data analysis.

6.4 Reports

RM RT11 Final report 2017

Wells, E., 2017. National Marine Biological Analytical Quality Control Scheme-Macroalgae Identification Module Report -RM RT11 2017. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

OMB RT08 Final Report 2017

Wells, E., 2017 National Marine Biological Analytical Quality Control Scheme-Macroalgae Biomass Module Report -OMB RT08 2017. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

OMC RT08 Final Report 2017

Wells, E., 2017 National Marine Biological Analytical Quality Control Scheme-Macroalgae Biomass Module Report -OMC RT08 2017. Report to the NMBAQC Scheme participants. Wells Marine Surveys.

7 Epibiota component

Component Administrator: Hayley Hinchen, JNCC.

7.1 Summary of activities

Discussions have been ongoing on developing a ring test for final guidelines for quality control. The last test by Envision was a few years back and NMBAQC had considered an in-house test before to reduce costs. However, other priorities have prevented this from happening. NMBAQC are currently considering a small ring test, e.g. have 10 images you need to identify or short videoclips, with arrows showing what needs to be identified. The test would run to standardise levels of identification and not forcing them. Another consideration is a workshop on habitat classification from given datasets.

8 Zooplankton component

Component Administrator: David Johns & Astrid Fischer, SAHFOS.

8.1 Summary of activities

A ring test containing 10 actual zooplankton specimens from the Northeast Atlantic, 10 written questions and a bead enumeration test were sent out in November 2016 to 19 participants from 12 laboratories. Participants were given 8 weeks to complete their test, and results were consequently judged by one of SAHFOS' senior taxonomists.

8.2 Summary of results

The competent monitoring agencies all achieved a level of at least 89% in both tests. For the specimen test, the most difficult to ID proved to be the invasive species *Pseudodiaptomus marinus*. For the written test the most difficult question was to specify the taxonomic order of the single celled organisms, *Spumellaria*. The participants enjoyed the test, saying that it challenged them and that it was gauged at the right level of expertise.

The bead enumeration test was considered to be challenging, but it was unclear to the participants how a species was defined. In future test it was recommended that the bead enumeration would have a taxonomic key that defined bead species. The results of the test did show that most analysts agree on the differentiation in the beads, however, for the individual scoring of participants the enumeration was not taken into account.

8.3 Issues and recommendations

For future ring tests it was recommended that the results of the tests get sent out before the workshop, so that participants can prepare better for the workshop and ask questions on the day. Other suggestions that were made were:

- Include juveniles of common copepods
- Include two of the same species
- Include more species in the specimens' test
- Include Echinodermata
- More focus on copepods other than Calanoida, include e.g. Cyclopoida
- Develop a taxonomic discrimination protocol (to which level should a species be taken)
- Include higher numbers in the enumeration test
- Have a two-day workshop with more time for participants' specimens
- Have better quality microscopes at the venue
- Hold the workshop at a venue that has better travel connections

All recommendations will be taken into account in the next zooplankton ringtest.

8.4 Reports

Zooplankton UK Ring Test 2016/2017

A. Fischer, M. Wootton and D. Johns, Zooplankton component - Zooplankton Ring Test. Report to the NMBAQC Scheme committee and participants. 39pp, August 2017

Appendix 1 - NMBAQC Co-ordinating Committee – 2016/2017

Name	Organisation	Position
David Johns	Sir Alister Hardy Foundation for Ocean Science (SAHFOS)	Chair
Tim Mackie	Department of Agriculture, Environment and Rural Affairs, Northern Ireland (DAERA-NI)	CMA Representative
Graham Phillips	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
Claire Young	Department of Agriculture, Environment and Rural Affairs, Northern Ireland (DAERA-NI)	Macroalgae Contract Manager
Grant Rowe	Fugro EMU Ltd	Contractors' Representative
Amy Ridgeway (until 23/5/16) Paul Whomersley (from 23/5/16)	Joint Nature Conservation Committee (JNCC)	Epibiota Contract Manager
Jim Ellis	Centre for Environment, Fisheries & Aquaculture Science (CEFAS)	Fish Contract Manager
Claire Mason	CEFAS	PSA Contract Manager
Keith Cooper	CEFAS	CMA Representative
Paul Brazier	Natural Resources Wales (NRW)	CMA Representative
Adele Boyd (to 6/2/17) Annika Clements (from 6/2/17)	Agri-Food Biosciences Institute, Northern Ireland (AFBI)	CMA Representative
Astrid Fischer	SAHFOS	Technical Secretary

Appendix 2 - NMBAQC Scheme – Component Participation for 2016/2017 (Participants from UK unless otherwise stated)

PARTICIPANT COMPONENT →	BENTHIC INVERTS	PSA	FISH	PHYTOPLANKTON	MACROALGAE	ZOOPLANKTON
AGQ PERU S.A.C, Peru				√		
Agri Food and Biosciences Institute (AFBI)	√	√	√	√		
APEM Limited	√	√	√	√	√	√
Aristotle University of Thessaloniki, Greece				√		
ARPA FVG, Italy				√		
ARPA Puglia - DAP Bari - U.O.S. Biologia delle Acque , Italy				√		
ARPA Puglia Dap Brindisi, Italy				√		
Benthic Solutions Limited	√	√				
Biofar, Faroe Islands	√					
Biologia delle Acque - DAP Taranto - ARPA Puglia, Italy				√		
Biotikos Limited	√	√				
Bureau Waardenburg – Koemen en Bijkerk bv, Netherlands	√					
Cawthron Institute, New Zealand				√		
Centre for Environment, Fisheries & Aquaculture Science (CEFAS)	√	√		√		√
Certificaciones del Peru S.A., Peru				√		
CMACS Limited	√	√				
Department of Agriculture, Environment & Rural Affairs (DAERA), Northern Ireland	√	√	√	√	√	
DHI Water & Environment Ltd, Singapore						√
Dipartimento Provinciale di Lecce - ARPA Puglia, Italy				√		
eCoast Marine Research, Netherlands	√					
Ecospan Environmental Limited	√					
Environment Agency (EA)	√	√	√		√	
Environmental Protection Agency, Ireland				√		
Estonian Marine Institute, Estonia					√	
Eurofins Aquasense, Netherlands	√					
Fish Vet Group Limited	√	√				
Fondazione Centro Ricerche Marine, Italy				√		
Food Safety and Veterinary Institute, Albania				√		
Fugro EMU Limited	√	√	√		√	√

PARTICIPANT COMPONENT →	BENTHIC INVERTS	PSA	FISH	PHYTOPLANKTON	MACROALGAE	ZOOPLANKTON
Gardline Limited		√				
Hebog Environmental Limited	√					
Hunter Biological & Sue Hamilton		√				
IFREMER, France				√		
ILVO (Institute for Agricultural and Fisheries Research)-ANIMALAB, Belgium	√					
IMARES, The Netherlands	√			√		
Institut National de Recherche Halieutique, Morocco				√		
Institut za oceanografiju i ribarstvo (IOR), Croatia				√		
Institute of Estuarine & Coastal Studies, (IECS), University of Hull	√	√	√			
Institute of Marine Biology, Montenegro				√		
Integrative Marine Ecology Department Stazione Zoologica Anton Dohrn Napoli, Italy						√
IPMA - Fitoplâncton Lab, Portugal				√		
IRTA, Spain				√		
Isle of Man Government Laboratory, Isle of Man				√		
Istituto Zooprofilattico Sperimentale delle Venezie , Italy				√		
Joint Nature Conservation Committee (JNCC)	√	√				
Kenneth Pye Associates Limited		√				
Koeman en Bijkerk bv, Netherlands				√		
Laboratorio de Control de Calidad de los Recursos Pesqueros, Spain				√		
LIENSS / CNRS, France				√		
Marine Ecological Surveys Limited	√					
Marine Institute, Oranmore/Bantry, Ireland				√		
Marine Invertebrate Ecological Services	√					
Marine Scotland Science - Marine Laboratory (MSS)		√		√		√
MEA-nl , Netherlands				√		
Microalgal Services, Australia				√		
Myriad Taxonomy	√					
Natural England (NE)	√	√				
Natural Resources Wales - Cyfoeth Naturiol Cymru (NRW)	√	√	√		√	
Nautica Environmental Associates, Abu Dhabi						√
NIEA - (DAERA Environment, Fisheries and Marine Group Laboratory)	√	√	√	√	√	

PARTICIPANT COMPONENT →	BENTHIC INVERTS	PSA	FISH	PHYTOPLANKTON	MACROALGAE	ZOOPLANKTON
Ocean Ecology Limited	√	√	√			√
Orbicon, Denmark				√		
Organismo Nacional De Sanidad Pesquera, Peru				√		
Plymouth Marine Laboratory				√		
Polo specializzazione Biologia avanzata Acque, Italy				√		
Precision Marine Survey Limited	√	√	√			
Rijkswaterstaat CIV, Netherlands	√					
Scottish Association for Marine Science (SAMS)				√		√
Seastar Survey Limited	√					
Scottish Environment Protection Agency (SEPA)	√	√	√	√	√	√
Shetland Seafood Quality Control (SSQC) Ltd	√					
Sir Alister Hardy Foundation for Ocean Science (SAHFOS)				√		√
Swedish Meteorological and Hydrological Institute (SMHI), Sweden				√		
Sydney Water, Australia				√		
Thomson Unicomarine Limited	√		√			
Wells Marine Limited					√	

Appendix 3 - NMBAQC Scheme Benthic Invertebrate Taxonomic Workshop

Field Studies Council – Millport, Isle of Cumbrae, October 2016

Day	Session	Discussion / Demonstration / Practical	Aims	Session Leader
Tuesday 11th Oct. 2016		Arrival. Registration. (From 8:30 AM to 10:00 AM)	Register participants.	Nic Pennisi (APEM Ltd.)
	10:00 AM	Introduction. General information.	Welcome participants. Q&A session regarding workshop. Outline timetable.	Nic Pennisi (APEM Ltd.)
	10:15 AM	Introduction — Millport Marine Station. Brief details. Local information. Lab. rules (H&S issues).	To give brief history of Millport and facilities. Areas of local interest. Pub guide.	Dr. Daniel Moncrieff (FSC, Millport)
	10:30 AM	Discussion / Demonstration - Introduction to selected Paraonidae. Literature. Problem areas. Identification techniques.	To introduce the major features / terminology used for identification of Paraonidae.	João Gil (Centre D'Estudis Avançats de Blanes)
	12:00 PM	Laboratory set-up.	Laboratory setup.	Nic Pennisi (APEM Ltd.)
	1:00 PM	**Buffet lunch.**		
	PM	Practical - Examination & identification of range of Paraonidae taxa from reference material.	To obtain identification experience. View / verify reference material.	João Gil (Centre D'Estudis Avançats de Blanes)
Wednesday 12th Oct. 2016	9:00 AM	Discussion / Demonstration - Introduction to selected Paraonidae. Literature. Problem areas. Identification techniques.	To introduce the major features / terminology used for identification of Paraonidae.	João Gil (Centre D'Estudis Avançats de Blanes)
	AM	Practical - Examination & identification of range of Paraonidae taxa from reference material.	To obtain identification experience. View / verify reference material.	João Gil (Centre D'Estudis Avançats de Blanes)
	1:00 PM	**Lunch.**		
	PM	Discussion / Demonstration - Introduction to selected Paraonidae. Literature. Problem areas. Identification techniques.	To introduce the major features / terminology used for identification of Paraonidae.	João Gil (Centre D'Estudis Avançats de Blanes)
	PM	Practical - Examination & identification of range of Paraonidae taxa from reference material.	To obtain identification experience. View / verify reference material.	João Gil (Centre D'Estudis Avançats de Blanes)
Thursday 13th Oct. 2016	9:00 AM	Discussion / Demonstration – Spionidae. Literature. Problem areas. Identification techniques.	To introduce the major features / terminology used for identification of Spionidae.	Vasily Radashevsky (Far Eastern Branch of the Russian Academy of Sciences)
	AM	Practical - Examination & identification of range of Spionidae taxa from reference material.	To obtain identification experience. View / verify reference material.	Vasily Radashevsky
	1:00 PM	**Lunch.**		
	PM	Discussion / Demonstration – Spionidae. Literature. Problem areas. Identification techniques.	To introduce the major features / terminology used for identification of Spionidae.	Vasily Radashevsky
	PM	Practical - Examination & identification of range of Spionidae taxa from reference material.	To obtain identification experience. View / verify reference material.	Vasily Radashevsky
Friday 14th Oct. 2016	9:00 AM	Discussion / Demonstration – Spionidae. Literature. Problem areas. Identification techniques.	To introduce the major features / terminology used for identification of Spionidae.	Vasily Radashevsky (Far Eastern Branch of the Russian Academy of Sciences)
	AM	Practical - Examination & identification of range of Spionidae taxa from reference material.	To obtain identification experience. View / verify reference material.	Vasily Radashevsky
	1:00 PM	**Lunch.**		
	2:00PM	History of NMBAQC scheme and role of the benthic invertebrate component contract manager	To provide an overview of the NMBAQC scheme since inception and introduce the contract manager to the group.	Myles O'Reilly (SEPA, NMBAQC BI Component Contract Manager)
	PM	Practical - Examination & identification of range of Spionidae taxa from reference material.	To obtain identification experience. View / verify reference material.	Vasily Radashevsky (Far Eastern Branch of the Russian Academy of Sciences)
	7:00 PM	**Workshop Dinner - Royal George Hotel, Millport**		
Saturday 15th Oct. 2016	9:00 AM	Workshop feedback. Equipment pack up.	Distribute / collect workshop feedback forms. Pack up equipment & prepare for departure	Nic Pennisi (APEM Ltd.)
	9:00 AM	Tea & coffee; Departure with packed lunch	-	-

Appendix 4 - IPI/NMBAQC Scheme Phytoplankton Taxonomic Workshop

Agenda 'International Phytoplankton Intercomparison' (IPI) workshop

Danhostel, Hillerød, Denmark. 27 Nov – 1 Dec 2016

	Morning 9.00-12.00	Afternoon 13.30-17.00
Sunday **27 Nov**		Arrival to Danhostel at 16.00, Light evening meal, sandwich
Monday, **28 Nov**	International phytoplankton intercomparison (IPI) exercise 2016 in abundance and composition of marine microalgae **Rafael Salas** and **Jacob Larsen** Ocean teacher online HABs quiz, exercise results **Rafael Salas** and **Jacob Larsen**	Development and Improvement of Standards in support of the Water Framework Directive. CEN mandate M/424- Work package 7: Guidance on the estimation of algal biovolume Dr. Claus-Dieter Dürselen Presentations by the participants: An unusual bloom of *Dinophysis acuta* in Scottish coastal waters linked to a change in diarrhetic shellfish toxin profiles **Sarah Swan** SAMS Biotoxin Monitoring in England and Wales **Charlotte Mitchell** Cefas
Tuesday, 29 Nov	Lecture and microscope demonstration: Dinoflagellates with focus on species of the ***Tripos***-group	Presentations by the participants: Habs Bulletin. The journey so far…

	Jacob Larsen and **Rafael Salas** 	**Tara Chamberlain** Phytoplankton Laboratory: Portuguese Institute for the Sea and Atmosphere **Alexandra Silva** Imaging FlowCytoBot (IFCB) Tångesund observatory. **Malin Mohlin** Microscopy of participants' samples / mixed samples
Wednesday 30 Nov	<u>Lecture and microscope demonstration</u>: Dinoflagellates with focus on ***Protoperidinium*** **Jacob Larsen** and **Rafael Salas** 	<u>Presentations by the participants</u>: Analysis of the potential impact of ocean acidification on the pelagic gastropod community in the North East of Scotland **Pablo Diaz** Fish mortality: Swamps of L'Houmeau **Christophe Arnaud** <u>Lecture and microscope demonstration</u>: Dinoflagellates with focus on ***Protoperidinium*** continue. **Jacob Larsen** and **Rafael Salas** 
Thursday 1 Dec	10 am, departure	