NMBAQC

The National Marine Biological Analytical Quality Control Scheme

Benthic Invertebrate Component Annual Report
Scheme Operation 2014/2015 (Year 21)

Author: Carol Milner, NMBAQCS Benthic Invertebrate Administrator

Reviewer: David Hall, NMBAQCS Project Manager

Approved by: Myles O'Reilly, Contract Manager, SEPA

Contact: nmbaqc@apemltd.co.uk

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BENTHIC INVERTEBRATE COMPONENT ANNUAL REPORT FROM APEM Ltd

SCHEME OPERATION – 2014/15 (Year 21)

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Linked Documents (hyperlinked in this report):

Ring Test Bulletin – RTB#47
Ring Test Bulletin – RTB#48
Macrobenthic Exercise Results – MB22
Own Sample Module Summary Report – OS56, 57 & 58
Description of the Scheme Standards for the Benthic Invertebrate Component
Guidelines for Processing Marine Macrobenthic Invertebrate Samples

1. Introduction

The National Marine Biological Analytical Quality Control (NMBAQC) Scheme addresses three main areas relating to benthic biological data collection:

- The processing of macrobenthic invertebrate samples;
- The identification of macrofauna;
- The determination of physical parameters of sediments.

Scheme year 2014/2015 (Year 21) followed the format of Year 20. A series of components, modules and exercises involved the distribution of test materials to participating laboratories and the centralised examination of returned data and samples. The labelling and distribution procedures employed previously have been maintained. Specific details can be found in previous Scheme annual reports.

Thirty-nine laboratories participated in the benthic invertebrate component of the NMBAQC Scheme in 2014/2015 (Year 21). Fourteen participants were Competent Monitoring Authorities (CMAs) and twenty-five were private consultancies. One of the participants was a consortium of sole traders. Seven of the CMA participants were responsible for the Clean Seas Environment Monitoring Programme (CSEMP) or Water Framework Directive (WFD) sample analysis. Laboratory Codes were assigned in a single series for all laboratories participating in the benthic invertebrate components of the NMBAQC Scheme. Separate Laboratory Codes were assigned for the particle size component laboratories.

As in previous years, some laboratories elected to be involved in limited aspects of the Scheme. CSEMP/WFD laboratories were no longer required to participate in all components of the Scheme.

In this report, performance targets have been applied for the OS module only (see Description of the Scheme Standards for the Benthic Invertebrate Component). These targets have been applied to the results from laboratories and 'Pass' or 'Fail' flags assigned accordingly. These flags are indicated in Table 1 of the Own Sample Module Summary Report – OS56, 57 and 58 (Year 21 OS Module Summary) presenting the comparison of laboratory results with the standards.

1.1 Summary of Performance

This report presents the findings of the Benthic Invertebrates Component for year 2014/2015 (Year 21) of the National Marine Biological Analytical Quality Control (NMBAQC) Scheme.

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

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

The analytical procedures of the various modules were the same as for Year 20 of the Scheme, which includes the specification that the Macrobenthic Sample module and CSEMP/WFD samples within the Own Sample module should be conducted using the NMBAQC guidance for macrobenthic invertebrate sample analysis (Worsfold, Hall & O'Reilly (Ed.) 2010). The results for each of the Scheme exercises are presented and discussed. Comments are provided on the performance for each of the participating laboratories in each of the exercises.

Two **Ring Tests (RT)** of 25 specimens were distributed (RT47 and RT48). Both sets contained 25 invertebrate specimens, the second (RT48) was targeted at the polychaete family Syllidae and similar taxa. A draft version of San Martin & Worsfold, 2015 was included with the circulation data sheets and protocol.

For RT47 each participating laboratory (a total of 20 participants) recorded on average 3.4 generic differences and 6.7 specific differences. Seven taxa (three annelids, two crustaceans, one mollusc and one echinoderm) were responsible for almost two thirds (64%) of the specific differences.

For RT48 each participating laboratory (a total of 18 participants) recorded on average 2.7 generic differences and 7.8 specific differences. Eight taxa (all syllids) were responsible for almost two thirds (64%) of the specific differences.

Laboratory Reference (LR): Five laboratories submitted their specimens for confirmation. Most misidentifications were found to be for Annelida, Gastropoda and Crustacea belonging to genera which are either speciose, or for which the taxonomy has yet to be finalized. The majority of taxonomic errors could be attributed to the submitted polychaetes (53%) and molluscs (18%).

Four laboratories signed up for the **Macrobenthic module (MB)** but the exercise was completed by only two laboratories. Analysis of the sample by the two participating laboratories and subsequent re-analysis by APEM Ltd. provided information on the efficiency of extraction of the fauna, accuracy of enumeration and identification and the reproducibility of biomass estimations. For MB22, natural marine samples from the south west coast of England were distributed. Results for this macrobenthic exercise showed an extraction efficiency (of individuals) was on average 96.95%. Comparison of the results from the laboratories with those from analysis by APEM Ltd. (following the NMBAQC macrobenthic analysis guidelines) was made using the Bray-Curtis similarity index (untransformed). The value of the index varied between 84% and 89% meaning both laboratories failed when Own Sample standards were applied. Both failures were due to identification differences which ranged from 10 to 12 total errors.

The revised protocols of Scheme Year 10 for 'blind' **Own Sample (OS)** audits were continued in this Scheme year. Laboratories were asked to submit full completed data matrices from their previous year's CSEMP/WFD, or similar alternative sampling programmes. The OS 'Pass/Fail' flagging system, introduced in Scheme Year 8, was continued (see Description of the Scheme Standards for the Benthic Invertebrate Component). In OS56-58, extraction efficiency was better than 90% in 83% of the comparisons and better than 95% in 71% of all comparisons. 100% of countable taxa were extracted from the sample residues in 48% of samples. No residue was submitted for checking in the case of two samples and residue had been discarded on the instruction of the client for a further two samples. The Bray-Curtis similarity index ranged from 52% to 100% with an average figure of 92%. The Bray-Curtis

similarity index was greater than 95% in 58% of comparisons and in 77% of cases the value of the index was greater than 90% and, therefore, achieved 'Pass' flags. Twelve samples (15%) achieved 'Pass-Excellent' flags with Bray-Curtis similarity scores of 100%.

1.1.1 Statement of Performance

Each participating laboratory was supplied with a 'Statement of Performance', which included a summary of results for each of the Scheme modules and details of the resulting flags where appropriate. These statements were first circulated with the Year 5 annual report (1998/1999) for the purpose of providing evidence of Scheme participation and for ease of comparing year on year progress.

2. Summary of Benthic Invertebrate Component

2.1 Introduction

There are four modules within the Benthic Invertebrate Component: Invertebrate Ring Test (RT), Invertebrate Laboratory Reference (LR), Macrobenthic Sample (MB) and Own Sample (OS) Modules.

Each of these modules is described in more detail below. A summary of their performance with respect to standards determined for the CSEMP/WFD is presented. A brief outline of the information to be obtained from each module is given, together with a description of the preparation of the necessary materials and brief details of the processing instructions given to each of the participating laboratories.

2.1.1 Logistics

The labelling and distribution procedures employed previously have been maintained and specific details can be found in the Scheme's annual reports for 1994/95 and 1995/96 (Unicomarine 1995 & 1996).

2.1.2 Data Returns

Return of data to APEM Ltd. followed the same process as in previous Scheme years. Spreadsheet based forms (tailored to the receiving laboratory) were distributed to each laboratory via email. All returned data have been converted to Excel 2010 format for storage and analysis. In this, and previous, Scheme years slow or missing returns for

exercises lead to delays in processing the data and resulted in difficulties with reporting and rapid feedback of results to laboratories. Reminders were distributed shortly before each exercise deadline.

2.1.3 Confidentiality

In July 2014 each participant was given a confidential, randomly assigned Scheme Year 21 LabCode. Codes are prefixed with the component initials, for example, BI for benthic invertebrates, the Scheme Year and a unique number (between 01 and 39) *e.g.* Laboratory number one in Scheme Year 2014/2015 (Year 21) was recorded as BI_2101. Laboratory codes, with a PSA_ prefix, were assigned separately for the particle size component (also administered by APEM Ltd.).

2.2 Invertebrate Ring Test (**RT**) Module

2.2.1 Description

The invertebrate ring test module is a training module which examines inter-laboratory variation in the participants' ability to identify fauna and attempts to determine if errors were the result of inadequate keys, lack of reference material or the incorrect use of satisfactory keys.

Two sets of 25 benthic invertebrate specimens were distributed in 2014/15. The first circulation (RT47) was a general invertebrate ring test and included 11 (44%) annelids, 7 (28%) molluscs, 6 (24%) crustaceans, and 1 (4%) echinoderms. The second circulation (RT48) was a targeted syllid ring test. This test included 24 (96%) syllids and 1 (4%) other annelids. Details of substratum, salinity, depth and geographical location were provided for all ring test specimens to assist identification.

2.2.1.1 Preparation of the Samples

The specimens distributed were obtained from a range of surveys from around the UK. Specimens were also donated by Scheme participants and other organizations. Every attempt was made to provide animals in good condition and of similar size for each laboratory. Each specimen was uniquely identifiable by means of a coded label and all material has been retained for subsequent checking. Where relevant, every effort was made to ensure all specimens of a given species were of the same sex. For both ring tests the

specimens were taken from replicate trawls, grabs or cores within a single survey and in most cases they were replicates from a single sampling station.

2.2.1.2 Analysis Required

The participating laboratories were required to identify each of the RT specimens to species level. If a laboratory had not routinely identified the specimen to species level, they were asked to state this in the 'confidence level' field. Laboratories could also add brief notes and information detailing the keys, or other literature used, to determine their identifications. Specimens were to be returned to APEM Ltd. for verification, resolution of any disputed identifications and potential reuse in future Scheme exercises. The implementation of this part of the Scheme was the same as in previous years. Participating laboratories were permitted to supply multiple data entries (*i.e*. different sets of results from different analysts) for each exercise to enhance the training value of this module. No laboratories chose to utilise this option in Scheme year 21. The protocols followed for the two circulations, in particular the method of scoring results, were the same as for previous circulations. Approximately six weeks were allowed for the analysis of both RT exercises (RT47 and RT48).

2.2.2 Results

2.2.2.1 General Comments

A number of laboratories use the ring tests for training purposes and have selected them preferentially over other modules. The results are not used to assign 'Pass' or 'Fail' flags. In total 21 laboratories subscribed to RT47 and RT48. For RT47, 20 laboratories returned data (20 individual data sets). For RT48, 18 laboratories returned data (18 individual data sets).

2.2.2.2 Returns from Participating Laboratories

Identifications made by the participating laboratories were compared with those made by APEM Ltd. to determine the number of differences. Where identification deviated from the APEM Ltd. identification due to the use of synonyms, or incorrect spellings of the name, the difference was ignored for the purpose of calculating the total number of differences.

Tables 1 and 2 of the Ring Test Bulletins (RTB) 47 and 48 show identifications made by each of the participating laboratories for the twenty-five specimens, arranged by specimen and by

laboratory respectively. For clarity, the name is given only in those instances where the generic or specific name given by the laboratory differed from the APEM Ltd. identification. Where it was considered that the name referred to the same species as the APEM Ltd. identification, but differed for one of the reasons indicated above, the name was presented in brackets: "[name]". A dash, "-", in the tables indicates that the name of the genus (and / or species) given by the laboratory was considered to be the same as the APEM Ltd. identification. A pair of zeros, "0 0", in the Tables indicates that the subscribing laboratory did not return data.

2.2.2.2.1 Scoring of RT Results

The laboratory's score was increased by one for each difference between their identification and the APEM Ltd. identification, *i.e*. for each instance where text other than a dash or a bracketed name appears in the appropriate column in the tables (Tables 1 and 2 in RTB47 and RTB48). Two separate scores were maintained for differences at genus and species level.

2.2.2.3 Ring Test Results

The intention of this training module is to discover where particular difficulties lie within specific common taxa. Results for Year 21 were presented in the Ring Test Bulletins (RTB) along with the reasons for each individual identification discrepancy. These bulletins contained images of the test material and the alternative, incorrectly recorded taxa, where these taxa where available. Participating laboratories were advised to retain their ring test specimens for a few weeks after receiving their results, in order that they could review their identifications, if necessary. Participants are encouraged to question APEM Ltd. identifications if they still believe their original identifications to be correct. On completion of each exercise, specimens were required to be returned to APEM Ltd. for potential future circulation.

2.2.2.3.1 Ring Test 47 (Type: General)

The results discussed below are given in Table 1 of RTB47 which displays the data arranged by species to enable quick reference to the range of answers received and in Table 2, which presents the results arranged by laboratory (see Ring Test Bulletin – RTB47).

Eleven of the 25 specimens circulated were annelids, seven were molluscs, six were crustaceans and one was as an echinoderm. The agreement at generic level was generally good; 67 differences, or 13% of all genus identifications, were recorded in the 20 data sets received from 21 participating laboratories. There was less agreement at species level, with 133 differences recorded, equal to 27% of all species identifications.

Seven of the specimens circulated were incorrectly identified at species level by almost two-thirds (61%) of the participants. These were the annelids *Loimia medusa*, *Pista mediterranea* and *Phyllodoce groenlandica*; the crustaceans *Tanaissus danica* and *Cymodoce truncata*; the mollusc *Abra nitida*; and the echinoderm *Ophiocten affinis*.

Five of the 25 specimens circulated, the molluscs *Turritella communis* and *Goodallia triangularis*, the annelids *Sabellaria alveolata* and *Paramphinome jeffreysii* and the crustacean *Photis longicaudata* were correctly identified by all participants.

Further details and analysis of results can be found in the Ring Test Bulletin RTB47 which was circulated to each laboratory that supplied results for this exercise and was also posted on the Scheme's website (www.nmbaqcs.org/RTB47).

2.2.2.3.2 Ring Test 48 (Type: Targeted on Syllidae)

The results discussed below are given in Table 1 of RTB48 which displays the data arranged by species to enable quick reference to the range of answers received and in Table 2 which presents the results arranged by laboratory (see Ring Test Bulletin – RTB48).

All 25 of the specimens circulated were annelids. The agreement at genus level was good; 49 differences, or 11% of all genus identifications, were recorded in the 18 data sets received from 18 participating laboratories. There was less agreement at species level, with 141 differences recorded, equal to 31% of all species identifications.

Five of the specimens circulated were incorrectly identified at species level by more than two thirds of the participants (72%). These specimens were *Erinaceusyllis c.f. belizensis, Parapionosyllis c.f. macaronesiensis, Prosphaerosyllis c.f. tetralix*, *Syllis variegata/alternata* and *Sphaerosyllis c.f. taylori*.

Two of the twenty-five specimens circulated (*i.e*. the polychaetes *Plakosyllis brevipes* and *Trypanosyllis coeliaca*) were correctly identified by all participants.

Further details and analysis of results can be found in the Ring Test Bulletin RTB48 which was circulated to each laboratory that supplied results for this exercise and was also posted on the Scheme's website (www.nmbaqcs.org/RTB48).

2.2.2.4 Differences between Participating Laboratories

Differences recorded at genus and species level for each of the participating laboratories are summarised in the graph related to Table 2 in RTB47 and RTB48 respectively. The laboratories are ordered by increasing number of differences at species level. The division of laboratories into three bands (Low, Medium and High) on the basis of the number of differences at the level of species is also shown.

2.2.2.5 Differences by Taxonomic Group

The total differences by taxonomic group for both exercises are shown below:

Taxon	**No. species**	**Generic differences**		**Specific differences**	
Polychaeta	36	78	67%	204	74%
Crustacea	6	21	18%	35	13%
Mollusca	7	5	4%	23	8%
Echinodermata	1	12	10%	12	4%
Total	50	116	100%	274	100%

Most of the specific differences in the two ring test exercises can be attributed to polychaete species followed by crustaceans, molluscs and echinoderms.

2.2.3 Discussion

The results were in general comparable with those from previous exercises, with an average of 3 generic and 7-8 specific differences across the participating laboratories. The RT component is considered a valuable training tool and can be an indicator of problem groups. It can highlight possible areas for further 'targeted' ring test exercises or for inclusion at

taxonomic workshops. The ability of participants to submit multiple data entries and the inclusion of images in the Ring Test Bulletins have enhanced the training value of this component. No participants chose to submit multiple datasets for the Ring Test exercises in this Scheme year. All participating laboratories have been made aware of the variety of problems encountered during these ring tests via Ring Test Bulletins RTB47 and 48, which also include a list of useful literature which they can then source. A draft version of the Syllidae key (San Martin and Worsfold, 2015) was included with the RT48 circulation e-mails and soon after the 'final' version of the key was made available, funded by the NMBAQC committee, on the NMBAQC website (San Martin and Worsfold, 2015).

The best results were obtained by BI_2101, BI_2108, BI_2112, BI_2129, BI_2107 and BI_2109 for RT47 with between zero and one differences at genus level and zero to three differences at species level. In RT48 the best participants were BI_2101, BI_2108, BI_2112, BI_2129, BI_2105 with zero differences at generic level and between one and five differences at specific level.

2.3 Invertebrate Laboratory Reference (**LR**) Module

2.3.1 Description

The Laboratory Reference Module is a training module which encourages laboratories to build extensive, verified reference collections to improve identification consistency. The value of reference material in assisting the process of identification cannot be over-emphasized; the creation and use of reference collections is viewed as best practice. Accordingly, the Laboratory Reference (LR) module of the Scheme was introduced in Scheme Year 3 (1996/97). This module can help assess the ability of participating laboratories to identify material from their own area, or material with which they are familiar. Laboratories are also able to use this exercise to verify identifications of difficult or problematic taxa about which they are unsure. Specimens were, wherever possible, representatives from CSEMP/WFD reference collections. This was the nineteenth Laboratory Reference exercise (LR19). The participants were able to submit up to 25 specimens for re-examination by APEM Ltd.

2.3.1.1 Preparation of samples

A prepared results sheet was distributed with the exercise's instructions and attached labels for the laboratories to identify each of the specimens. Participating laboratories were asked to prepare and submit their reference specimens within 6 weeks. All specimens were re-identified and the identification made by APEM Ltd. compared with that made by the participating laboratories. All specimens were returned to the laboratories after analysis.

2.3.2 Results

In total, nine laboratories signed up for this exercise (LR19) but only five laboratories submitted specimens for examinations. Detailed results have been separately reported to each of the participating laboratories. Misidentifications were usually found for polychaete, amphipod and gastropod mollusc species and belonging to genera which are either speciose or for which keys are inadequate. The majority of taxonomic errors could be attributed to the submitted polychaetes (57 %) and molluscs (18 %).

2.3.3 Discussion

In view of the different species that were sent by laboratories for identification it is inappropriate to make detailed inter-laboratory comparisons. Most laboratories elected to obtain a 'second opinion' on more difficult species.

2.4 Macrobenthic Sample (**MB**) Module

2.4.1 Description

The Macrobenthic Sample Module is a training module which assesses the participants' ability to process macrobenthic samples from the same habitat. In the case of MB22, natural marine samples from the South West of England were distributed in order to examine differences in sample processing efficiency and identification plus their combined influence on the results of multivariate analysis. In addition, an examination of the estimates of biomass made by each of the participating laboratories was undertaken.

2.4.1.1 Analysis Required

Each participating laboratory was required to carry out sorting, identification, enumeration, and biomass estimations of the macrobenthic fauna contained in the sample. Precise protocols were provided (see Appendix 1 of Macrobenthic Sample Results – MB22 and

Worsfold, Hall & O'Reilly (Ed.) 2010). The participating laboratories were required to complete a Macrobenthic Sample Details Form, which specified their processing methodology. The extracted fauna were to be separated, identified and stored in individually labelled vials. Labels were provided and cross-referenced to the recording sheets.

In addition, measurements of the biomass of the recorded taxa were requested. Detailed instructions were provided for this exercise; measurements were to be blotted wet weights to 0.0001g for each of the enumerated taxa.

Participants were asked to complete sample analysis within 6 weeks. All sorted and unsorted sediments and extracted fauna were to be returned to APEM Ltd., together with the data on counts and biomass determinations.

2.4.1.2 Post-return Analysis

Upon return to APEM Ltd., the various components of the MB samples were re-examined. All extracted fauna was re-identified and re-counted for comparison with the participating laboratory's own counts. The sample residues were re-sorted and any missed fauna removed, identified and counted. All fauna weighed by the participating laboratories were re-weighed to 0.0001g by the same APEM Ltd. individual, using a standard technique.

Prior to analysis of the differences found between the participants' and APEM Ltd.'s results, some minor adjustments were made to allow direct comparisons, *e.g.* separating / combining adults and juveniles, ignoring typing errors and name changes, in order to reflect a common identification policy and remove artificial differences in the data.

2.4.2 Results

2.4.2.1 General Comments

The distributed macrobenthic sample (MB22) was a natural marine sample from the South West of England. Two laboratories returned fauna and enumeration and biomass data for re-analysis. An interim report for this exercise was distributed to the participating laboratories and a final report was also posted on the Scheme's website (www.nmbaqcs.org/MB22).

2.4.2.2 *Efficiency of Sample Sorting*

Table 1 of the MB22 Report presents a summary of the numbers of taxa and individuals counted by each of the participating laboratories for sample MB22, together with the corresponding count made by APEM Ltd. after re-analysis. Comparison of the respective counts is given as a percentage. Table 2 shows the composition of fauna missed by each participating laboratory.

2.4.2.3 *Number of Taxa*

Column 5 in Table 1 (Estimation of taxa - % missed) shows the variation between the two laboratories in the percentage of taxa identified in the samples. Compared to the number of taxa found by APEM Ltd. one of the two laboratories (BI_2110) recorded fewer taxa (due to extra taxa being found in the residue and when the identification was checked), and the other laboratory recorded more taxa, the reason for this was due to mis-identifications and dead mollusc specimens being recorded as live by the laboratory. Column 4 of Table 5 shows the total number of taxa missed in the taxa pots and the residues when the samples were reanalysed by APEM Ltd.

The values presented for the number of individuals not extracted (Table 2) refer to taxa not extracted from the residue. Laboratory BI_2110 missed 6.1% of individuals overall, and Bi_2111 missed 0.3% of individuals.

2.4.2.4 *Number of Individuals*

Re-analysis of the sample residues showed that Laboratory BI_2110 missed 6.1% of individuals overall, and BI_2111 missed 0.3% of individuals (Table 2), showing the extraction efficiency of both laboratories was high.

2.4.2.5 *Uniformity of Identification*

Neither of the participating laboratories correctly identified all taxa (Table 1, column 7). Numbers of taxonomic errors ranged from 10 to 12 in total.

2.4.2.6 Comparison of Similarity Indices (Bray-Curtis)

The faunal list for each sample analysed by the participating laboratory was compared with the list after re-analysis by APEM Ltd. The comparison was made by calculating the Bray-Curtis similarity index (non-transformed) for the original and audit data. The results of this calculation are presented in Table 1. The BCSI values ranged from 84.47% to 89.91%, with an average value of 87.19%. The participating laboratories achieved 'Fail' sample flags under NMBAQC standards, if applied. Further details of each participating laboratory's performance are given in the MB22 report.

2.4.2.7 Biomass Determinations

A comparison of the biomass estimates made by the participating laboratories and APEM Ltd., broken down by major taxonomic group for the MB22 sample, is presented in Table 3. Both laboratories supplied biomass data. The average difference between the two total weight values was +9.8% (*i.e.* heavier than the weight values made by APEM Ltd.), with variable measurements by major faunal groups. The overall biomass percentage differences between participating laboratories and APEM Ltd. ranged from +5% to +14%. The average difference varied greatly between faunal groups, ranging from -3.14% to +38% (from others to echinoderms, respectively).

2.4.3 Discussion

Extraction efficiency (of individuals) was between 96.5 and 99.9%. Comparison of the results from the laboratories with those from analysis by APEM Ltd. (following the NMBAQC macrobenthic analysis guidelines) was made using the Bray-Curtis similarity index (untransformed). The value of the index varied between 84.47% (BI_2110) and 89.914% (BI_2111). The average BCSI of 87% for this natural marine sample is similar to the one achieved for MB10 (estuarine) and MB07 (coastal) which were 88% (see table below).

Summary of average Bray Curtis similarity indices achieved overall:

MB22 (fully marine)	87%
MB21 (estuarine)	98%
MB20 (intertidal)	93%
MB19 (fully marine)	92%
MB18 (artificial estuarine)	78%
MB17 (coastal)	93%
MB16 (estuarine)	95%
MB15 (coastal)	92%
MB14 (estuarine)	90%
MB13 (coastal)	97%
MB12 (estuarine)	77%
MB11 (artificial coastal)	93%
MB10 (estuarine)	88%
MB09 (coastal)	93%
MB08 (estuarine)	95%
MB07 (coastal)	88%
MB06 (estuarine)	91%
MB05 (coastal)	85%
MB04 (estuarine)	82%

The 'blot-drying' procedure employed by APEM Ltd. for the determination of biomass was as specified in the Green Book and the NMBAQC Processing Requirements Protocol (Worsfold, Hall & O'Reilly (Ed.) 2010), *i.e.* avoiding excessive pressure when blotting specimens dry. The estimates of total biomass made by the participating laboratories and APEM Ltd. show some variation with both laboratories recording weights an average of 9.79% greater than the audit weight. BI_2110 recorded weights on average, 14.28% greater than the weight recorded by APEM Ltd. As discussed in the reports for MB18 and MB19, it is difficult to see a pattern in the variance of biomass estimations. The main reason for the observed differences between the measurements is probably due to variable drying times utilised by laboratories prior to weighing.

2.5 Own Sample (**OS**) Module

2.5.1 Description

The Own Sample Module examines laboratory analytical performance on material from each participating laboratory's annual CSEMP/WFD or other sample analysis batches. Following a review of the Own Sample Module (Hall and Worsfold, 2001), several changes to sample selection and scoring were implemented in Scheme Year 8 (2001/02). All participants must meet these new Own Sample requirements. Own Sample participants must supply their previous year's CSEMP/WFD data matrices, where relevant, for Own Sample selection, *i.e.* 2013/2014 CSEMP/WFD data. This is to ensure that all processing is completed (prior to selection of samples for audit), preventing reworking of the selected Own Samples and enabling samples to be audited earlier in the Scheme year. Each participating laboratory was requested to send data from which three samples were selected. The selection was, in turn, notified to the laboratories. Laboratories responsible for CSEMP/WFD samples were advised to use these samples if possible, otherwise there was free choice, provided a minimum of twelve samples were included in the submitted data matrix.

2.5.1.1 Analysis Required

Participating laboratories were instructed to have conducted macrobenthic analysis of the samples using their normal procedures. A summary of these in-house sample processing procedures was to be provided, on a standard form, with each Own Sample. Samples requiring sub-sampling were to be avoided where possible. All procedures were to be documented and details returned with the sample components. All material from the sample was to be sent to APEM Ltd. broken down as follows:

- Sorted residue - material from which all animals had been removed and counted;
- Separated taxa - individually labelled vials containing the identified fauna; and
- Other fractions - *e.g*. material containing fauna which had been counted *in situ*.

Identification was to be to the normal taxonomic level employed by the laboratory (presumed to be usually species), except for CSEMP/WFD samples where the NMBAQC guidelines for macrobenthic sample analysis were to be followed (Worsfold, Hall & O'Reilly (Ed.) 2010). The names and counts of specimens were to be recorded on a matrix and linked to the vials through a specimen code number. Biomass analysis was to be carried out in the same manner as for the MB exercise.

Two weeks were allowed for the submission of data; and a further four weeks was allowed for the preparation and submission of the Own Samples selected for re-analysis. The sorted residue was re-examined and any countable material extracted. Identified fauna was checked for the accuracy of enumeration and identification and, in cases where biomass was provided by the participant, all taxa were re-weighed using the same procedure as for the MB exercise.

2.5.2 Results

2.5.2.1 General Comments

Following the request to participating laboratories to submit data of suitable samples for re-analysis, 93 selected Own Samples were received from 32 laboratories, together with descriptions of their origin and the collection and analysis procedures employed. Samples were identified as OS56, OS57 and OS58 and labelled with LabCodes. As would be expected, the nature of the samples varied considerably. Samples were received from estuarine and marine locations, both intertidal and subtidal. The sediment supplied for resorting varied from mud to gravel in various volumes of residue. The number of taxa per sample ranged from 1 to 206, with the number of countable individuals from 1 to 5550. Of the 93 submitted Own Samples, 12 had to be audited externally by Fugro EMU Ltd. due to the initial processing being carried out by APEM Ltd. Interim reports have been submitted to the participating laboratories. A summary of results from this module is presented in the Own Sample Module Summary Report – OS56, 57 & 58.

2.5.2.2 Efficiency of Sample Sorting

Table 1 of the OS Summary Report displays a summary of the data obtained from the OS analysis. All taxa identified and enumerated by the participating laboratory were included in the analysis, except in instances where the fauna had been damaged and rendered unidentifiable and uncountable. In 34 samples out of the total 93, the number of taxa recorded by the participating laboratories was identical to that obtained by the auditing laboratory (column 3). In the remaining 59 cases, the difference was on average 3.8 with a maximum of 21 taxa. Data for the numbers of individuals recorded (columns 16 and 17, Table 1) show a range of differences from re-analysis of 0% to 49%. The average difference between the samples with recorded differences was 8.2% (and 5% across the 93 samples), with 16 samples exceeding this average.

35 of the 93 samples reported showed 100% extraction of fauna from the residue (column 16), and in 58 samples between 1 and 453 individuals had been missed during processing. In just 13 of these samples only individuals attributed to taxa already recorded in the sample were found. In the other 45 samples new taxa, as well as individuals attributed to already recorded taxa were recorded. Numbers of previously unrecorded taxa found in the residue ranged from 1 to 21 with an average of 3.5 new taxa per sample. The poorest extraction records were a total of 21 missed taxa and 57 individuals and 11 missed taxa and 453 individuals. A breakdown of the missed individuals by taxonomic group is presented in Table 2 of the OS Summary Report. The average number (across all 93 samples) of missed individuals found upon re-sorting the residue was approximately 25, and the average number of missed taxa was 1.8.

2.5.2.3 Uniformity of Identification

Taxonomic differences between the auditor and participating laboratories' results were found in 56 (60%) of the 93 own samples. A summary of mis-identified taxa is presented in Table 3 of the OS Summary Report. An average of 7 taxonomic errors per laboratory was recorded; in the worst instance, 20 identification errors occurred. A large variety of samples (and fauna) was received. Polychaetes accounted for 45%, Mollusa and Crustacea for 17%, Others for 10%, Oligochaeta for 5% and Echinodermata for 2% of the taxonomic errors, with a variety of species responsible for these errors.

2.5.2.4 Comparison of Similarity Indices (Bray-Curtis)

The procedure for the calculation of the similarity index was as used for the MB exercise. The Bray-Curtis similarity index figures (Table 1, column 23) ranged from 52% to 100%, with an average figure of 92%. Twenty one samples from eleven laboratories achieved a similarity figure of less than 90%. Twelve samples produced a similarity figure of 100%; these were submitted by eight different laboratories (BI_2101, BI_2103, BI_2106, BI_2107, BI_2113, BI_2114, BI_2115, BI_2125). The best overall result was achieved by BI_2214 with 100% similarity across all three Own Samples. The lowest overall result was achieved by BI_2127 with an average similarity index of less than 62.5% over all three samples.

2.5.2.5 Biomass Determinations

It was not possible to make an accurate comparison of the biomass determination in all cases; 69 samples had not been supplied with species blotted wet weight biomass data. Consequently, only 24 of the 93 samples received were used for comparative analysis. Table 4 of the OS Summary Report shows the comparison of the participating laboratory and APEM Ltd. biomass figures by major taxonomic groups. The total biomass values obtained by the participating laboratories varied greatly compared to those obtained by APEM Ltd. Differences in the recorded biomass ranged from +1.16% to +60%. The reason for these large differences is presumably a combination of variations in apparatus (*e.g.* calibration) and operator technique (*e.g.* period of and effort applied to drying). These figures are not comparable to those produced by the same module in each of the previous years due to the variability in the duration and method of drying and the consistency of results within each major taxonomic group. The APEM Ltd. biomass data were achieved using a non-pressure drying procedure as specified in the Green Book and the NMBAQC guidelines for macrobenthic sample analysis (Worsfold, Hall & O'Reilly (Ed.) 2010).

2.5.3 Discussion

The total numbers of samples for which the participating laboratories submitted data to APEM Ltd to chose audit Own Samples ranged from 11 (less than the requested minimum of 12) to 493. The average number of samples data for selection was 67. It is evident that some laboratories use the Scheme as a complete audit check of their entire year's work, whereas some laboratories chose certain projects for submission, and may even do so prior to analysis.

The average Bray-Curtis similarity index of 92% achieved for this Own Sample Module shows that the agreement between the participating laboratories and APEM Ltd. was generally acceptable.

There were 93 samples submitted for the Own Sample Module, including the 12 processed by the Scheme's external auditor. Of the 93 samples, 72 (77%) exceeded the 90% Bray-Curtis Pass mark and 54 (58%) of the samples exceeded 95% BCSI. Since the beginning of this module in Year 02 of the Scheme, only the results of Years 03, 05, 06, 07, 09, 15, 20 and now 21 achieved 76% or less of the samples exceeding the 90% Bray-Curtis Pass mark (see Table 5 of the OS Summary Report).

Since the beginning of the Own Sample Module, 1211 admissible samples have been received (OS01-58). Of these, 230 samples (21%) have fallen below the 90% Pass mark. Overall, these results are acceptable and show the efficacy of the OS module, although a dip in quality has been noticed in year 20 and 21 compared with the previous four years. Some participating laboratories should be able to improve their results by reviewing their extraction methods and their use of taxonomic literature and identification keys.

2.5.4 Application of NMBAQC Scheme Standards

One of the key roles of the Benthic Invertebrate Component of the NMBAQC Scheme is to assess the reliability of data collected as part of the CSEMP or WFD monitoring programmes. With this aim, performance target standards were defined for certain Scheme exercises and applied in Scheme Year 3 (1996/97). These standards were the subject of a review in 2001 (Hall and Worsfold, 2001) and were altered in Scheme Year 8; each performance standard is described in detail in the Description of the Scheme Standards for the Benthic Invertebrate Component document. Laboratories meeting or exceeding the required standard for a given exercise would be considered to have performed satisfactorily for that particular exercise. A flag indicating a 'Pass' or 'Fail' would be assigned to each laboratory for each of the exercises concerned. It should be noted that, as in previous years, only the Own Sample Module have been used in 'flagging' for the purposes of assessing data for the CSEMP/WFD.

As the Scheme progresses, additional exercises may be included. In the meantime, the other exercises of the Scheme as presented above are considered of value as more general indicators of laboratory performance, or as training exercises.

2.5.4.1 Laboratory Performance

The target values for each Own Sample and the corresponding laboratory results, including the assigned flags are presented in Table 1 of the OS Summary Report. Comparisons between results are not applicable due to the diversity of samples and processing methodologies exhibited throughout this module.

It can be seen from Table 1 (column 26) that 39% (12 of 31) of participating laboratories met or exceeded the required standard for three of the OS targets - the enumeration of taxa, enumeration of individuals and the Bray-Curtis comparison, for all three samples submitted

as part of this exercise. Nineteen laboratories achieved a Bray Curtis of >90% for all three of their Own Samples.

Overall, 69% of the comparisons were considered to have passed the enumeration of taxa standard, 81% exceeded the enumeration of individuals standard and 77% passed the Bray-Curtis comparison standard (>90%). NMBAQC Scheme sample flags have been applied to each of the Own Samples in accordance with the performance flagging criteria introduced in Scheme Year 08 (Table 1, column 26); one sampled was flagged as a 'Fail' due to it being incomplete as a submission (both taxa and residue were missing), 15 samples (16.3%) are flagged as 'Fail - Bad', 6 (6.5%) as 'Fail – Poor', 18 (19.6%) as 'Pass - Acceptable', 41 (44.6%) as 'Pass - Good' and 12 (13%) as 'Pass - Excellent' for their Bray-Curtis similarity indices. 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 (see 2.5.4.3 Remedial Action below).

Performance with respect to the biomass standard was poor (Table 1, column 22) with only 58% of the samples with submitted biomass values meeting the required standard.

2.5.4.2 Comparison with Results from Previous Years

A comparison of the overall results for recent years is presented in Table 5 of the OS Summary Report (Own Sample Module Summary Report – OS56, 57 & 58). The table shows the number of laboratories assigned 'Pass' and 'Fail' flags for the OS exercises over the past twenty one years based upon the current NMBAQC Scheme standards (see Description of the Scheme Standards for the Benthic Invertebrate Component). This year's 93 Own Samples resulted in a pass rate of 76% (the highest being 100% achieved in exercise OS01 that involved just fourteen samples; the lowest being 67% recorded in Year 7 from 45 samples).

2.5.4.3 Remedial Action

It is imperative that failing CSEMP/WFD samples, audited through the Own Sample Module, are addressed. Remedial action should be conducted upon the associated CSEMP/WFD replicates to improve the flagged data. For a CSEMP/WFD sample, the associated samples are the five sample replicates or the five dispersed samples in the same water body. For a WFD sample, the associated samples would normally be the samples (5-10 in number)

collected from the same water body. The revised NMBAQC Scheme OS standards, introduced in Scheme Year 08, give clear methods for discerning the level of remedial action required (see Description of the Scheme Standards for the Benthic Invertebrate Component). A failing Own Sample is categorised by a Bray-Curtis similarity indices of <90%. The performance indicators used to determine the level of remedial action required are % taxa in residue (missed taxa), % taxonomic errors, % individuals in residue (missed individuals) (see Table 1, columns 7, 10 and 17 in OS Summary Report) and % count variance. Own Samples not achieving the required standards are monitored by the NMBAQC committee. The participating laboratories are expected to initiate remedial action according to the advice of APEM Ltd. APEM Ltd. or the NMBAQC Scheme Contract Manager should be notified when this has been completed. Any remedial action undertaken should be audited externally where required. The NMBAQC Contract Manager and Scheme's contractor, APEM Ltd., will provide clarification on specific details of remedial action or consider appeals relating to the remedial action process.

Below is a summary of the samples that have been flagged with 'Fail' flags in Scheme Year 21. Twenty two samples 'failed' (some of these may include data that is reported to the CMA's e.g. WFD samples). Remedial action, outlined below, was required for associated replicates of the following Own Samples:

Non-CMA samples:

LabCode	OS no.	Remedial action	Notes
BI_2113	OS57	Review separation of taxa. All taxa to be separated to ensure high audit resolution. Separate bryozoans for future Own Sample submissions; Extract, record and quantify anthropogenic material (metal splatter, plastics, facilitated seeds, etc.) for disposal sites; Assign faunal fragments to taxon vials where possible	Remedial action completed 8/12/14
BI_2118	OS57	High counting discrepancy; review possible transcription error with smp B1-17	Remedial action recommended
BI_2121	OS57	Reprocess residues and review taxonomic errors for associated samples	Remedial action recommended
	OS58	Reprocess residues for associated samples	Remedial action recommended
BI_2126	OS56	Reprocess residues for associated samples	Remedial action recommended
	OS57	Reprocess residues for associated samples	Remedial action recommended
	OS58	Reprocess residues for associated samples	Remedial action recommended
BI_2127	OS56	Reprocess all taxonomic errors in associated samples; ensure all taxa are supplied for future audit samples	Remedial action undertaken; to be evaluated/audited.
	OS57	Reprocess taxonomic errors and resort residues for associated samples	Remedial action undertaken; to be evaluated/audited.
	OS58	Reprocess taxonomic errors and review biota extraction methods for associated samples	Remedial action undertaken; to be evaluated/audited.
BI_2128	OS56	Review taxonomic errors and reprocess residues for associated samples	Remedial action recommended
	OS57	Review taxonomic errors for associated samples	Remedial action recommended
	OS58	Review taxonomic errors and reprocess residues for associated samples	Remedial action recommended

CMA samples:

LabCode	OS no.	Remedial action	Notes
BI_2106	OS56	Reprocess residues for all associated samples	Remedial action recommended
	OS57	Reprocess residues and review taxonomic errors for all associated samples	Remedial action recommended
BI_2115	OS56	Reprocess taxonomic errors and reprocess associated residues	Remedial action completed 3/7/15
	OS57	Reprocess taxonomic errors and reprocess associated residues	Remedial action completed 3/7/15
BI_2131	OS58	Fail based upon failure to retain & supply full sample for audit (taxa & residue); Review taxonomic errors for all associated samples	Fail due to incomplete sample submission (taxa & residue); Remedial action recommended
BI_2132	OS58	Fail due to failure to retain & supply sample residue for audit; detail extent of available smp residues & supply further sample for audit	Fail due to incomplete sample submission (taxa & residue); Remedial action recommended
BI_2133	OS56	Reprocess taxonomic errors in affected samples	Remedial action recommended
BI_2138	OS57	Reprocess residues of all affected samples	Remedial action completed 11/12/15
	OS58	Reprocess residues and taxonomic errors in all affected samples	Remedial action completed 11/12/15

3. Conclusion and Recommendations

A number of observations may be made from the results of the exercises described above. The following is a summary of the major points of importance:

1. 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. Of the results submitted 31% of RT, 60% of LR, 50% of MB and 52% of all Own Samples were late. Late submission ranged from a day to four months late (Laboratories BI_2101, BI_2106, BI_2125, BI_2132, BI_2133 for the Own Sample component). Laboratories should endeavour to report their results within the requested time, according to the deadlines circulated at the beginning of each Scheme year; this would greatly facilitate the analysis of results and effective feedback.

2. The range in numbers of sample data sets provided for selection of the Own Samples ranged from 11 to 493 and averaged 67 samples available for Own Sample selection. The number of project data sets submitted ranged from 1 to 9 (with only 11 samples in total) with the average percentage audit being 12% of the submitted data. Best practice for commercial laboratories should be to use the Scheme as an external auditor and no 'cherry picking', pre-analysis selection, or pre-submission re-working of samples should be undertaken.

3. Several samples submitted as Own Samples were submitted without residues and with missing taxa. While some samples comprised very small volumes of sorted residues and no faunal fragments. Participants are reminded that Own Samples must include all sorted residues, including all extracted materials deemed 'unrecordable' during the initial processing; and all recorded taxa must be submitted to the auditing laboratory. Failure to supply all sample components according to the NMBAQC OS Protocol will result in the assignment of a 'Fail' audit flag.

4. There were continued problems associated with the measurement of biomass for individual species. 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. The initial processing of a CSEMP/WFD sample should

in no way compromise the effectiveness of an audit. Biomass procedures should not render the specimens unidentifiable; trials would help to derive the best protocol for the blotted weighing technique. Biomass must be reported to four decimal places with nominal weights recorded as 0.0001g. A standardised protocol is available and must be followed for CSEMP/WFD analysis.

5. 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. Laboratories are strongly recommended to implement and expand in-house reference collections of fauna. The inclusion of growth series material is extremely useful for certain faunal groups, *e.g.* identifying certain molluscs. All surveys should have an associated reference collection to enable ease of cross-checking or adopting future taxonomic developments.

6. Participants submitting data for laboratory reference exercises should add a note on location of sample to aid identification. A similar 'Habitat Notes' section to that distributed with the ring test exercises will be distributed for completion from Scheme Year 23 (2016/2017).

7. Participants submitting data for the ring test exercises should complete the 'literature used' section to enable additional information to be gathered regarding incorrect identification. In some cases this information could result in a laboratory being marked correctly for what could be perceived as a mis-identification without that information *e.g.* with the *Pista* identification in RT47.

8. 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 datasheets to enable additional information to be gathered regarding the difficulty of ring test specimens.

9. All Own Sample submissions must be accompanied with a 'processing details sheet' to ensure that the re-analysis (audit) matches that of the initial processing. Laboratories should also ensure that these sheets are completed accurately. Own

Samples processed for CSEMP/WFD must be processed according to the NMBAQC guidelines (Worsfold, Hall & O'Reilly (Ed.) 2010).

10. 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.

11. 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. There may also be a problem within certain taxonomic groups (*e.g.* crustaceans floating within sample or molluscs settled within the coarser sediment fractions). 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.

12. It is apparent that some laboratories are not utilizing the NMBAQC guidelines for processing macrobenthic samples (Worsfold, Hall & O'Reilly (Ed.), 2010) issued with MB18 in Scheme Year 17 to improve the consistency of analysis*, i.e.* all analysts extracting and recording all biota. 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.

13. 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 LR, OS and MB Modules, detailed results have been forwarded to each participating laboratory as soon after the exercise deadlines as practicable. After each RT exercise a bulletin was circulated, reviewing the literature used and detailing the correct identification of the taxa circulated. Participants are encouraged to

review their exercise reports and provide feedback concerning content and format wherever appropriate. Valuable feedback was received from participants for RT48, which led to further collaboration with International experts and an extremely detailed and taxonomically valuable RT48 bulletin. Hopefully the feedback and additional discussions will result in further revision of the literature. APEM Ltd. wish to thank all participants that submitted feedback, photos and were involved in further discussions regarding RT48.

14. Positive, constructive feedback has been received from participants during Scheme Year 21 (2014/2015). As in previous years, participants have expressed the benefits of the modules, especially RT and OS. The primary aim of the Benthic Invertebrate Component of the Scheme is to improve the quality of biological data via training and audit modules. An informal constructive reporting system exists to assist in the overall improvement of data quality. For example, laboratories struggling with particular faunal groups in their Own Samples often receive additional support, as well as receiving their returned OS faunal material separated, according to the AQC identifications, for future reference. Three of the twenty two 'failing' Own Samples in Scheme Year 21 have already been rectified via the recommended remedial action.

15. If participants have queries, or wish to raise issues regarding Own Sample or Ring Test specimen identifications this must be done in a timely manner. Issues have been raised up to two months after the interim reports which led to delays with exercise, module and Annual Report.

16. APEM Ltd. strives to ensure smooth running and transparency of the Scheme at all times. Consideration should be given by participants as to the tone of correspondence with APEM Ltd. Participants should remember that APEM Ltd. must log and make available all correspondence to the Benthic Invertebrate Contract Manager (Myles O'Reilly, SEPA). As such participants should not communicate anything regarding the Scheme or Scheme Contractor that they would not wish to be shared with the Contract Manager. Participants can be assured that their anonymity will be protected if this correspondence is required to be shared with the Committee.

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