



BEQUALM NATIONAL MARINE BIOLOGICAL ANALYTICAL QUALITY CONTROL SCHEME

Benthic Invertebrate Component Annual Report: Year 20 - 2013/2014

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BENTHIC INVERTEBRATE COMPONENT ANNUAL REPORT FROM APEM Ltd

SCHEME OPERATION - YEAR 20 - 2013/14

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Linked Documents (hyperlinked in this report): Ring Test Bulletin RT45 Ring Test Bulletin RT46 Macrobenthic Report MB21 Own Sample Module Summary Report – OS53, 54 & 55 Description of the Scheme Standards for the Benthic Invertebrate Component Guidelines for Processing Marine Macrobenthic Invertebrate Samples

1. Introduction

The NMBAQC) Scheme Invertebrate Component 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.

Year 20 of the Benthic Invertebrate Component (2013/14) followed the format of Year 19. A series of modules and exercises involved the distribution of test materials to participating laboratories and the centralized examination of returned data and samples. 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).

Thirty-eight laboratories participated in the benthic invertebrate component of the NMBAQC Scheme in Year 20, including nine Environment Agency Labs. Sixteen participants were Competent Monitoring Authorities (CMAs) and twenty were private consultancies, including five non UK consultancies. One of the participants was a consortium of sole traders. Six of the CMA participants were responsible for the Clean Seas Environment Monitoring Programme (CSEMP) sample analysis. To reduce potential errors and simplify administration, LabCodes were assigned in a single series for all laboratories participating in the benthic invertebrate, fish and particle size components of the NMBAQC Scheme.

As in previous years, some laboratories elected to be involved in limited aspects of the Scheme. CSEMP laboratories were required to participate in all relevant components of the Scheme, although this was not strictly enforced.

In this report, performance targets have been applied for the OS module only (see <u>Description of the Scheme Standards for the Benthic Invertebrate Component</u>). 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 – OS53, 54 and 55 (<u>Own Sample Module Summary Report – OS53, 54 & 55</u>) 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 20 of the National Marine Biological Analytical Quality Control (NMBAQC) Scheme.

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

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

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

Initially the Year 20 Benthic Invertebrate component administrator was Thomson Unicomarine Ltd. However they only completed the RT and MB modules and failed to undertake the LR and OS modules. The samples for the latter modules were transferred in their entirety to the new contractor APEM Ltd.

Two **Ring Tests (RT)** of 25 specimens were distributed (RT45 and RT46). Both sets contained 25 invertebrate specimens. RT45 was targeted on crustaceans and RT46 contained mixed species. In general, there was fairly good agreement between the identifications made by the participating laboratories and those made by Thomson Unicomarine Ltd.

For RT45 each participating laboratory recorded on average 1.08 generic differences and 2.04 specific differences. One taxon (the isopod *Lekanesphaera levii*) was responsible for more than a fifth of the specific differences.

For RT46 each participating laboratory recorded on average 1.2 generic differences and 2.6 specific differences. Three taxa (two polychaetes *Malmgrenia andreapolis* and *Eclysippe vanelli*, and one crustacean, *Aora gracilis*) were responsible for more than one third of the specific differences.

Analysis of the **Macrobenthic Sample (MB)** by the eight participating laboratories, and subsequent re-analysis by Thomson Unicomarine, provided information on the efficiency of extraction of the fauna, accuracy of enumeration and identification and the reproducibility of biomass estimations. For MB21, natural estuarine samples from the Medway River were distributed. Results for this macrobenthic exercise showed a high degree of agreement to the re-analysis by Thomson Unicomarine. Extraction efficiency (of individuals) was on average 96.37% with one laboratory achieving 100%, four laboratories achieving over 99%, and only one lab extracting less than the required 90% of individuals. Comparison of the results from the laboratories with those from analysis by Thomson Unicomarine (following the NMBAQC macrobenthic analysis guidelines) was made using the Bray-Curtis similarity index (untransformed). The value of the index varied between 93.6% and 100%. It was better than 90% in all eight labs and better than 95% in six labs.

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

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

1.1.1 Statement of Performance

Each participating laboratory was supplied with an SOP 'Statement of Performance', which included a summary of results of component modules and details of the resulting flags where appropriate. Previously the results of the Benthic Invertebrate, Particle Size Analysis, and Fish Components were includes in a single SOP. For Year 20 the results of each of these components were provided in separate SOP. Moreover the Year 20 Benthic Invertebrate Component SOPs were prepared by APEM Ltd who completed the OS and LR modules only. To minimise delays the results of earlier RT and MB exercises carried out under Thomson Unicomarine Ltd. were not included in the SOPs unless subsequently specifically requested by participants. These statements have been circulated since Year 5 (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.

The Ring Test (RT) and Macrobenthic Sample (MB) exercises were administered by Thomson Unicomarine Ltd. The Lab Reference (LR) and Own Sample (OS) exercises were administered by APEM Ltd. A summary of the module performances 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 along with a description of the preparation of the necessary materials and brief details of the processing instructions given to each of the participating laboratories.

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). Following termination of the administration contract with Thomson Unicomarine Ltd., the unaudited Lab Reference (LR) and Own Samples (OS) were uplifted by the Environment Agency before transferal to the new administrator of the component contract, APEM Ltd.

2.1.1 Data Returns

Return of data to Thomson Unicomarine Ltd. followed the same process as in previous years. Spreadsheet based forms (tailored to the receiving laboratory) were distributed to each laboratory via email, with additional hard copies where appropriate. All returned data have been converted to Excel 2003 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. Following termination of the administration contract with Thomson Unicomarine Ltd., the data for the unaudited Lab Reference (LR) and Own Samples (OS) was e-mailed to the Benthic Invertebrate Component contract, APEM Ltd.

2.1.2 Confidentiality

To preserve the confidentiality of participating laboratories, each are identified by a fourdigit Laboratory Code. In September 2013 each participant was given a confidential, randomly assigned Scheme Year 20 LabCode. Codes are prefixed with the Scheme Year to reduce the possibility of obsolete codes being used inadvertently by laboratories, *e.g.* Laboratory number four in Scheme Year 20 will be recorded as LB2004.

To further reduce potential errors and simplify administration, LabCodes were assigned in a single series for all laboratories participating in the benthic invertebrate, fish and particle size analysis components of the NMBAQC Scheme (as Thomson Unicomarine Ltd. was initially administering these three components).

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 whether any errors were the result of inadequate keys, lack of reference material or the incorrect use of satisfactory keys. The Year 20 RT module was administered by Thomson Unicomarine Ltd.

Two sets of 25 benthic invertebrate specimens were distributed in 2013/14. The first circulation (RT45) was targeted on crustacean and included 28% amphipods, 20% decapods, 20% isopods, 12% mysids, 8% cumaceans, 8% tanaids, and 4% Cirripedia.

The second circulation (RT46) was a general invertebrate test and included 32% polychaetes, 20% amphipods, 20% bivalves, 12% pycnogonids, 12% echinoderms, and 4% Gastropoda. 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 organisations. Every attempt was made to provide animals in good condition and of similar size for each laboratory. Each specimen sent 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.

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 to the keys, or other literature used, to determine their identifications. Specimens were to be returned to Thomson Unicomarine 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. The

protocols followed for the two circulations, in particular the method of scoring results, were the same as for previous circulations. Approximately eight weeks were allowed for the analysis of both RT exercises (RT45 and RT46).

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. CSEMP laboratories are required to participate in this exercise though the results are not used to assign 'Pass' or 'Fail' flags. In total 20 laboratories subscribed to RT45 and RT4. For RT45, 19 laboratories returned data (24 individual data sets). For RT46, 20 laboratories returned data (24 individual data sets).

2.2.2.2 Returns from Participating Laboratories

Identifications made by the participating laboratories were compared with those made by the AQC to determine the number of differences. In the case of an identification deviating from the AQC identification through the use of synonyms, or the misspelling of names, the difference was ignored for the purpose of calculating the total number of differences.

Tables 1 and 2 of the Ring Test Bulletins RTB45 and RTB46 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 AQC identification. Where it was considered that the name referred to the same species as the AQC identification, but differed for one of the reasons indicated above, the name was presented in brackets: "[name]". Errors of spelling or the use of a synonym are not bracketed in this way, if the species to which the laboratory was referring to was not the same as the AQC identification. 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 AQC 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 AQC 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 RTB45 and RTB46). 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 20 were presented in the Ring Test Bulletins (RTB) outlining the reasons for each individual identification discrepancy. These bulletins contained images of the test material and the alternative, incorrectly recorded taxa, where available. Participating laboratories were advised to retain their ring test specimens for a few weeks after receiving their results, in order to review their identifications, where necessary. On completion of each exercise, specimens were required to be returned to Thomson Unicomarine Ltd. for potential future circulation.

2.2.2.3.1 Ring test 45 (Type: Targeted)

The results discussed below are given in Table 1 of RTB 43 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 <u>Ring Test Bulletin – RTB45</u>).

Seven of the 25 specimens were amphipods, five were decapods, five were isopods, three were mysids, two were cumaceans, two were tanaids, and one was a cirriped. The agreement at generic level was generally good; 26 differences (from a potential 600) were recorded in the 24 data sets received from 19 participating laboratories. There was less agreement at species level, with 49 differences recorded, equal to 8% of all species identifications.

One of the specimens circulated was incorrectly identified at species level by more than onethird (46%) of the participants. This was the isopod Lekanesphaera levii. Nine of the 25 specimens circulated (i.e. the decapods *Hippolyte varians, Palaemon varians, Pandalus montagui, Crangon crangon,* the amphipods *Leptocheirus hirsutimanus, Jassa* falcata/herdmani, the isopods *Idotea linearis, Cyathura carinata* and the cirriped *Verruca stroemia*) were correctly identified by all participants.

Further details and analysis of results can be found in the Ring Test Bulletin RTB45 which was circulated to each laboratory that supplied results for this exercise and was also posted on the Scheme's website (<u>www.nmbaqcs.org</u>).

2.2.2.3.2 Ring test 46 (Type: General)

The results discussed below are given in Table 1 of RTB46 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 <u>Ring Test Bulletin – RTB46</u>).

Eight of the 25 specimens circulated were polychaetes, five amphipods, five were bivalves, three were pycnogonids, three were echinoderms and one was a gastropod. The agreement at genus level was again rather good; 27 errors (from a potential 600) were recorded in the 24 data sets received from 20 participating laboratories. Agreement at specific level was less than to RT45, with 63 differences recorded - equal to 10.5 % of all specific identifications.

Two of the specimens circulated were incorrectly identified at species level by nearly half and around a third of the participants. These were the polychaete, *Malmgrenia andreapolis,* and the amphipod, *Aora gracilis,* mis-identified in 11 (46%) and 8 (33%) of returns respectively.

Seven of the twenty-five specimens circulated (i.e. the polychaete, *Lepidonotus squamatus*, the bivalves, *Mytilus edulis, Abra tenuis,* and *Crenella decussata*, the amphipods, *Lysianassa ceratina, Socarnes erythrophthalmus*, and *Perrierella audouiniana*, and the ophiuroid, *Ophiura ophiura*) were correctly identified by all participants.

Further details and analysis of results can be found in the Ring Test Bulletin RTB46 which was circulated to each laboratory that supplied results for this exercise and was also posted on the Scheme's website (www.nmbagcs.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 RTB45 and RTB46 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:

<u>RT45</u>

Taxon	Nbr. species	Generic differences		Specific differences	
Amphipoda	7	7	26.9%	11	22.4%
Decapoda	5	0	0%	2	4.1%
Isopoda	5	1	3.8%	14	28.6%
Mysidacea	3	6	23.1%	10	20.4%
Cumacea	2	5	19.2%	5	10.2%
Tanaidacea	2	7	26.9%	7	14.3%
Cirripedia	1	0	0%	0	0%
TOT.	25	26	99.9%	49	100%

<u>RT46</u>

Taxon	Nbr. species	Generic differences		Specific differences	
Polychaeta	8	11	40.7%	26	41.3%
Bivalvia	5	2	7.5%	5	7.9%
Amphipoda	5	5	18.5%	14	22.2%
Pycnogonida	3	1	3.7%	8	12.7%
Echinodermata	3	5	18.5%	7	11.1%
Gastropoda	1	3	11.1%	3	4.8%
TOT.	25	27	100%	63	100%

2.2.3 Discussion

In RT45 most of the differences were attributable to three taxa – the isopod *Lekanesphaera levii*, the mysid *Schistomysis kervillei*, and the cumacean *Cumella pygmaea* with eleven, five and five specific differences respectively. Regarding the isopod *L. levii* most participants appeared to be unaware of the review by Jacobs (1987). In RT46 most of the differences were attributable to three taxa – the polychaetes *Malmgrenia andreapolis* and *Eclysippe vanelli*, and the amphipod *Aora gracilis*. Participants struggled with species level identification for *Malmgrenia*, and genus level identification for *Eclysippe*. For the *Aora* many participants used the incorrect name "*Aora typica*" cited in Lincoln's monograph, perhaps unaware that this has been subsequently corrected. It is surprising that some participants were unable to correctly identify the dogwhelk (*Nucella lapillus*) which is one of our most widespread intertidal species. The identification of the polycheate *Owenia* specimens circulated in this exercise was reviewed as it has only recently been realised that

more than one species occurs in British waters. APEM was the only ring test participant to identify their *Owenia* specimen as *Owenia* borealis, which prompted Thomson Unicomarine to revise the AQC identification for this taxon. Similarly the polychaete *Polygordius* was identified at genus level only as this taxon is currently the subject of taxonomic investigations which are not yet completed.

The results were in general comparable with those from previous exercises, with a high level of agreement between participating laboratories for the majority of distributed species. The RT component is considered to provide a valuable training mechanism and to be an indicator of problem groups and possible areas for further 'targeted' exercises for inclusion at taxonomic workshops. Multiple data entries from each 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 variety of problems encountered during these ring tests via Ring Test Bulletins RTB45 & RT46, which include also a list of useful literature.

The best results were obtained by LB2004, LB2026a-e, LB2054a, LB2060 and LB2062 for RT45 with zero differences at genus level and species level. In RT46 the best participants were LB2004, LB2008a, LB2031, LB2054, LB2062 and LB1908 with zero differences at generic level and species 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 overemphasized; 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 or WFD reference collections. This was the eighteenth Laboratory Reference exercise (LR18). Participants submitted up to 25 specimens for re-examination by Thomson Unicomarine Ltd. However, Thomson Unicomarine Ltd. were unable to complete analysis of seven out of eight of the sets received so the specimens were sent to APEM Ltd for analysis.

2.3.1.1 Preparation of samples

A results sheet including labels for each taxon pot was distributed with the protocol for the exercise. Participating laboratories were asked to prepare and submit their reference specimens according to instructions from Thomson Ecology. 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, eight laboratories participated in the Laboratory Reference exercise (LR18). Detailed results have been separately reported to each of the participating laboratories. Misidentifications were found from a variety of faunal groups, often where species belong to genera which are either speciose or for which keys are complex or were inadequate at the time the specimens were submitted (for example, scale-worms, syllids and sabellids). The majority of taxonomic errors could be attributed to the submitted polychaetes (32% of the total polychaete taxa submitted and 55% of the errors over all) and molluscs (44% of the total mollusc taxa submitted and 23% of the errors over all).

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. Some laboratories sent well known species while others 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. The Year 20 MB module (with sample MB21) was administered by Thomson Unicomarine Ltd. In the case of MB21, natural estuarine samples from the Medway River 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 <u>Macrobenthic Exercise Results – MB21</u> and <u>Worsfold, Hall & O'Reilly (Ed.) 2010</u>). 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 12 weeks. All sorted and unsorted sediments and extracted fauna were to be returned to Thomson Unicomarine Ltd., together with the data on counts and biomass determinations.

2.4.1.2 Post-return Analysis

Upon return to Thomson Unicomarine Ltd., the various parts of the MB samples were reexamined. 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 member of Thomson Unicomarine staff, using a standard technique.

Prior to analysis of the differences found between the participants' and Thomson Unicomarine'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 (MB21) was a natural estuarine sample from the Medway River, Kent. Eight laboratories returned fauna and data for re-analysis. Two participating laboratories did not supply biomass data. A report for this exercise was distributed to the participating laboratories (<u>Macrobenthic Sample Results – MB21</u>) and was also posted on the Scheme's website (<u>www.nmbaqcs.org</u>).

2.4.2.2 Efficiency of Sample Sorting

Table 1 of the MB21 Report presents a summary of the numbers of taxa and individuals counted by each of the participating laboratories for sample MB21, together with the corresponding counts made by Thomson Unicomarine 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 shows the variation between laboratories in the percentage of taxa identified in the samples. Compared to the number of taxa found by Thomson Unicomarine five of the eight laboratories (LB2026, LB2027, LB2029, LB2061 and LB2062) extracted the same number of taxa. Three other laboratories (LB2033, LB2034 and LB2035) extracted fewer taxa, the re-analyses showing that new taxa were present in the residue and each lab missed one taxon. No laboratory recorded more taxa than Thomson Unicomarine.

2.4.2.4 Number of Individuals

Re-analysis of the sample residues showed that four out of eight participants missed individuals (see columns 11 and 12 of Table 1). The proportion of missed individuals in the samples was less than 5%, except for Lab LB2033 where 15.8% were missed. Of the latter, 15 individuals were the gastropod *Peringia ulvae*.

In the worst case (LB2033), 19 out of 120 individuals (15.8%) were not extracted during the initial sample processing and in the best case, no individuals from 528 (0.0%) were missed in the residue. One sample, LB2027, only had four taxa and 10 individuals present.

A breakdown of the missed individuals by taxonomic group is presented in Table 2. Of the four major taxa present, Polychaeta and Crustacea were the best 'picked' faunal group, with many of the participants picking all individuals. The worst extracted faunal group was Crustacea and "Other" with 100% missed but in reality these were only a single specimen missed from each group. In practical terms the picking of Mollusca was poorest with LB 2033 missing 76% (16 individual specimens).

2.4.2.5 Uniformity of Identification

Seven of the participating laboratories correctly identified all taxa (Table 1, column 15), and one laboratory (LB2035) misidentified only one taxon.

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 Thomson Unicomarine. The comparison was made by calculating the Bray-Curtis similarity index for the pair of samples using non-transformed data. The results of this calculation are presented in column 14 of Table 1. The variation among laboratories in the values calculated for the index ranged from 93.6% to 100%, with an average value of 97.9%. All labs exceeded 90% and the index for the majority of laboratories (6 of 8) was better than 95%; these six laboratories would achieve 'Pass Good' sample flags under NMBAQC / CSEMP standards, if applied. Further details of each participating laboratory's performance are given in the MB21 report.

2.4.2.7 Biomass Determinations

A comparison of the biomass estimates made by the participating laboratories and Thomson Unicomarine, broken down by major taxonomic group for the MB20 sample, is presented in Table 3. Two laboratories did not supply biomass data. For five of the labs with The difference between the two total weight values for taxon groups ranged between -2.4% and +16.7% (*i.e.* latter heavier than the weight values made by Thomson Unicomarine) with the sixth lab recording -409.5% due a transcription error. The overall biomass percentage differences between participating laboratories and Thomson Unicomarine ranged from +0.7% to +6.7% for the five labs (and -343% for the sixth lab with the transcription error!)

2.4.3 Discussion

As in the previous year, results indicated a high degree of agreement to the re-analysis by Thomson Unicomarine Ltd. Extraction efficiency (of individuals) was on average 97.55%, with four laboratories achieving 100% and all laboratories (except LB2033) extracting more than the required 90% of individuals. Comparison of the results from the laboratories with those from analysis by Thomson Unicomarine Ltd. (following the NMBAQC macrobenthic analysis guidelines) was made using the Bray-Curtis similarity index (untransformed). The value of the index varied between 93.6% (LB2033) and 100% (LB2027), was better than 90% in all of the comparisons and lower than 95% in only two laboratories. The average BCI of 97.9% for this natural estuarine sample is the highest achieved over the last 18 years.

Summary of average Bray Curtis similarity indices achieved overall:

MB21 (estuarine)	98%
MB20 (intertidal)	93%
MB19 (fully marine)	92%
MB18 (artificial)	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%
MB06 (estuarine) MB05 (coastal)	91% 85%

The 'blot-drying' procedure employed by Thomson Unicomarine for the determination of biomass was as specified in the <u>Green Book</u>, *i.e.* avoiding excessive pressure when blotting specimens dry. The estimates of total biomass made by three of the participating

laboratories and Thomson Unicomarine show some variation. As discussed in the previous MB reports, 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 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 project load. 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

All instructions were provided to participating laboratories by the previous contractor, Thomson Unicomarine Ltd. Available material from the selected samples was delivered from the previous contractor to APEM Ltd. on 15th October 2014. All material from the sample was provided 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 (<u>Worsfold, Hall & O'Reilly</u> (<u>Ed.), 2010</u>). 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.

Data submission, sample preparation and submission times were defined by the previous contractor. 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 that utilised for the Year 19 MB exercise.

2.5.2 Results

2.5.2.1 General Comments

A total of 102 Own Samples were received from 34 laboratories, together with descriptions of their origin and the collection and analysis procedures employed. The OS submission included samples from the Mediterranean which were processed in the same manner as all other Own Samples. APEM Ltd. are capable of auditing macrobenthic samples from any biogeographical region. Samples were identified as OS53, OS54 and OS55 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 2 to 145, with the number of countable individuals from 1 to 5542. Of the 102 submitted Own Samples, 16 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 – OS53, 54 & 55.

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 44 samples out of the total 102, the number of taxa recorded by the participating laboratories was identical to that obtained by APEM Ltd. (column 3). In the remaining 58 cases, the difference was on average 3.4 with a maximum of

14 taxa. Data for the numbers of individuals recorded (columns 12 and 13, Table 1) show a range of differences from re-analysis of 0% to 100%. The average difference was 6.1%, with 19 samples exceeding this average.

35 of the 102 samples reported showed 100% extraction of fauna from the residue (column 6), and in 65 samples between 1 and 568 individuals had been missed during processing. One sample was provided without residue and one sample missed 2 taxa which could not be enumerated. In just 21 of these 65 samples only individuals attributed to taxa already recorded in the sample were found. In the other 44 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 14 with an average of 1.3 new taxa per sample (across all 102 samples). The poorest extraction record for taxa was a total of 14 missed taxa (and 185 individuals). The poorest extraction record for individuals was a total of 568 missed individuals (and 5 missed taxa). A breakdown of the missed individuals by taxonomic group is presented in Table 2 of the OS Summary Report. The average number (across all 102 samples) of missed individuals found upon re-sorting the residue was 19.39.

2.5.2.3 Uniformity of Identification

Taxonomic differences between APEM Ltd. and participating laboratories' results were found in 68 (67%) of the 102 own samples. A summary of mis-identified taxa is presented in Table 3 of the OS Summary Report. An average of 2.5 taxonomic errors per sample (or 3.7 errors in the affected samples) was recorded; in the worst instance, 18 identification errors occurred. A large variety of samples (and fauna) was received. Polychaetes accounted for 57%, Mollusca for 17% and Crustacea for 12% 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 previous OS exercises. The Bray-Curtis similarity index figures (Table 1, column 23) ranged from 15.9% to 100%, with an average figure of exactly 90%. Twenty nine samples from sixteen laboratories achieved a similarity figure of less than 90%. Fourteen samples produced a similarity figure of 100%; these were submitted by fourteen different laboratories (BI_2001, BI_2005, BI_2007, BI_2009, BI_2029, BI_2031, BI_2036, BI_2044, BI_2045, BI_2049, BI_2050, BI_2052, BI_2054, BI_2059). The best overall result was achieved by BI_2051 with an average

similarity index of 99.68% across all three Own Samples. The lowest overall result was achieved by BI_2071 with an average similarity index of 35.5% over all three samples. This is the poorest performance ever of any lab for the Own Sample module.

2.5.2.5 Biomass Determinations

It was not possible to make an accurate comparison of the biomass determination in all cases; 22 samples (21.6%) were supplied with species blotted wet weight biomass data and could be 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 Overall biomass ranged from -0.1% to +55.9%. 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 <u>Green Book</u> and the NMBAQC guidelines for macrobenthic sample analysis (<u>Worsfold</u>, Hall & O'Reilly (Ed.) 2010).

2.5.3 Discussion

The average Bray-Curtis similarity index of 90% achieved for this Own Sample Module shows that the agreement between the participating laboratories and APEM Ltd. was generally acceptable.

There were 102 samples submitted for the Own Sample Module, including the 16 processed by the Scheme's external auditor. Of the 102 samples, 73 (72%) exceeded the 90% Bray-Curtis Pass mark and 55 (54%) 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 and 07 achieved less than 72% exceeding the 90% Bray-Curtis Pass mark (see Table 5 of the OS Summary Report).

Since the beginning of the Own Sample Module, 1012 admissible samples have been received (OS01-55). Of these, 208 samples (17%) 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 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.

It is worth noting that the results of LB2071 were the worst witnessed in the 19 years of the Own Sample module. As a result of these poor results the average BCSI% has been reduced and would have given a result of 91.65% without the results of this participant.

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 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 exercises have been used in 'flagging' for the purposes of assessing benthic invertebrate data for the CSEMP/WFD programmes.

As the component progresses, additional exercises may be included for flagging. In the meantime, the other exercises of the component 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 less than half (44%) of participating laboratories (15 of 34) 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. Eighteen laboratories (<50%) achieved a Bray Curtis of >90% for all three of their Own Samples.

Overall, 77% of the comparisons were considered to have passed the enumeration of taxa standard, 83% exceeded the enumeration of individuals standard and 72% passed the Bray-Curtis comparison standard (>90%). Performance with respect to the biomass standard was acceptable (Table 1, column 22) with 73% of the samples with submitted biomass values meeting the required standard.

NMBAQC Scheme / CSEMP/WFD 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); 19 samples (18.6%) are flagged as 'Fail - Bad', 10 (9.8%) as 'Fail – Poor', 18 (17.6%) as 'Pass - Acceptable', 41 (40%) as 'Pass - Good' and 12 (11.8%) 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 2.3.4.3 Remedial Action below).

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 <u>OS</u> <u>Summary Report</u>. 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 <u>Description of the Scheme Standards for the Benthic Invertebrate</u> <u>Component</u>). This year's 102 Own Samples resulted in a pass rate of 72% (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 Water Framework Directive (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 the current component administrator, APEM Ltd. Completion of remedial action is mandatory for labs contributing to CSEMP/WFD programmes or other UK national programmes or provision of data under licence to UK Competent Monitoring Authorities (CMAs e.g. EA, CEFAS, MSS, NRW, SEPA, NIEA, NE, SNH). APEM Ltd. or the NMBAQC Scheme Contract Manager should be notified when remedial action 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 20. Twenty nine 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:

Lab Code	OS no.	Remedial action	Notes
	OS53	Reprocess associated residues and taxonomic errors.	Remedial action completed 8/4/15
BI_2016	OS54	All residues & taxa must be retained until external QA complete; reprocess taxonomic errors; provide details of project residues available & resubmit revised data for further auditing	Remedial action recommended
BI_2029	OS53	Correct identification errors throughout project; correction of Lumbrinerids increases BCSI for this sample to 93.939%	Remedial action completed 4/6/15

CMA samples:

	OS54	Correct identification errors throughout project; correction of Lumbrinerids increases BCSI for this sample to 92.226%	Remedial action completed 4/6/15
BI_2046	OS53	Review extraction methods.	Remedial action completed 18/3/15
	OS54	Review taxonomic errors	Remedial action completed 2/7/15
BI_2047	OS55	Reprocess residue and taxa of all associated samples; Where residues are subsampled, ensure that all processing components are retained in separate, clearly labelled vials	Remedial action complete 2/7/15 but to be evaluated
	OS53	Reprocess residues and taxonomic errors for all associated samples	Remedial action complete 2/7/15 but to be evaluated
BI_2048	OS54	Reprocess taxonomic errors and review residue sorting methods for all associated samples	Remedial action complete 2/7/15 but to be evaluated
	OS55	Reprocess residues and taxonomic errors for all associated samples	Remedial action complete 2/7/15 but to be evaluated
BL 2050	OS53	Reprocess taxonomic errors for all associated samples	Remedial action recommended
ві_2059	O\$55	Review taxonomic errors and review residue sorting methods	Remedial action recommended

Non-CMA samples:

Lab Code	OS no.	Remedial action	Notes
BI_2001	OS55	Review Hydrobidae / Cochliopidae identification	Remedial action recommended
BI_2002	OS53	Review taxonomic errors	Remedial action completed 2/4/15
BI_2017	OS55	Reprocess associated sample residues; review taxonomic errors	Remedial action recommended
	OS54	Re-sort of residues; Check Bathyporeia identification through batch	Remedial action recommended
BI_2019	OS55	Re-sort of residues; Check Phyllodoce and Mactra identification through batch	Remedial action recommended
BI_2023	OS53	Report revised following external review of Paradialychone filicaudata; Review identification errors through project	Remedial action completed 29/4/15
BI_2030	OS55	Review Nereididae identification policy, especially for juvenile specimens	Remedial action completed 16/7/15
BI_2033	OS53	Reprocess sediments and review taxonomic errors	Remedial action recommended

	O\$54	Reprocess sediments and review taxonomic error; check biomass for transcription errors	Remedial action recommended
	OS55	Reprocess sediments and review taxonomic errors	Remedial action recommended
	OS53	Reprocess taxonomic errors throughout project; review brittlestar & phoronid enumeration policy	Remedial action recommended; review undertaken and discussions ongoing.
BI_2056	OS54	Reprocess taxonomic errors throughout project; review extraction methods	Remedial action recommended; review undertaken and discussions ongoing.
	OS55	Reprocess taxonomic errors throughout project	Remedial action recommended; review undertaken and discussions ongoing.
BI_2058	OS54	Review Cirratulidae identification through the batch	Remedial action recommended
	OS53	Reprocess residues and review taxonomic errors in all affected samples	Remedial action recommended
BI_2071	OS54	Reprocess residues and review taxonomic errors in all affected samples	Remedial action recommended
	OS55	Reprocess residues and review taxonomic errors in all affected samples	Remedial action recommended

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:

- 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).
- 2. 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.
- **3.** 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.
- 4. 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 intercomparable as possible.

- 5. 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. Eight of the twenty nine 'failing' Own Samples in Scheme Year 20 have already been rectified via the recommended remedial action. Four others samples have had remedial action completed and are to be evaluated by APEM Ltd.
- 6. 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 leads to delays to the component reports.
- 7. 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.
- 8. Year 20 included a laboratory from Italy. This is the first year that samples from the Mediterranean have been included in the Own Sample Module. The samples were received and processed in the same manner as samples from the North East Atlantic. It is recommended that the Scheme be advertised more widely. Participants and prospective participants are reminded that APEM Ltd. are able to accept and audit samples from all geographic regions.

4. References

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