NMBAQC

NE Atlantic Marine Biological Analytical Quality Control Scheme

Benthic Invertebrate Component Annual Report
Scheme Operation 2022 / 2023 (Year 29)

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BENTHIC INVERTEBRATE COMPONENT ANNUAL REPORT FROM APEM Ltd

SCHEME OPERATION – 2022 / 2023 (Year 29)

Linked Documents (hyperlinked in this report):

Annual Report for 2018/2019 (Year 25)
Annual Report for 2019/2020 (Year 26)
Annual Report for 2020/2021 (Year 27)
Annual Report for 2021/2022 (Year 28)
Ring Test Bulletin – RTB#63
Ring Test Bulletin – RTB#64
Laboratory Reference Module Summary Report – LR27
Own Sample Module Summary Report – OS80, 81 & 82
Description of the Scheme Standards for the Benthic Invertebrate Component
Guidelines for Processing Marine Macrobenthic Invertebrate Samples
Ring Test Protocol
Laboratory Reference Protocol
Own Sample Exercise Protocol
Own Sample Interim Report Review and Remedial Action Processes
Review of recording policy differences

1. Introduction

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

- The processing of macrobenthos samples;
- The identification of macrobiota;
- The determination of physical parameters of sediments.

Scheme year 2022 / 2023 (year 29) followed the format of year 2020 / 2021. 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.

Fifty-one laboratories (with multiple participants from some organizations counted separately) participated in the Benthic Invertebrate Component of the NMBAQC Scheme in 2022 / 2023 (year 29). Nineteen of the participants were UK Competent Monitoring Authorities (CMAs), responsible for the Clean Seas Environment Monitoring Programme (CSEMP) or Water Framework Directive (WFD) sample analysis/data; seventeen were UK private consultancies. Fifteen of the participants were non-UK laboratories (including eight government organizations and seven private consultancies); five institutions from three countries responsible for collective monitoring of the Black Sea region joined the scheme under a BRIDGE-BS consortium (bridgeblacksea.org). Laboratory Codes were assigned in a single series for all laboratories participating in the Benthic Invertebrate component. Separate Laboratory Codes were assigned for the other scheme components, such as the particle size component.

As in previous years, some laboratories elected to be involved in limited aspects of the scheme. UK Competent Monitoring Authorities (CMAs) completing benthic biological analyses for monitoring programmes, including the assessment of MPAs (Marine Protected Areas), as evidence under MSFD (Marine Strategy Framework Directive), WFD (Water Framework Directive) and the CSEMP (Clean Seas Environmental Monitoring Programme),

must participate in the Benthic Invertebrate component. CSEMP / WFD laboratories are no longer required to participate in all components / modules of the scheme.

In this report, performance targets have been applied for the Own Sample module only (see Hall, 2010: 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 – OS80, 81 and 82 (Own Sample Module Summary Report – OS80, 81 & 82) presenting the comparison of laboratory results with the standards.

1.1 Summary of Year

This report presents the findings of the Benthic Invertebrate component for year 2022 / 2023 (year 29) of the North East Atlantic Marine Biological Analytical Quality Control (NMBAQC) Scheme.

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

- Own Sample module (OS) - re-analysis by APEM Ltd. of three samples supplied by participating laboratories;
- Invertebrate Ring Test module (RT) - identification of two sets of twenty-five invertebrate specimens; and
- Laboratory Reference module (LR) - re-identification by APEM Ltd. of a set of up to twenty-five specimens supplied by participating laboratories.

The analytical procedures of the various modules were the same as for 2021 / 2022 (year 28) of the Scheme (Worsfold et al., 2023a). The results for each of the Scheme exercises are presented and discussed. Comments are provided on the performance of participating laboratories in each of the exercises.

Two **Ring Tests (RT)**, each of 25 specimens, were distributed (RT63 and RT64). The second (RT64) was targeted on Peracarida, excluding amphipods. The methods and policies used in the module followed the Ring Test Protocol (Worsfold & Hall, 2017a).

For RT63, the average numbers of differences per participating laboratory (for a total of 23 laboratories with 21 submissions) were 2.4 generic differences and 4.8 specific differences. Four species (two polychaetes a nudibranch and a hydrozoan) were responsible for just over half (51%) of the specific differences.

For RT64, the average numbers of differences per participating laboratory (for a total of 23 participants with 21 submissions) were 1.9 generic differences and 3.2 specific differences. Four specimens were responsible for just under 40% of the specific differences.

Laboratory Reference (LR): Six laboratories signed up for the LR27 module and four laboratories submitted specimens for confirmation. Most misidentifications were for Annelida (45%), followed by Arthropoda (29%). The methods and policies used in the module followed the Laboratory Reference Protocol (Hall & Worsfold, 2017).

The methods and policies used in the **Own Sample (OS)** module followed the recent Own Sample Exercise Protocol (Worsfold & Hall, 2017b), produced to explain and standardise policies, including details of audit sample selection and determination of 'associated samples' for subsequent remedial actions. 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 Hall, 2010: Description of the Scheme Standards for the Benthic Invertebrate Component). In OS80-82, extraction efficiency (of individuals) was better than 90% in 90% of the comparisons and better than 95% in 80% of all comparisons. 100% of countable taxa were extracted from the sample residues in 69% of samples. The Bray-Curtis similarity index ranged from 27.8% to 100% with an average of 94.08%. The Bray-Curtis similarity index was greater than 95% in 73% of comparisons; in 85% of cases, the value of the index was greater than 90% and, therefore, achieved 'Pass' flags. Nineteen samples (18%) achieved 'Pass-Excellent' flags with Bray-Curtis similarity scores of 100%.

Taxonomic Discrimination Protocol (TDP) development: Progress was made through Years 27, 28 and 29 towards a TDP at family level for all biota, to allow better standardisation of recording policies and identification levels between laboratories for different taxa. Comments were received from participants and NMBAQC Committee members on a draft version of the report. These comments were compiled and included in a version posted on

the NMBAQC scheme website in September 2023 (Worsfold et al., 2023b). Comments are now invited from the wider benthic analysis community, in addition to continued input from participants.

Workshops: APEM presented two **beginners' workshops** in Year 29. A small-capacity workshop was presented at SEPA from 12th - 16th December 2022. The main beginners' taxonomic workshop for benthic invertebrates was held from 26th - 30th June 2023, at the University of Galway (UoG). The workshops provided introductory training in the identification of major benthic groups, followed by 1-day sessions on each of: polychaetes, crustaceans, molluscs and echinoderms. The workshop was delivered by five APEM/AQUAFACT benthic specialists. In total, including AQUAFACT and UoG attendees, there were twenty-three participants from ten organisations representing academic, government and private laboratories. Ten participants were based in the Republic of Ireland, five travelled from Northern Ireland, four from England, two from the Netherlands, and two from Belgium. Progress was made towards a possible **experts' workshop** in spring 2024. Peter Barry (CEFAS) provisionally agreed to present on thyasirid bivalves and Magdalena Błażewicz (University of Łódź) provisionally agreed to present on tanaid crustaceans. Guidelines for Experts' workshop requirements were circulated to potential presenters and also published as a Scheme document (Worsfold, 2023).

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. Review of Benthic Invertebrate Component

2.1 Introduction

There are three modules within the Benthic Invertebrate component: Invertebrate Ring Test (RT), Invertebrate Laboratory Reference (LR) 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 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. Specific details can be found in the Scheme's Benthic Invertebrate component protocols: Laboratory Reference Protocol (Hall & Worsfold, 2017), Ring Test Protocol (Worsfold & Hall, 2017a) and Own Sample Exercise Protocol (Worsfold & Hall, 2017b).

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. 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 October 2022, each participant was given a confidential, randomly assigned 2022 / 2023 (Scheme year 29) LabCode. Codes are prefixed with the component initials (*e.g.*, BI for Benthic Invertebrates), the Scheme Year and a unique number (between 01 and 46); *e.g.* Laboratory number one in Scheme Year 2022 / 2023 (Year 29) was recorded as BI_2901. 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 variation in participants' identifications of different species and attempts to determine whether differences are the result of literature deficiencies, lack of reference material or misinterpretation of identification resources. Details are explained in the recent Ring Test Protocol (Worsfold & Hall, 2017a)

Two sets of 25 benthic invertebrate specimens were distributed in 2022 / 2023. The first circulation (RT63) was a general invertebrate ring test. It included 12 (48%) annelids, 5 (20%) molluscs, 3 (12%) arthropods, 1 (4%) echinoderm and 4 (16%) taxa belonging to other phyla. An effort was made to include a proportion of species that had not previously been circulated through the module (25 - 100%, for RT63; 4 - 16%, for RT64) and that would highlight taxonomic problems. The second circulation (RT64) was targeted on peracarid crustaceans other than amphipods. Basic notes on substratum, salinity, depth and geographical region 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 North-East Atlantic. Care was taken to provide animals of similar size and condition for each laboratory. Each specimen was uniquely identifiable by means of a coded label and all material has been retained for subsequent checking. For both ring tests, the specimens were taken from samples within a single survey and, in many cases, they were from a single sample, or replicates from a single sampling station.

2.2.1.2 Analysis Required

The participating laboratories were asked to identify each of the RT specimens to species level and they were also asked to complete a 'confidence level' field to indicate whether they would ordinarily have left the specimen at a higher taxonomic level. Laboratories could also add brief notes and information detailing the 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 returns (*i.e*. different sets of results from different analysts for the same set of circulated specimens) for each exercise to enhance the training value of the module. One laboratory chose to submit two returns for the same set of specimens for both RT63 or RT64 and two laboratories requested multiple circulations (3 and 2) for each. The protocols followed for the two circulations, particularly the method of counting differences, were the same as for previous circulations. Approximately eight weeks were allowed for the analysis of RT63; approximately ten weeks were allowed for RT64.

2.2.2 Results

2.2.2.1 General Comments

Several laboratories use the ring tests for training purposes and select them preferentially over other modules. The results are not used to assign 'Pass' or 'Fail' flags. In total, 23 laboratories subscribed to RT63 and 23 laboratories subscribed to RT64. For RT63, 20 laboratories returned data (21 individual data sets). For RT64, 20 laboratories returned data (21 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 numbers of differences. Where identifications 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 Ring Test Bulletins (RTB) 63 and 64 show identifications made by each of the participating laboratories for the twenty-five specimens in each ring test, arranged with laboratories as rows and specimens in columns in Table 1; specimens as rows and laboratories as columns in Table 2. For clarity, the participant's identification is given only where the 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 is presented in brackets: "[name]". A dash, "-", in the tables indicates that the name of the genus (and / or species) given by the laboratory was 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 Counting RT Result differences

For each laboratory, a count was made of 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 Tables 1 and 2 for RTB63 and RTB64). Separate counts 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 difficulties lie in the identification of certain taxa. Results for Scheme Year 29 (2022 / 2023) were presented in the Ring Test Bulletins (RTB) along with the reasons for each identification discrepancy. These bulletins contain images of the test material and of all available taxa that were named as alternative identifications by participants. Participating laboratories were advised to retain 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 reference and / or potential future circulation.

2.2.2.3.1 Ring Test 63 (Type: General)

The results discussed below are given in Table 1 of RTB63, which displays the data arranged with columns for species to enable quick reference to the range of answers received and in Table 2, which presents the results arranged with columns for laboratories (see Worsfold et al., 2023c; Ring Test Bulletin RTB63).

Twelve (48%) of the 25 specimens circulated were annelids, five (20%) were molluscs, three (12%) were arthropods (two crustaceans and one chelicerate), 1 (4%) was an echinoderm and four (16%) were from other phyla (two Bryozoa, one Cnidaria and one foraminiferan). RT63 included twenty-five species never previously sent (i.e. the entire circulation).

There were 50 generic level differences (10% of all genus identifications received from participants) recorded in the 21 data sets received from 23 participating laboratories and 100 species level differences (19% of all species identifications received from participants).

Four of the specimens circulated were responsible for just over half (51%) of participants' species level identification differences. These were the polychaete worms *Harmothoe extenuata* and *Dipolydora flava*, the hydrozoan *Lovenella clausa* and the nudibranch mollusc *Polycera quadrilineata*.

Five of the 25 specimens circulated: the polychaete worms *Pholoe pallida*, and *Travisia forbesii*, the sea spider (pycnogonid) *Pycnogonum litorale*, the bivalve mollusc *Magallana gigas* and the bryozoan *Eucratea loricata* were correctly identified by all participants.

Further details and analysis of results can be found in the Ring Test Bulletin RTB63, which was circulated to each laboratory that supplied results for this exercise and was also posted on the Scheme's website (www.nmbaqcs.org).

2.2.2.3.2 Ring Test 64 (Type: Targeted on Peracarida, excluding amphipods)

The results discussed below are given in Table 1 of RTB64, which displays the data arranged with species as columns to enable quick reference to the range of answers received and in Table 2 which presents the results with laboratories as columns (see Worsfold et al., 2023d; Ring Test Bulletin (RTB64).

RT64 included 25 circulations of peracarid Crustacea, (10 cumaceans, 10 isopods, 4 tanaids and a mysid), including two (cumacean) species circulated as both males and females and four species never previously sent.

There were 39 generic level differences (7% of all genus identifications received from participants) recorded in the 21 data sets received from 23 participating laboratories and 68 species level differences (13% of all species identifications received from participants).

Four of the specimens circulated were responsible for just under 40% of participants' species level identification differences. These were the isopod *Lekanesphaera levii*, the cumaceans *Pseudocuma longicorne* (male) and *Bodotria scorpioides* (male) and the tanaid *Tanaissus danica*.

Five of the twenty-five specimens circulated: the isopods *Gnathia oxyuraea* and *Cyathura carinata* and the cumaceans *Cumella pygmaea*, *Iphinoe trispinosa* and *Lamprops fasciatus*, were correctly identified by all participants.

Further details and analysis of results can be found in the Ring Test Bulletin RTB64, which was circulated to each laboratory that supplied results for this exercise and was also posted on the Scheme's website (www.nmbaqcs.org).

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 graphs related to Table 2 in RTB63 and RTB64 respectively. The laboratories are ordered by increasing number of differences at species level. The division of laboratories into three bands (Low, Mid and High) on the basis of the number of differences at species level is also shown.

2.2.2.5 Differences by Taxonomic Group

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

Major taxon	Species circulation	Generic differences		Specific differences	
Annelida	12	9	10%	50	30%
Arthropoda	28	40	45%	70	42%
Mollusca	5	16	18%	24	14%
Others	5	24	27%	24	14%
Total	**50**	**89**	**100%**	**168**	**100%**

The percentage differences are the proportions of total differences across the two ring tests that are attributed to each major taxonomic group. The specific differences were mainly from Arthropoda, as expected due to the RT64 target, with about twice as many for annelids as for molluscs or others.

2.2.3 Discussion

The results for RT63 were in general comparable with those from previous exercises, with an average of 2.4% generic and 4.8% specific differences across the participating laboratories. The results for RT64 were also within the range of those from previous exercises, but with fewer errors than most: 1.9% generic and 3.2% specific differences across the participants; the only past ring tests with fewer errors were RT38 (targeted on easiest), RT60 (targeted on biotope-defining spp.) and RT45 and RT46 (both of which were from within a period that may not have prioritized difficult taxa).

Most RT63 differences were due to inherent difficulties in seeing defining features. The scaleworm *Harmothoe extenuata* is easily damaged and loss of scales makes identification difficult (though all circulated specimens had some scales) and small specimens may have less clear features (many laboratories have juvenile and damaged categories for higher level identifications) The hydroid *Lovenella clausa* is widely misidentified due to confusion in the most commonly used key. The nudibranch *Polycera quadrilineata* is prone to contraction and loss of colour on preservation (some laboratories do not routinely identify nudibranchs beyond order level) and has been subject to recent taxonomic revisions.

Many RT64 differences were also due to difficulty in discerning features. Male *Pseudocuma longicorne* have a reduced second pleopod that would lead to the most commonly recorded error if missed and the uropod articulation of *Bodotria scorpioides* is often difficult to see. There were also difficulties with taxonomic revisions (such as for *Lekanesphaera levii*) and subtidal species missing from the standard (intertidal) identification guide (such as *Pseudarachna hirsuta*).

We consider the RT component to be a valuable training tool that can be an indicator of problem groups. It can highlight possible taxa for further 'targeted' ring test exercises or for inclusion at taxonomic workshops and provide data for the development of taxonomic discrimination policies. The allowance of multiple submissions per laboratory and the inclusion of images in the Ring Test Bulletins have enhanced the training value of this component. All participating laboratories have been made aware of the problems identified by these ring tests via Ring Test Bulletins RTB63 and RTB64, which also include literature citations that relate to the problem taxa.

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

2.3.1 Description

The Laboratory Reference module is a training module which encourages laboratories to build reference collections to improve identification consistency and to seek additional opinions for difficult specimens. The value of reference material in assisting 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 / 1997). This module can help participating laboratories to assess their ability to identify material from their own samples. Laboratories are also able to use this exercise to obtain second opinion identifications for difficult or problematic taxa of which they are unsure. This was the twenty-seventh Laboratory Reference exercise (LR27). 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 by APEM Ltd., with comparisons to the original identifications. All specimens were returned to the laboratories after analysis.

2.3.2 Results

Six laboratories signed up for this exercise (LR27) and four submitted specimens for examination. Detailed results have been separately reported to each participating laboratory. Taxonomic edits were made for submitted polychaetes (14; 45%), crustaceans (9; 29%) and molluscs (6; 19%). In addition, differences were noted for taxonomic resolution, recording notation and spelling for many specimens. A report summarising the results from this module is presented in the Laboratory Reference Module Summary Report – LR27 (Worsfold & Hall, 2023).

2.3.3 Discussion

As with all training exercises, detailed inter-laboratory comparisons are of limited value. Two of the differences were for recently described amphipods (*Pontocrates moorei*, *Cheirocratus pseudosundevallii*) that were be confused with similar species from older

literature. Others were from inherently problematic groups, including polychaetes (Polynoidae, Syllidae, Cirratulidae) and caenogastropods with internal (*Lamellaria*) or partially covered. (*Trivia*, *Simnia*) shells, which were confused with heterobranch families. The taxonomic resolution and recording policy differences were defined according to the current standardized format designed for these exercises (Worsfold, 2017), with a view to the development of a taxonomic discrimination protocol.

2.4 Own Sample (**OS**) Module

2.4.1 Description

The Own Sample module examines 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 & Worsfold, 2001), several changes to sample selection and scoring were implemented in Scheme Year 8 (2001 / 2002). All participants must meet these Own Sample requirements. The Own Sample Exercise Protocol (Worsfold & Hall, 2017b) was updated in August 2017 and circulated to all OS participants ahead of the module for the following scheme year (Year 24). Own Sample participants must supply their previous year's CSEMP / WFD data matrices, where relevant, for Own Sample selection, *i.e.* 2020 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 and the selection 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 twenty samples were included in the submitted data matrix.

Five institutions from three countries responsible for collective monitoring of the Black Sea region joined the scheme under a BRIDGE-BS consortium (bridgeblacksea.org). These laboratories participated in the Own Sample module with a modified sample/data selection process to align with existing field and laboratory sample processing methods for monitoring in this region.

2.4.1.1 Analysis Required

Participating laboratories were instructed to have conducted macrobenthic analysis of the samples using standard procedures. A summary of sample details, including codes, area and

sample processing procedures was to be provided, on a standard form, for each Own Sample. Samples requiring sub-sampling were to be avoided where possible. All procedures were 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 biota had been removed and counted;
- Separated taxa - individually labelled vials containing the identified biota; and
- Other fractions - *e.g*. material containing biota that had been counted *in situ*.

Recording and identification were assumed to have followed NMBAQC guidelines for macrobenthic sample analysis (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. In addition, measurements of the biomass of the recorded taxa were submitted where required; measurements were to be blotted wet weights to 0.0001g for each of the enumerated taxa.

The Own Sample Module was separated into two batches, with participants selecting a submission batch to align with their workflow. Participants were given a number of weeks to submit their data; a further period of several weeks were allowed for the preparation and submission of the Own Samples selected for re-analysis. The sorted residue was re-examined and any countable material or new non-countable taxa extracted. Identified biota were checked for accuracy of enumeration and identification and, in cases where biomass was provided, all taxa were re-weighed using the procedure outlined in the NMBAQC Sample Processing Protocol (Worsfold, Hall & O'Reilly (Ed.) 2010).

2.4.2 Results

2.4.2.1 General Comments

Following the request to participating laboratories to submit data of suitable samples for re-analysis, 108 selected Own Samples were received from 36 (of the 37 subscribing) laboratories, together with descriptions of their origin and the collection and analysis procedures employed. One of the 108 samples has been excluded from the module's summary statistics, as it was supplied without sorted residues and is therefore deemed incomparable. Samples were identified as OS80, OS81 and OS82 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, from UK and mainland European waters. 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 141, with the number of countable individuals from 1 to 2,970. Of the 108 submitted Own Samples, seven were audited externally by Marine Invertebrate Ecological Services (MIES), as the initial processing had been carried out by APEM Ltd. Interim reports were submitted to participating laboratories. A summary of results from this module is presented in the Own Sample Module Summary Report – OS80, 81 & 82.

2.4.2.2 Efficiency of Sample Sorting

Table 1 of the OS Summary Report displays a summary of the data obtained from the OS analysis. All taxa recorded by the participating laboratory were included in the AQC analysis (if required to be recorded by the NMBAQC PRP/TDP). In 51 samples out of the total 107 comparable samples, the number of taxa recorded by the participating laboratories was identical to that obtained by the auditing laboratory (columns 2 and 3). For the remaining 56 cases, the difference was on average 2.5 with a maximum of 7 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 21%. The average difference between the 74 samples with recorded differences was 4.26% (and 2.95% across all 107 samples), with 24 samples exceeding this average.

33 of the 107 applicable samples reported showed 100% extraction of individuals from the residue (column 16) and, in 74 samples, between 1 and 163 individuals had been missed during processing. In 26 samples, only individuals attributed to taxa already recorded in the sample were found. In 49 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 0 to 7, with an average of 0.98 new taxa per sample. Amongst the poorest extraction sample records were: a total of 3 missed taxa and 163 individuals, 3 missed taxa and 67 individuals, 4 taxa and 60 individuals, 5 taxa and 45 individuals, and 7 missed taxa and 27 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 107 samples) of missed individuals found upon re-sorting the residue was approximately 8.8 and the average number of missed taxa was less than 1.

2.4.2.3 Uniformity of Identification

Taxonomic differences (columns 10 and 11) between the auditor and participating laboratories' results were found in 58 (54%) of the 108 applicable Own Samples. A summary of misidentified taxa is presented in Table 3 of the OS Summary Report. For the samples with taxonomic errors, an average of 3 taxonomic errors per laboratory was recorded; in the worst instance, 14 identification errors occurred. A large variety of samples (and biota) was received. Polychaeta accounted for 43%, Mollusca for 27%, Crustacea for 18%, 'others' for 8%, Oligochaeta for 3%, and Echinodermata for 1% of the taxonomic errors (approximately), with a variety of species responsible for these errors.

2.4.2.4 Comparison of Similarity Indices (Bray-Curtis)

The procedure for the calculation of the similarity index was as used for the Own Sample exercise in Year 2017 / 2018 (Year 24). The Bray-Curtis similarity index figures (Table 1, column 23) ranged from 27.8% to 100%, with an average of 94.08%. Sixteen samples from seven laboratories achieved a similarity figure of less than 90%. Nineteen samples produced a similarity figure of 100%; these were submitted by twelve different laboratories. The best overall result was achieved by laboratories BI_2906, with 100% similarity across all three Own Samples. The lowest overall result was achieved by BI_2950 with an average similarity index of less than 60% over all three samples.

2.4.2.5 Biomass Determinations

It was not possible to make an accurate comparison of biomass determinations in all cases; 57 samples had not been supplied with species blotted wet weight biomass data. Consequently, only 51 of the 108 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 some of the participating laboratories varied greatly compared to those obtained by APEM Ltd. Differences in the recorded biomass ranged from -27% to +39%. The reason for these large differences is likely to be a combination of variations in apparatus (*e.g.* calibration), operator technique (*e.g.* period of and effort applied to drying), and data transcription errors. 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 (MARG, 2020) and the NMBAQC guidelines for macrobenthic sample analysis (Worsfold, Hall & O'Reilly (Ed.) 2010).

2.4.3 Discussion

It is evident that some laboratories use the Scheme as a complete audit check of their entire year's work, whereas others chose certain projects for submission, and may even do so prior to analysis. The latter approach would undermine the purpose of auditing, if the analyst(s) know beforehand which surveys, projects or samples are to be audited.

The average Bray-Curtis similarity index of 94.08% achieved for this Own Sample module shows that the agreement between the participating laboratories and APEM Ltd. was generally good.

There were 108 samples submitted for the Own Sample module, including the seven processed by the Scheme's external auditor. One sample was submitted without sorted residues (discarded post-primary processing) and was therefore deemed to have failed; it was only possible to conduct a partial audit, unobtainable audit metrics are excluded from various OS summary statistics. Of the 107 applicable samples, 91 (85%) exceeded the 90% Bray-Curtis Pass mark and 78 (73%) exceeded 95% BCSI. Since the beginning of this module in Scheme Year 02, 84% of the samples received have exceeded the 90% Bray-Curtis Pass mark (see Table 5 of the OS Summary Report).

Since the beginning of the Own Sample module, 2,023 admissible samples have been received (OS01-82). Of these, 316 samples (16%) have fallen below the 90% Pass mark. Overall, these results are good and show the efficacy of the OS module; although a dip in quality was noticed in years 20 and 21 (2013/14 and 2014/15) compared with the previous four years, there was a marked improvement in year 22 (2015 / 2016) and this has been maintained to year 28 (2021 / 2022). The increased failures in year 29 (2022 / 2023) can be attributed to a significant number of new participants joining the Own Sample module and pass rates will likely improve in future years following the application of procedural and taxonomic remedial actions. Some participating laboratories should be able to further improve their results by reviewing their extraction methods and their use of taxonomic literature and identification aids.

2.4.4 Application of NMBAQC Scheme Standards

One of the original roles of the Benthic Invertebrate component of the NMBAQC Scheme was to assess the reliability of data collected as part of the CSEMP or WFD monitoring programmes; this has since been expanded to other data sets. With this aim, performance target standards were defined for certain Scheme exercises and applied in Scheme Year 3 (1996 / 1997). These standards were the subject of a review in 2001 (Hall & 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 (Hall, 2010). Laboratories meeting or exceeding the required standard for a given exercise would be considered to have performed satisfactorily for that 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, 'flagging' has been applied only to the Own Sample module. A review of the formats used in recording identification differences was produced recently (Worsfold, 2017).

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 primarily as training exercises or to inform policy and future developments.

2.4.4.1 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. Although laboratories are requested to follow NMBAQC guidance, detailed comparisons of results between different laboratories are generally not applicable, due to the diversity of samples analysed and some minor inter-laboratory variations in processing methodologies, especially in relation to identification. Development of more detailed taxonomic discrimination protocols may help resolve some of the latter discrepancies.

Table 1 (columns 5, 15 and 26) shows 'pass / fail' results for three of the OS targets: the enumeration of taxa, enumeration of individuals and the Bray-Curtis comparison. Twenty-eight of the thirty-six participating laboratories achieved a Bray Curtis of >90% ('pass' flag) for all three of their Own Samples. Overall, 82% of the comparisons were considered to have passed the enumeration of taxa standard, 89% passed the enumeration of individuals standard and 85% 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); 11 samples (10%) are flagged as 'Fail - Bad', 5 (5%) as 'Fail - Poor', 13 (12%) as 'Pass - Acceptable', 59 (55%) as 'Pass - Good' and 19 (18%) 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 2.4.4.3 Remedial Action below).

Performance with respect to the biomass standard was generally good (Table 1, column 22), with 73% of the samples with submitted biomass values meeting the required standard.

2.4.4.2 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 – OS80, 81 & 82). The table shows the number of laboratories assigned 'Pass' and 'Fail' flags for the OS exercises over the past twenty-seven years based upon the current NMBAQC Scheme standards (see Description of the Scheme Standards for the Benthic Invertebrate Component). This year's 107 applicable Samples resulted in a pass rate of 93% (see Table 5 in the Own Sample Module Summary Report), which is an 8% decrease from the previous scheme year. However, this still represents maintained high standards in macrobenthic processing quality, as a large number of new laboratories joined the scheme this year and, typically, they take 1 or 2 rounds of Own Sample exercises to attain overall Own Sample pass flags. Historically, the highest pass rate achieved was 100% in exercise OS01 (1995 / 1996; Year 2) that involved just fourteen samples; the lowest pass rate was 67% recorded in 2000 / 2001 (Year 7) from 45 samples.

2.4.4.3 Remedial Action

It is important that failing samples audited through the Own Sample module, are addressed (mandatory for CSEMP/WFD samples). Remedial action should be conducted upon the associated samples to improve the flagged data. The mechanism for identifying associated samples is described in the Own Sample Exercise Protocol. For a CSEMP/WFD sample, the associated samples would normally be those collected from the same station, stratum or 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 index 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 the OS Summary Report) and % count variance. Own Samples not achieving the required standards are monitored by the NMBAQC committee. Participating laboratories are expected to initiate remedial action according to the advice of the Scheme's contractor. APEM Ltd. or the NMBAQC Scheme Invertebrate Component Technical Manager should be notified when this has been completed. Any remedial action undertaken should be audited externally where required. The Invertebrate Component Technical 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 were assigned 'Fail' flags in Scheme Year 2022 / 2023 (Year 29). Eight laboratories were responsible for seventeen 'failed' samples, including a deemed fail for residue disposal ahead of audit selection (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:

Lab Code	OS no.	Remedial action	Notes
BI_2932	**OS80**	Review taxonomic errors and reprocess residues for 2 'Associated Samples'	Remedial action not completed
BI_2932	**OS81**	Review taxonomic errors and reprocess residues for 2 'Associated Samples'	Remedial action not completed
BI_2936	**OS81**	Reprocess associated sample residues	Remedial action completed and evaluated 13th July 2023
BI_2938	**OS80**	Sample residue discarded ahead of AQC. Review sample storage instructions and chain of custody data to ensure that sample residues are stored until auditing is complete	Remedial action completed 19th May 2023
BI_2945	**OS81**	Reprocess taxonomic errors and reprocess associated sample residues (1x associated sample)	Remedial action not completed
BI_2950	**OS80**	Reprocess associated sample residues (sample replicates, if possible) and reprocess taxonomic errors	Optional remedial action; training workshop completed 21/22nd November 2023

BI_2950	**OS81**	Review extraction methods and reprocess taxonomic errors	Optional remedial action; training workshop completed 21/22nd November 2023
BI_2950	**OS82**	Reprocess associated sample residues (sample replicates, if possible) and reprocess taxonomic errors	Optional remedial action; training workshop completed 21/22nd November 2023
BI_2951	**OS80**	Reprocess associated sample residues (sample replicates, if possible) and review taxonomic errors	Optional remedial action; training workshop completed 21/22nd November 2023
BI_2951	**OS81**	Review extraction methods and review taxonomic errors	Optional remedial action; training workshop completed 21/22nd November 2023
BI_2951	**OS82**	Reprocess associated sample residues (sample replicates, if possible) and review taxonomic errors	Optional remedial action; training workshop completed 21/22nd November 2023
BI_2952	**OS80**	Review extraction methods and reprocess taxonomic errors	Optional remedial action; training workshop completed 21/22nd November 2023
BI_2952	**OS81**	Reprocess taxonomic errors	Optional remedial action; training workshop completed 21/22nd November 2023
BI_2952	**OS82**	Review extraction methods and reprocess taxonomic errors	Optional remedial action; training workshop completed 21/22nd November 2023
BI_2953	**OS80**	Reprocess associated sample residues (sample replicates, if possible) and reprocess taxonomic errors	Optional remedial action; training workshop completed 21/22nd November 2023
BI_2953	**OS81**	Reprocess taxonomic errors	Optional remedial action; training workshop completed 21/22nd November 2023
BI_2953	**OS82**	Reprocess associated sample residues (sample replicates, if possible) and reprocess taxonomic errors	Optional remedial action; training workshop completed 21/22nd November 2023

Data captured 12th December 2023

3. Conclusions and Recommendations

Several 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. Laboratories should endeavour to report their results within the requested time, according to the deadlines circulated at the beginning of each Scheme year.

2. The number of samples in **data sets provided for selection of Own Samples** varied considerably, with some laboratories offering less than the minimum 20 samples for audit selection (due to low volumes of sample processing) and other laboratories offering a fully year's benthic data across multiple projects. Best practice for commercial laboratories should be to use the Scheme as an external auditor for most or all of their samples and no 'cherry picking', pre-analysis selection, or pre-submission re-working of samples should be undertaken. **Retention of sample residues** will be required to facilitate this and to ensure that any subsequent remedial actions can be adequately completed.

3. Revised data request and sample submission forms were introduced for the 2017 / 2018 OS module to capture **data / sample ownership**. Where data belong to CMAs, the submitting participant was required to declare this so that audit results could be shared accordingly and CMA data auditing could be tracked and co-ordinated.

4. 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. The initial processing of a sample should in no way compromise the effectiveness of an audit. Biomass procedures should not render the specimens unidentifiable. Biomass must be reported to four decimal places with nominal weights recorded as 0.0001g. A standardised protocol is available in the NMBAQC guidance document (Worsfold, Hall & O'Reilly (Ed.) 2010) 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 biota**. The inclusion of growth series material is extremely useful for certain groups, *e.g.* 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 habitat / location** of samples, to aid identification. A similar 'Habitat Notes' section to that currently distributed with the ring test exercises would be appropriate.

7. Laboratories participating in the ring test exercises should attempt to identify all 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.

8. 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.

9. There are 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 biota 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 samples 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.

10. 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*, e.g.* analysts to extract

and record all biota, and sample residues to be subsampled if the specified criteria are met. Own Samples have been received that were processed in full despite meeting the NMBAQC subsampling criteria. A detailed **taxonomic discrimination policy (TDP) is available on the NMBAQC website** (Worsfold et al., 2023b) to accompany the processing requirement protocol (PRP) to ensure that macrobenthic data from multiple analysts are as consistent and inter-comparable as possible. 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.

11. Since the beginning of the scheme, continual improvement to the learning structure of the Scheme reports has been maintained. For the LR and OS modules, detailed results have been forwarded as **individual exercise reports** to each participating laboratory as soon after the exercise deadlines as practicable. The **Laboratory Reference Module Summary Reports introduced in 2017** show identification problems found in all LR submissions and should benefit all participants. In the RT module, after each RT exercise a bulletin was circulated, reviewing the literature used, detailing the accepted identification of the taxa circulated, and including images of relevant specimens. Participants are encouraged to review their exercise reports and **provide feedback concerning content and format** wherever appropriate.

12. 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 taxonomic groups in their Own Samples often receive additional support, as well as receiving their returned OS material separated, according to the AQC identifications, for future reference. Two of the seventeen 'failing' Own Samples in Scheme Year 2022 / 2023 (Year 29) have already been rectified via the recommended remedial action. Twelve failing samples resulted in optional 'review' remedial actions and these actions are deemed to have been completed. Three samples remain with pending remedial actions (one is a CMA sample). This year there has been an increase in the number of failed samples, along with a decrease in the average BCSI% score. However, the quality of sample processing observed this year remains in line with the general performance over recent scheme years, as the 'dip' is the result of a number of new participants joining the scheme.

APEM will continue to proactively chase outstanding remedial actions from previous scheme years to enable these data to be NMBAQC scheme quality assured. **Participants are reminded that completion of remedial action is mandatory for CMA labs and labs submitting data to CMAs. Participants are encouraged to provide feedback and request further information for any of the scheme exercises to improve the quality and consistency of their data.**

13. **Additional guidance for Own Sample 'next steps' following audit results** has been created to ensure that all participants and other stakeholders are aware of the route to quality assured data (Hall, 2016; *Own Sample Interim Report Review and Remedial Action Processes*).

14. There remain some misconceptions about the nature of the Scheme and the services it provides. It is not an accreditation scheme but provides quality assurance for the UK's CSEMP/WFD programme. In addition, the Scheme can provide **audits of samples** for any marine biological programme or development. It also provides **project-level audits** by applying the OS and LR protocols to examine project data. These services require more extensive communication (Scheme website, information note etc.) to notify all potential users and maintain consistent quality assurance for European marine data. A best practice guidance protocol for NMBAQC project-level audits needs to be produced and published on the scheme website. Meanwhile, it should be understood that a project level audit includes a review of data and check of reference collection specimens for the whole project, as well as for selected samples. Audits of samples from a project without more extensive reviews of data and other material do not constitute quality control of the whole project through the Scheme.

15. Despite protocol documents being produced for a recent Scheme year (Year 21, 2015-2016), misconceptions still exist regarding the purpose and methods for some of the Scheme's modules. **Protocol documents for all modules were reviewed and re-issued in 2017 (Ring Test Protocol, Laboratory Reference Protocol, Own Sample Exercise Protocol).**

16. APEM Ltd. strives to ensure smooth running and **transparency of the Scheme** at all times. APEM Ltd. log and make available all correspondence to the Benthic

Invertebrate Component Technical Manager (Myles O'Reilly, SEPA). 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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