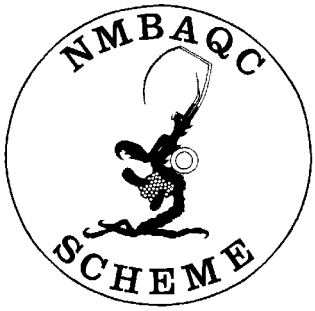

The National Marine Biological Analytical Quality Control Scheme

Benthic Invertebrate Component Report from the Contractor Scheme Operation – Year 16 2009/10

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BENTHIC INVERTEBRATE COMPONENT REPORT FROM THE CONTRACTOR

SCHEME OPERATION – YEAR 16 – 2009/10

Linked Documents (hyperlinked in this report)

Ring Test Bulletin – RTB#37

Ring Test Bulletin – RTB#38

Macrobenthic Exercise Results – MB17

Own Sample Module Summary Report – OS41, 42 & 43

Description of the Scheme Standards for the Benthic Invertebrate Component

1. Introduction

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

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

The sixteenth year of the Scheme (2009/10) followed the format of the fifteenth year. 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 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. Fifteen participants were government laboratories; twenty-three were private consultancies. Thirteen of the participants were responsible for CSEMP (Clean Seas Environment Monitoring Programme) sample analysis (excluding subcontracted samples). To further reduce potential errors and simplify administration, LabCodes were assigned in a single series for all laboratories participating in the benthic invertebrates, fish and particle size components of the NMBAQC Scheme (due to Unicomarine administering these three components).

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

In this report performance targets have been applied for the OS module only (see Description of the Scheme Standards for the Benthic Invertebrate Component). These targets have been applied to the results from laboratories and "Pass" or "Fail" flags assigned accordingly. As these data have been deemed the basis for quality target assessment, where laboratories failed to fulfil these components through not returning the data, a "Fail" flag has been assigned. These flags are indicated in the Tables presenting the comparison of laboratory results with the standards (see Table 5 in Own Sample Module Summary Report – OS41, 42 & 43).

1.1 Summary of Performance

This report presents the findings of the Benthic Invertebrates component for the sixteenth year of operation of the National Marine Biological Analytical Quality Control (NMBAQC) Scheme.

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

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

The analytical procedures of the various modules were the same as for the fifteenth year of the Scheme. The results for each of the Scheme exercises are presented and discussed. Comments are provided on the performance for each of the participating laboratories in each of the exercises.

Two **Ring Tests (RT)** of twenty-five animal specimens were distributed. One set contained twenty-five general invertebrate fauna (RT37) and a second set consisted of 'targeted' specimens from taxa that recorded no errors in previous ring tests (RT38; 'Beginners' Training Pack'). For the general set of fauna (RT37) there was fairly good agreement between the identifications made by the participating laboratories and those made by Unicomarine Ltd. On average each participating laboratory recorded 3.8 generic errors and 7.5 specific errors. Over half of the generic errors can be attributed to five

polychaete, one mollusc and one crustacean taxa. The 'targeted' ring test (RT38; 'Beginners' Training Pack'), as expected posed very few problems for species identification. On average each participating laboratory recorded just 1.9 generic errors and 2.5 specific errors. Six specimens were misidentified by six or more of the thirty-five participants and were responsible for 51% of all generic and 51% of specific errors recorded.

Laboratory Reference (LR): The identification of a set of twenty-five species selected and supplied by the participating laboratories was generally accurate. No clear problem areas were identified. However, there were differences in the approach to this exercise by the individual laboratories. For example, some laboratories used this as a test for confirming voucher specimens whilst others sought a means of having 'unknowns' identified.

Analysis of the **Macrobenthic sample (MB)** by the participating laboratories and subsequent re-analysis by Unicomarine Ltd. provided information on the efficiency of extraction of the fauna; accuracy of enumeration and identification and the reproducibility of biomass estimations. MB17 samples were artificially created by Unicomarine Ltd. to include set volumes of residue and known quantities of pre-identified fauna. These replicate samples have been based upon subtidal marine fauna and sediment from Torbay. Agreement between the laboratories and Unicomarine Ltd. was variable with generally good results, which were similar to those achieved in comparable previous MB exercises. The samples posed few problems associated with faunal extraction and identification of the taxa. Extraction efficiency (of individuals), irrespective of sorting, was on average 94.9%; eleven of the thirteen participating laboratories extracted greater than 90% of the individuals from the residue; two of the laboratories extracted all fauna from their residue. Comparison of the results from the laboratories with those from analysis by Unicomarine Ltd. (following the processing protocols specified by the participating laboratories) was made using the Bray-Curtis similarity index (untransformed). The value of the index varied between approximately 75.6% and 99.2% and was better than 90% in 85% of comparisons and greater than 95% in 23% of comparisons. As observed in all previous MB exercises, a variety of sample processing methodologies were followed by the participants (*e.g.* one participant identified to family level only and over half of the participants exclude specified faunal groups from their MB analyses); such differences reduce the comparability of results.

The Scheme year ten revised protocols for 'blind' **Own Sample (OS)** audits were continued in this Scheme year. Laboratories were to submit full completed data matrices from their previous year's Clean Seas Environment Monitoring Programme (CSEMP 2008; formerly NMMP) samples or alternative sampling programmes (if not responsible for CSEMP samples). The OS 'pass/fail' flagging system, introduced in Scheme year eight, was continued (see Description of the Scheme Standards for the Benthic Invertebrate Component). The results for the Own Samples were generally as good or better than those from the Macrobenthic sample. Agreement between the laboratories and Unicomarine Ltd. was generally very good. Extraction efficiency, irrespective of sorting, was better than 90% in 89% of comparisons and better than 95% in 82% of all comparisons. All countable faunal specimens were extracted from the sample residues in 48% (45) of the samples. The Bray-Curtis similarity index ranged from 47.1% to 100% with an average figure of 93.7%. The Bray-Curtis similarity index was greater than 95% in 60% of comparisons and in most cases (81%) the value of the index was greater than 90%, these samples all achieved 'pass' flags. Eighteen samples (19%) achieved 'excellent' pass flags with Bray-Curtis similarity scores of 100%.

1.1.1 Statement of Performance

Each participating laboratory was supplied with a 'Statement of Performance', which included a summary of results for each of the Scheme modules and details the resulting flags where appropriate. These statements were first circulated with the 1998/1999 annual report, for the purpose of providing proof 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 identification (RT), Laboratory Reference voucher specimen identification (LR), Macrobenthic sample analysis (MB) and Own Sample (OS) reanalysis modules.

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

2.1.1 Logistics

The labelling and distribution procedures employed previously have been maintained and specific details can be found in the Scheme's annual reports for 1994/95 and 1995/96 (Unicomarine, 1995 & 1996). Email was the primary means of communication for all participating laboratories. This has considerably reduced the amount of paper required for the administration of the Scheme.

2.1.2 Data Returns

Return of data to Unicomarine Ltd. followed the same process as in previous years. Spreadsheet based forms (tailored to the receiving laboratory) were distributed for each circulation 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.

2.1.3 Confidentiality

To preserve the confidentiality of participating laboratories, each are identified by a four-digit Laboratory Code. In July 2009 each participant was given a confidential, randomly assigned Scheme year sixteen 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 sixteen will be recorded as LB1604.

In this report all references to Laboratory Codes are the post-August 2009 codes (Scheme year sixteen), unless otherwise stated. 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 (due to Unicomarine administering these three components).

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

2.2.1 Description

This training module examined inter-laboratory variation in the participants' ability to identify fauna and attempted to determine whether any errors were the result of inadequate keys, lack of reference material (*e.g.* growth series), or the incorrect use of satisfactory keys.

Two sets of twenty-five benthic invertebrate specimens were distributed in 2009/10. The first of the year's RT circulations (RT37) was a general invertebrate ring test. The specimens included representatives of the major phyla and approximately 52% of the taxa were annelids, 32% were molluscs and 16% were crustaceans. The second circulation (RT38) comprised 'targeted' specimens of taxa without errors from previously ring tests (a 'beginners' training pack'); participants were not aware of the theme of this exercise. The specimens included representatives of the major phyla and approximately 44% of the taxa were annelids (36% polychaetes and 8% oligochaetes), 36% were molluscs, 16% were crustaceans and 4% were echinoderms. 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.

For the standard RT (RT37) and the 'targeted' RT (RT38), all specimens were taken from replicate trawls, grabs or cores within a single survey and in most cases they were replicates from a single sampling station.

2.2.1.2 Analysis Required

The participating laboratories were required to identify each of the RT specimens to species and provide the Species Directory code (Howson & Picton, 1997) for the specimen (where available). If a laboratory would not routinely have identified the specimen to the level of species then this should be detailed in the 'confidence level' field. Laboratories can also add brief notes and information on the keys or other literature used to determine their identifications. Specimens were to be returned to Unicomarine Ltd. for verification, resolution of any disputed identifications and potential re-use in future Scheme exercises. The implementation of this part of the Scheme was the same as previous years. The two RT circulations were accompanied by details of each specimen's habitat details (depth, salinity, substratum, and geographical location). Participating laboratories were permitted to supply multiple data entries for each exercise to maximise results and enhance the training aspect 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 **nine weeks** were allowed for the analysis of the both RT exercises (RT37 and RT38).

2.2.2 Results

2.2.2.1 General Comments

A number of laboratories use these modules of the Scheme for training purposes and have selected them preferentially over other modules. CSEMP laboratories are required to participate in this component though it is not used when assigning 'pass' or 'fail' flags. In total twenty-four laboratories were distributed with RT37 and RT38 specimens. For RT37, all twenty-four laboratories returned data (thirty individual data sets). For RT38, all twenty-four laboratories returned data (thirty-five individual data sets).

2.2.2.2 Returns from Participating Laboratories

Each laboratory returned a list of their identifications of the taxa. The identifications made by the participating laboratories were then compared with the AQC identifications to determine the number of differences. A simple character-for-character comparison of the text of the two names (the AQC identification and the laboratory identification) was the starting point for this determination and provided a pointer to all those instances where (for whatever reason) the names differed. Each of these instances was examined to determine the reason for the difference.

As previously found, the main cause of an identification being different from the AQC identification was through differences in spelling of what was clearly intended to be the same species or the use of a valid synonym. There were several examples of these differences:

- Use of a different synonym for a taxon, *e.g. Portlandia philippiana* for *Yoldiella philippiana*.
- Simple mis-spelling of a name, *e.g. Macolma baltica* for *Macoma balthica*.

NB. For the purposes of calculating the total number of differences in identification made by each laboratory a difference was ignored if it was clearly a result of one of the above.

Tables 1 and 2 (Ring Test Bulletin – RTB#37) presents the identifications made by each of the participating laboratories for the twenty-five specimens in circulations RT37, arranged by specimen and by laboratory respectively. Tables 1 and 2 (Ring Test Bulletin – RTB#38) present the identifications made by each of the participating laboratories for the twenty-five specimens in circulations RT38, 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, then the name is presented in brackets "[name]". Errors of spelling or the use of a different synonym are not bracketed in this way if the species to which the laboratory was referring 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 method of scoring was to increase a laboratory's score 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 RTB37 and RTB38). Two separate scores were maintained; for differences at the level of genus and species. These are not independent values, if the generic level identification was incorrect then the specific identification would normally also be incorrect, though the reverse is not necessarily the case.

2.2.2.3 Ring Test Distribution Results

The RT circulations are designed as a learning exercise to discover where particular difficulties lie within specific common taxa. Results were forwarded to the participating laboratories as soon as practicable. Each participant also received a ring test bulletin (RTB37 and RTB38), 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 instructed to retain their ring test specimens, for approximately three weeks after the arrival of their results, to facilitate an improved learning dimension via the essential 'second look'. RT37 specimens were required to be returned to Unicomarine for potential future circulation; participants were permitted to retain the RT38 specimens and utilise them for in-house testing or add them to their faunal reference collections.

2.2.2.3.1 Thirty-seventh Distribution – RT37

Table 2 (Ring Test Bulletin – RTB#37) presents the results for the RT37; Table 1 displays these data arranged by species to enable quick reference to the range of answers received. One of the specimens was donated by Lee Heaney (SEPA, Riccarton); another was donated by Carol Milner (APEM). Thirteen of the twenty-five specimens circulated were polychaetes; eight were molluscs; and four were crustaceans. The agreement at the generic level was generally good; one-hundred and fifteen errors (from a potential seven hundred and fifty) were recorded in the thirty data sets received from twenty-four participating laboratories. Agreement at the specific level was also generally good; two hundred and twenty-five errors were recorded. One laboratory only identified the ring test specimens to family level and consequently recorded twenty-five errors for both generic and specific identifications. Eight of the specimens circulated were incorrectly identified at species level by at least a third of the participants. These taxa, responsible for the majority of differences, are described briefly below.

The bulk of the errors recorded could be attributed to eight specimens. *Marenzelleria viridis* (medium, fair specimen; specimens verified by Dr Vasily Radashevsky), *Desdemona ornata* (medium, good / fair specimen), *Eusarsiella zostericola* (medium, good specimen), *Odostomia turrita* (medium, good specimen), *Yoldiella philippiana* (medium, good specimen), *Yoldiella philippiana* (small, good specimen), *Pholoe assimilis* (small, fair specimen) and *Parvicardium scabrum* (medium, good specimen) accounted for a total of 43% of all generic and 56% of all the specific differences recorded. One of these specimens, *Odostomia turrita*, was incorrectly identified at species level by all except eight participating laboratories, however just three generic errors were recorded. One of the twenty-five circulated specimens, *Ficopomatus enigmaticus* (small / medium, good specimen), was correctly identified by all participating laboratories (excluding results from the laboratory identifying to family level only). Further details and analysis of results can be found in the Ring Test Bulletin (Ring Test Bulletin – RTB#37) 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 Thirty-eighth Distribution – RT38

RT38 contained twenty-five specimens from taxa that recorded no errors in previous ring tests. The results from the circulation are presented in Table 2 (Ring Test Bulletin – RTB#38) in the same manner as for all previous RT circulations. Table 1 displays these data arranged by species to enable quick reference to the range of answers received. Participants were permitted to retain the test specimens for future in-house training or as voucher specimens. The agreement at the generic level was very good, as expected; sixty-seven errors (from a potential eight hundred and seventy-five; 8% of all the generic

identifications) were recorded in the thirty-five data sets received from twenty-four participating laboratories. Agreement at the specific level was also very good; Eighty-eight errors (10% of all species identifications) were recorded. One laboratory only identified the ring test specimens to family level and consequently recorded twenty-five errors for both generic and specific identifications. Six of the specimens circulated were incorrectly identified at species level by approximately 17% of the participants. These taxa, responsible for the majority of differences, are described briefly below.

The bulk of the errors recorded could be attributed to six specimens. *Heterochaeta costata* (medium, good specimen), *Spio martinensis* (medium / small, good specimen), *Lagis koreni* (medium, good specimen), *Magelona alleni* (medium / large, poor / fair specimen), *Pseudoprotella phasma* (small, poor specimen) and *Crepidula fornicata* (small, fair specimen) accounted for a total of 51% of all generic and 51% of all the specific differences recorded. Six of the twenty-five circulated specimens were correctly identified by all participating laboratories (excluding results from the laboratory identifying to family level only); these specimens comprise *Goodallia triangularis* (medium, good specimen), *Poecilochaetus serpens* (medium, good / fair specimen), *Pisione remota* (variable size, fair specimen), *Turritella communis* (medium / large, good specimen), *Caecum glabrum* (medium, good specimen) and *Echinocyamus pusillus* (medium, good specimen). Further details and analysis of results can be found in the ring test bulletin (Ring Test Bulletin - RTB#38) 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

The ring test bulletins (Figure 1 in RTB37 and Figure 1 in RTB38) present the number of differences recorded at the level of genus and species for each of the participating laboratories, for RT circulations RT37 and RT38. One laboratory only identified the ring test specimens to family level and consequently recorded twenty-five errors for both generic and specific identifications. The laboratories are ordered by increasing number of differences at the level of species. 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

Most of the differences of identification in the general RT37 were of polychaetes and crustaceans Polychaete specimens (thirteen specimens in total) were responsible for 63% of generic differences and 43% of the total number of specific differences. Eight of the total twenty-five specimens circulated were molluscs and these produced 23% of the generic and 44% of the specific differences recorded. Four crustacean specimens were responsible for 14% of generic differences and 13% of the total number of specific differences.

2.2.3 Discussion

The results were in general comparable with those from all 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 be an indicator of problem groups and possible areas for further 'targeted' exercises or inclusion at taxonomic workshops. Multiple data entries from each laboratory and the inclusion of images in the ring test bulletins (RTB) have further emphasised the learning aspect of this component.

RT37 identified potential discrepancies with literature used by some participating laboratories for their identification of the *Tharyx* sp. A, *Desdemona ornata*, *Eusarsiella zostericola*, *Chaetozone gibber*, *Pterolysippe vanelli*, *Yoldiella philippiana*, *Pholoe assimilis*, and *Caulleriella alata* specimens. One Laboratory (LB1606) identified all twenty-five RT37 specimens correctly. *Yoldiella philippiana* was distributed twice in RT37, one medium and one small specimen; both specimens proved to be difficult for the participants; the smaller specimen produced 75% more specific identification errors than the medium sized specimen. All participating laboratories have been made aware of the variety of problems encountered for these ring tests via the RT37 ring test bulletin (Ring Test Bulletin - RTB#37).

RT38 identified potential discrepancies with literature used by some participating laboratories for their identification of the *Kurtiella bidentata* specimen. RT38 was a targeted upon taxa without errors from previous ring tests and consequently fifteen of the thirty-five participants (LB1601, 1602, 1605a, 1606, 1607a, 1607b, 1607c, 1608b, 1608c, 1608d, 1621a, 1622, 1633, 1635 and 1637) identified all of the

twenty-five RT38 specimens correctly. It must be noted that several participating laboratories and analysts have changed over the previous Scheme years, contributing to more errors in this ring test than anticipated when observing previous ring test performances. All participating laboratories have been made aware of the variety of problems encountered for these ring tests via the RT38 ring test bulletin (Ring Test Bulletin - RTB#38).

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

2.3.1 Description

This training module encourages laboratories to build extensive, verified reference collections to improve identification consistency. The value of reference material in assisting the process of identification cannot be over-emphasised; the creation and use of reference collections are viewed as best practice. Accordingly the Laboratory Reference (LR) module of the Scheme was introduced in Scheme year three (1996/97). This module assesses the ability of participating laboratories to identify material from their own area, or with which they are familiar. This was the fourteenth Laboratory Reference exercise (LR14). The participants were required to submit a reference collection of twenty-five specimens for re-examination by Unicomarine Ltd. Laboratories are also permitted to use this exercise to verify identifications of difficult or problematic taxa about which they are unsure.

2.3.1.1 Selection of Fauna

The different geographical distributions of species meant that a request for a uniform set of species from all laboratories was unlikely to be successful. Accordingly a list of instructions was distributed to participating laboratories. The specimens were to broadly represent the faunal groups circulated in the general Ring Tests, *i.e.* mixed phyla. However, each laboratory was permitted to include any number of unidentified or problematic taxa. Specimens wherever possible were to be representatives from CSEMP reference collections.

2.3.1.2 Analysis

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 permitted **eleven weeks** to prepare and submit their reference specimens. All specimens were re-identified and the identification made by Unicomarine Ltd. compared with that made by the participating laboratories. All specimens were returned to the laboratories after analysis.

2.3.2 Results

2.3.2.1 General Comments

Of the sixteen laboratories participating in this exercise (LR14), ten laboratories supplied specimens for verification; six laboratories did not submit specimens or provide notification of abstention from this exercise.

2.3.2.2 Returns from Participating Laboratories

The identification of the specimens received from the participating laboratories was checked. Detailed results have been reported to each of the participating laboratories separately. Due to this component's emphasis upon training and the diversity of submissions, comparisons of results are not applicable and as such no summary statistics are provided in this report.

2.3.3 Discussion

In view of the different species that were sent by laboratories for identification it is inappropriate to make detailed inter-lab comparisons. In the majority of instances identifications made by Unicomarine Ltd. were in agreement with those made by the participating laboratories. Due to the range of species submitted it was not possible to identify a single taxon causing the majority of problems.

Despite the free format of specimen choice in this module, only approximately two thirds of the subscribing laboratories supplied specimens. The module deadlines and instructions will be reviewed to improve submission levels, if possible.

The results for this exercise should be viewed giving consideration to the different approaches by participant laboratories. Some laboratories appear to be sending well known species while others elect to obtain a 'second opinion' on more difficult species. Thus the scores are not comparable and it is not considered appropriate to assign any rank to the laboratories. Each participant should deliberate upon the aims of this component in terms of data quality assessment.

2.4 Macrobenthic Samples (**MB**) Module

2.4.1 Description

This training module examined the participants' ability to process macrobenthic samples from the same habitat. Artificial, uniformed grab samples containing 'known' marine fauna were created and distributed to each participating laboratory. This part of the Scheme examined 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 Preparation of the Samples

MB17 samples were artificially created marine samples based upon subtidal fauna and sediment from Torbay. The distributed samples were created using fauna donated by the Environment Agency from a previously audited WFD survey. Approximately 200ml of mud (<0.5mm), 200ml of grit / gravel (>2<4mm) and 100ml of sand (<1mm) were added to each replicate to produce a relatively accurate sample composition (0.5litres in total). The samples contained twenty-five taxa and fifty-nine individuals, covering a variety of phyla. Several faunal fragments were included to observe potential variances in their treatment. All specimens were checked for quality and size consistency, using the same protocols as the Scheme's RT module; enumeration of each taxon was carefully checked and verified prior to their addition to the replicate samples. The fauna and sediment were gently mixed in 70% IDA (Industrial Denatured Alcohol) prior to distribution to the participating laboratories. For further details of the samples components refer to the MB17 Report.

2.4.1.2 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 not provided, other than the use of a 1mm sieve mesh; participating laboratories were instructed to employ their normal methods. The participating laboratories were required to complete a Macrobenthic Sample Details Form, which specified their processing methodology (for example, stating whether nematodes are extracted). 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.

Thirteen weeks were allowed for completion of the sample analysis. All sorted and unsorted sediments and extracted fauna were to be returned to Unicomarine Ltd., together with the data on counts and biomass determinations.

2.4.1.3 Post-return Analysis

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

2.4.2 Results

2.4.2.1 General Comments

The distributed macrobenthic sample (MB17) was an artificial replicate marine sample based upon subtidal fauna and sediment from Torbay. All thirteen laboratories subscribing to this module returned fauna and data; one laboratory (LB1636) disposed of their sorted residues, the remaining laboratories all supplied full samples for re-analysis. None of the participating laboratories subsampled their residues. Seven participating laboratories did not supply biomass data. One laboratory (LB1636) processed their macrobenthic sample following their family level identification policy. A report for this exercise was distributed to the participating laboratories (Macrobenthic Exercise Results – MB17) and was also posted on the Scheme's website (www.nmbaqcs.org); additional comments are added below.

2.4.2.2 Efficiency of Sample Sorting

Table 1 (MB17 Report) presents a summary of the estimate of numbers of taxa and individuals made by each of the participating laboratories for sample MB17, together with the corresponding count made by Unicomarine Ltd prior to sample dispatch. Comparison of the number of taxa and number of individuals between the participating laboratory and Unicomarine Ltd. is given as a percentage. Prior to analyses of these data some minor adjustments (combination of juvenile taxa, spelling errors, removal of spaces, etc.) were made to allow direct comparisons to be made and remove artificial differences in these data. 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 variation between laboratories in the percentage of taxa identified in the samples. At most seven taxa (and 28% of the total taxa in the sample) were not extracted, lost during sieving or not recognised within the picked material. Two laboratories recorded the same number of taxa as Unicomarine prepared within the artificial samples (LB1607 and LB1608). Of the eleven remaining participants, nine recorded fewer taxa than Unicomarine Ltd.; two recorded more.

The simple comparison of numbers of taxa can be misleading as taxa counts are affected by inaccuracies in identification, *e.g.* 'over splitting' (separating a single taxon incorrectly into multiple taxa). For example, one laboratory (LB1608) produced the correct summary number of taxa for the sample but missed a taxon in the residue.

The values presented for the number of taxa not extracted (column 10) represent taxa not recorded or extracted (even if misidentified) elsewhere in the results, *i.e.* these were taxa completely missed or sieved from the artificial sample by the laboratory. Three laboratories (LB1607, LB1621 and LB1635) extracted representatives of all the taxa present in their samples. On average laboratories missed two taxa in their residues and in the worst instance six new taxa were missed during the picking stage of this exercise.

2.4.2.4 Number of Individuals

Re-sorting of the sample residues by Unicomarine Ltd. retrieved additional individuals from eleven of the thirteen participants' samples; these data are presented in columns 11 and 12 of Table 1. It must be noted that several specimens not extracted by the participating laboratories were also not found during the residue resorting, these specimens have be attributed to processing loss, *e.g.* passing through or over the 1 mm sieve. The number of individuals not extracted from the sample (column 11) is given as a percentage of the total number in the sample (including those missed) in column 12 (*i.e.* column 12 = column 11 / column 7 %). The proportion of missed individuals in all of the samples was less than 11% of the true total number in the sample. In the worst instances six individuals and 10.2% of the total number of individuals were not extracted during the initial sample processing. The average number of missed individuals found upon re-sorting the residue was three. A breakdown of the missed individuals by taxonomic group is presented in Table 2. Echinoderms and crustaceans were the best 'picked' faunal groups with all thirteen participating laboratories extracting all the specimens. Molluscs were the worst extracted faunal group, with one laboratory failing to record half of the specimens supplied in their artificial sample.

2.4.2.5 *Uniformity of Identification*

Most of the species in the distributed sample were identified correctly by the participating laboratories. Four of the participating laboratories had no taxonomic differences, *i.e.* disagreement with the AQC identification (Table 1, column 15 in the MB17 Report). In the worst instance eight taxonomic differences were recorded. On average 1.8 taxonomic differences were encountered per sample. These showed some correlation across the data set with *Eumida bahusiensis* (medium) producing three errors and *Ampelisca tenuicornis* producing four taxonomic errors.

2.4.2.6 *Comparison of Similarity Indices (Bray-Curtis)*

The fauna list for each sample obtained by the participating laboratory was compared with the list of fauna artificially created by Unicomarine Ltd. The comparison was made by calculating the Bray-Curtis similarity index for the pair of samples using non-transformed data. Prior to analyses of these data some minor adjustments were made to allow direct comparisons to be made, *e.g.* separating / combining adults and juveniles to reflect a common identification policy and remove artificial differences in these data. The results of this calculation are presented in column 14 of Table 1 (MB17 Report). There was variation among laboratories in the values calculated for the index, from 75.6% to 99.2%, with an average value of 92.7%. The index for the majority of laboratories (10 of 13) was above 90%; two of the participating laboratories would have achieved 'fail' sample flags if the NMBAQC / CSEMP standards were applied. Further details of each participating laboratory's performance are given in the Macrobenthic Exercise Results Report.

2.4.2.7 *Biomass Determinations*

A comparison of the estimates of the biomass made by the participating laboratories and Unicomarine Ltd. broken down by major taxonomic group for the MB17 circulation is presented in Table 3. Seven laboratories did not supply biomass data. The average difference between the two total weight values was -2.6% (*i.e.* lighter than that made by Unicomarine Ltd.), with extremely variable measurements by major faunal groups (*i.e.* a mixture of one exact biomass match, fifteen lighter and fourteen heavier than Unicomarine measurements). The range of overall biomass percentage difference results, between participating laboratories and Unicomarine Ltd., was from –18.4% (measurements by laboratory were lighter than those made by Unicomarine Ltd.) to +11.2% (measurements by laboratory were greater than those made by Unicomarine Ltd.). There was great variation in biomass estimations between participating laboratories and between taxonomic groups. The average difference between estimations varied greatly between faunal groups, ranging from –82.1% to +1.0% (from Crustacea to Mollusca, respectively).

2.4.2.8 *Uniformity of Samples*

MB17 was an artificial sample created by Unicomarine, the residue and faunal content of the samples distributed are shown in Sheet 1 and Table 4. The samples can be best assigned to the *Mysella bidentata* and *Abra* spp. in infralittoral sandy mud biotope (SS.SMu.ISaMu.MysAbr) (Connor *et al.* 2004). All fauna included in MB17 were obtained from samples previously analysed with a 1 mm sieve mesh. However, it was noted during MB17 preparation that a number of the specimens added to the residue could be lost according to the degree of sieving employed by the participating laboratories.

2.4.3 *Discussion*

The sample distributed as MB17 comprised an artificial, but typical, coastal marine muddy sample. The comprised relatively high numbers of *Sternaspis scutata*, a southern species. Several participants excluded taxa from their analysis on the basis of their in-house processing policies; a summary of processing methods is presented in Table 5 (MB17 Report). Direct comparisons between laboratories cannot be readily concluded due to the application of differing in-house processing methods, *i.e.* several participants followed their in-house methods and did not extract, identify or enumerate all biota present in the test sample. Two of the participating laboratories (LB1607 and LB1635) extracted all the countable material from the residue. In the worst instances six individuals, 10% of the total individuals, were not extracted from the residue. One laboratory identified the empty *Parvicardium scabrum* shell added to all the artificial replicates. Identification caused various problems for a minority of laboratories; four of the thirteen participating laboratories correctly identified their entire extracted fauna. There were a total of twenty-three taxonomic mistakes from nine of the participants, these

included misidentifications of *Ampelisca tenuicornis, Eumida bahusiensis, Abra nitida, Corbula gibba, Melinna palmate, Euclymene oerstedii, Clymenura* sp., *Heteromastus filiformis, Pholoe baltica, Pholoe longicaudata* and *Hydrallmania falcata* (in order of frequency). One laboratory (LB1636) disposed of their replicate sample residue and identified the fauna to family level. This laboratory incorrectly identified three of the families present in the artificial sample. Only two of the thirteen returning laboratories attained a Bray-Curtis similarity index lower than 90%. The highest Bray-Curtis similarity index achieved was a very high value, 99.17% (LB1607). The average Bray-Curtis figure achieved was 92.7%. This figure is very similar to that recorded for the previous marine sample in the MB module (MB15). The average Bray-Curtis similarity index achieved for MB16 (estuarine) was 95.5%, the average for MB15 (coastal) was 92.4%, the average for MB14 (estuarine) was 89.9%, MB13 (coastal) was 97%, MB12 (estuarine) was 77%, MB11 (an artificial coastal sample) was 93%, MB10 (estuarine) was 88%, MB09 (coastal) was 93%, MB08 (estuarine) was 95%, MB07 (coastal) was 88%, MB06 (estuarine) was 91%, MB05 (coastal) was 85% and MB04 (estuarine) was 82%.

Table 4 shows the variation, by major Phyla, between reported data from the participating laboratories for those artificial samples circulated for the macrobenthic exercise (MB17). All differences can be attributed to sample processing (sieving / extracting / identification) procedures. All samples were provided with exact components of residue and fauna, including faunal fragments. Some laboratories failed to return all of the residue material and faunal fragments. It is assumed that in such instances these components have been disposed during processing.

The need for a standard macrobenthic sample processing policy was clearly emphasised by this exercise. Thirteen exact replicate samples produced some relatively poor similarity figures from the participant laboratories prior to data adjustments to improve recording differences. The adjustments included several faunal groups; *Mysella bidentata* combined with *Kertiella bidentata*; *Nucula nitidosa* juveniles combined with *N. nitidosa*; *Clymenura johnstoni* combined with *Clymenura* sp.; *Phoronis muelleri* combined with *Phoronis* sp.; *Ampharete lindstroemi* agg. and *A. finmarchica* combined with *A. lindstroemi*; *Actinia* equina and *Actinia* sp. combined with Actiniaria. Also all spelling errors were corrected. Such adjustments could not be possible in 'normal' samples with data processed by 'remote' database managers and consequently, in the absence of a standard policy, these processing differences are etched into each individual laboratory's data.

The 'blot-drying' procedure employed by Unicomarine Ltd. for the determination of biomass was as specified in the Green Book, *i.e.* avoiding excessive pressure when blotting specimens dry. However, there remains a considerable variation between the estimates of total biomass made by the participating laboratories and Unicomarine Ltd. Six laboratories provided biomass data; three provided data that was lighter in total than Unicomarine Ltd.; three supplied data that was heavier than Unicomarine Ltd. estimations. The extremes recorded were 18.4% lighter (LB1638) and 11.2% heavier (LB1605) than the Unicomarine Ltd. estimations. Overall the average difference between the values determined by the participating laboratories and Unicomarine Ltd. was -2.6% (*i.e.* laboratory measurements were lighter than those made by Unicomarine Ltd.). Previous Scheme years have not shown any particular pattern of variance for biomass estimations; the last four year's average biomass difference figures were 3.4% lighter (MB16), 2.4% heavier (MB15), 2.3% lighter (MB14) and 9.9% heavier (MB13). It seems likely that the main reasons for the observed differences between the measurements are more thorough, or less consistent, drying by participating laboratories prior to weighing. A similar observation was made in previous years of the Scheme. The average percentage difference between Unicomarine Ltd. and participating laboratories biomass figures for MB12 was +2.2%, MB11 was -3.1%, MB10 was -13.3%, MB09 was –14.6%, MB08 it was +4.9%, MB07 it was –1.67%, MB06 it was +26%, MB05 it was +32% and for MB04 it was +20%. There are likely to be several reasons for the differences between years, though the nature of the fauna in the distributed samples is likely to be of particular importance.

Clearly, determination of biomass remains a problem area warranting further examination. Although all laboratories are following the same protocol it is apparent that different interpretations are being made of the degree of drying required. When single specimens of small species are being weighed (*e.g.* amphipods) very small differences in the effectiveness of drying will make large percentage differences in the overall weight recorded. It must be noted that the Green Book recommends that ash-free dry weights for biomass are derived from the blotted wet weights using published conversion factors. However the details of techniques used to determine initial wet weights for these conversion factors may vary from those specified in the Green Book. A series of trials should be commissioned to

ascertain the best methods for accurate and consistent 'blotted' dry weight figures which can in turn be reliably applied to existing or new conversion factors.

2.5 Own Sample (**OS**) Module

2.5.1 Description

This exercise examined laboratory analytical performance on material from each participating laboratory's 'home' area. Following a review of the Own Sample exercise (Unicomarine, 2001) several changes to sample selection and scoring were implemented in Scheme year eight (2001/02). All participants must meet the new Own Sample requirements. Own Sample participants must supply their previous year's CSEMP data matrices, where relevant, for Own Sample selection, *i.e.* 2008 CSEMP data. This is to ensure that all processing is completed, 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 a data matrices from which three samples were selected. The selection was in turn notified to the laboratories. Laboratories responsible for CSEMP samples were advised to use these samples if possible, otherwise there was free choice providing a minimum of twelve samples were included in the submitted data matrix.

2.5.1.1 Analysis Required

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

Approximately **seventeen weeks** were allowed for the submission of data and preparation of the Own Samples selected for reanalysis. Upon receipt at Unicomarine Ltd. all OS samples were re-analysed by the same operator. The sorted residue was re-examined and any countable material extracted. Identified fauna was checked for the accuracy of enumeration and identification and all specimens were re-weighed using the same procedure as for the MB exercise.

2.5.2 Results

2.5.2.1 General Comments

Following the request to participating laboratories to submit data of suitable samples for re-analysis, ninety-three selected Own Samples were received from thirty-one laboratories, together with descriptions of their origin and the collection and analysis procedures employed; two laboratories did not submit data for OS selection. Samples were identified as OS41, OS42 and OS43 and labelled with LabCodes. The nature of the samples varied considerably. Samples were received from estuarine and marine locations, both intertidal and subtidal. The sediment varied from mud to gravel and from 10 ml to 7 L of residue. The associated fauna of the samples was also very varied; the number of taxa recorded ranged from 0 to 102, with the number of countable individuals from 0 to 2906. Thirty-one of the thirty-three laboratories that subscribed to the OS module returned three Own Samples; sixteen of these Own Samples were audited externally by Aquatic Environments due to Unicomarine Ltd. being responsible for the initial sample processing; two laboratories did not supply data for sample selection or communicate their abstention. Detailed results have been reported to the participating laboratories. A summary of results from the Own Sample module is presented in Own Sample Module Summary Report (OS41, 42 & 43).

2.5.2.2 *Efficiency of Sample Sorting*

Table 1 (Own Sample Module Summary Report – OS41, 42 & 43) displays a summary of the data obtained from the analysis of the Own Sample exercise. 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 forty-eight samples (52% of all samples) the number of taxa recorded by the participating laboratories was identical to that obtained by Unicomarine Ltd. (column 4). In the forty-five exceptions, the difference was at most fifteen taxa and the average difference was approximately one taxon.

Data for the numbers of individuals recorded (columns 6 and 7, Table 1) shows a range of differences from re-analysis of between 0% and 27.5%. The average difference was 3.7% (twenty-five samples exceeded this average). Forty-four of the ninety-three samples reported showed 100% extraction of fauna from the residue (column 12), and in nineteen samples various numbers of individuals (but no new taxa) were missed during sorting (column 11). The remaining thirty samples contained taxa in the residue that were not previously extracted, the worst example being fifteen new taxa found in the residue (column 10). In the worst instance residue was found to contain fifty-seven individuals. A breakdown of the missed individuals by taxonomic group is presented in Table 2. The average number of missed individuals found upon re-sorting the residue was approximately five, and the average number of missed taxa was less than one.

2.5.2.3 *Uniformity of Identification*

Taxonomic differences between Unicomarine Ltd. and participating laboratories' results were found in forty-seven (51%) of the ninety-three samples re-analysed. A summary of mis-identified taxa is presented in Table 3 (Own Sample Module Summary Report – OS41, 42 & 43). An average of 1.4 taxonomic differences per laboratory were recorded; in the worst instance ten differences in identification occurred. A great variety of samples (and hence fauna) was received and no particular faunal group was found to cause significant problems. Some taxonomic errors were more frequently recorded, these included cirratulids, syllids, maldanids, *Abra* spp. and sipunculans. These recurring mis-identifications may be the result of repeat taxonomic errors from a few laboratories responsible for the analysis of several Own Samples, i.e. subcontractors processing samples for several Scheme participants.

2.5.2.4 *Comparison of Similarity Indices (Bray-Curtis)*

The procedure for the calculation of the similarity index was as used for the MB exercise. The Bray-Curtis similarity index figures (Table 1, column 14) ranged from 47.1% to 100%, with an average figure of 93.7%. Eighteen samples from twelve laboratories achieved a similarity figure of less than 90%. Eighteen samples produced a similarity figure of 100%; these were submitted by fourteen different laboratories (LB1601, LB1602, LB1605, LB1609, LB1611, LB1612, LB1615, LB1616, LB1617, LB1618, LB1621, LB1624, LB1626 and LB1628). The best overall results were achieved by laboratory LB1612 (results comprised 99.48%, 99.67% and 100%), which averaged 99.72% similarity. The worst overall results were achieved by laboratory LB1625, whose results comprised 77.74%, 52.56% and 50.00% (an average of 60.10%). It should be noted that a small number of differences between samples can result in a large difference in the Bray-Curtis index. This difference does not necessarily reflect the laboratory's interpretative ability.

2.5.2.5 *Biomass Determinations*

It was not possible to make an accurate comparison of the biomass determination in all cases; forty-six samples were not supplied with species blotted wet weight biomass data; four samples were reported to five decimal places (grammes to 4 decimal places is required). Consequently, only forty-four of the ninety-three samples received have been used for comparative analysis. Table 4 shows the comparison of the participating laboratory and Unicomarine Ltd. biomass figures by major taxonomic groups. The total biomass values obtained by the participating laboratories varied greatly with those obtained by Unicomarine Ltd. The average was a +15.4% difference between the two sets of results (*i.e.* heavier than Unicomarine Ltd.); the range was from –85.7% to +64.0%. 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). Further analysis of biomass results by major taxonomic groups indicated an average difference of +20.6% for polychaetes, +3.6% for oligochaetes, +32.2% for

nemerteans, -31.9% for Chelicerata, +21.3% for crustaceans, +15.8% for echinoderms, +13.2% for molluscs and +25.2% for all remaining faunal groups. These figures are vastly different to those produced by this same exercise in each of the previous years. This emphasises the variability caused by not only duration and method of drying but also the consistency of results within each major taxonomic group. The Unicomarine Ltd. biomass data was achieved using a non-pressure drying procedure as specified in the Green Book.

2.5.3 *Discussion*

Considering just the Bray-Curtis index, as a measure of similarity between the results obtained by the participating laboratories and those obtained from re-analysis, participating laboratories performed slightly better in the OS exercise compared to the MB17 exercise. The average value of the index was 93.7% for the OS, compared with 92.7% for MB17. Both modules have produced several good results and some instances of excellent sample processing.

There were ninety-three samples submitted for this module, including sixteen samples that were processed by the Scheme's external auditor. Two laboratories (LB1610 and LB1631) did not supply data for Own Sample selection; these laboratories are not directly responsible for CSEMP samples and are therefore not deemed to have failed the Scheme's standards.

Approximately 81% of the ninety-three samples reported exceeded the 90% Bray-Curtis pass mark and approximately 60% of the samples exceeded 95% Bray-Curtis similarity. The average Bray-Curtis similarity index achieved was 93.7%. These figures are generally in line with those from previous OS exercises (see Table 6; Own Sample Module Summary Report – OS41, 42 & 43).

Since the beginning of the OS component eight hundred and twelve admissible samples have been received (OS01-43), with an average Bray-Curtis similarity figure of 93.49%. One hundred and fifty-five samples (19%) have fallen below the 90% pass mark. One hundred and three samples have achieved a similarity figure of 100% (13% of all returns). Extraction of fauna is an area in which several participating laboratories could review their efficiency. All countable fauna must be extracted to record a truly representative sample, although this is rarely the case due to time restraints or inefficient methods used. A sample that has been poorly picked stands a high possibility of being unrepresentative regardless of the quality of subsequent faunal identifications, and should the sorted residue be disposed, this cannot be rectified. Laboratories should study their detailed OS and MB reports and target the particular taxon or groups of taxa that are being commonly overlooked during the picking stages of sample analysis. It must be resolved whether the individuals are either not recognised as countable or not scanned using the extraction methods employed. If it is the former, then training is appropriate. If the latter is the case then a review of current extraction methods should be conducted. Some instances of repeated taxonomic errors in Own Samples from previous Scheme years have been noted. Taxonomic errors should be investigated by participating laboratories even if the 'whole sample' has achieved a 'pass' flag. If a participating laboratory disagrees with any recorded taxonomic errors they should contact Unicomarine Ltd for further information (as they are invited to do so upon receipt of their Own Sample Interim Report).

2.5.4 *Application of NMBAQC Scheme Standards*

One of the key roles of the Invertebrate and Particle Size components of the NMBAQC Scheme is to assess the reliability of data collected as part of the Clean Seas Environment Monitoring Programme (CSEMP; formerly UK NMMP). With this aim performance target standards were defined for certain Scheme exercises and applied in Scheme year three (1996/97). These standards were the subject of a review in 2001 (Unicomarine, 2001) and were altered in Scheme year eight; 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 OS and PS exercise have been used in 'flagging' for the purposes of assessing data for the CSEMP.

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

Non-return of samples for the OS module resulted in the assignment of a "Fail" flag to the laboratory. The only exception to this approach has been in those instances where laboratories elected not to participate in the module.

2.5.4.1 Laboratory Performance

The target values for each Own Sample exercise and the corresponding laboratory results are presented in Table 5 (Own Sample Module Summary Report – OS41, 42 & 43). The assigned flags for each exercise are also given. An assessment is performed separately for each of the three OS samples. Comparisons between exercise results are commonly inapplicable due to the diversity of samples and processing methodologies exhibited throughout this module.

It can be seen from Table 5 (columns 4, 13 and 22) that for the OS exercise the majority of laboratories are considered to have met or exceeded the required standard for three of the OS targets - the enumeration of taxa and individuals and the Bray-Curtis comparison. Overall 84% of the comparisons were considered to have passed the enumeration of taxa standard; 83% exceeded the enumeration of individuals standard and 75% passed the Bray-Curtis comparison standard. NMBAQC Scheme / CSEMP sample flags have been applied to each of the Own Samples in accordance with the performance flagging criteria introduced in Scheme year eight (Table 5, column 23); ten of the ninety-three applicable samples are flagged as 'Fail - Bad'; eight are flagged as 'Fail - Poor'; nineteen are flagged as 'Pass - Acceptable'; thirty-eight are flagged as 'Pass - Good'; and eighteen are flagged as 'Pass - Excellent' for achieving 100% Bray-Curtis similarity indices. All the laboratories with 'Poor' or 'Bad' sample flags have been provided with specific recommendations of remedial actions to quality assure their Own Sample data sets (see 2.5.4.3 Remedial Action below).

Performance with respect to the biomass standard was poorer (Table 5, column 19) with just 50% of the eligible samples meeting the required standard. It should be noted that there were laboratories for which the results from the biomass exercise should be considered unsuitable for comparison with the standard (expressed as five decimal places instead of the requested four, and fauna rendered dry or damaged by initial biomass procedures).

2.5.4.2 Comparison with Results from Previous Years

A comparison of the overall results for recent years is presented in Table 6 (Own Sample Module Interim Summary Report – OS41, 42 & 43). The Table shows the number of laboratories assigned 'Pass' and 'Fail' flags for the OS exercises over the past fifteen years based upon the current NMBAQC Scheme standards (see Description of the Scheme Standards for the Benthic Invertebrate Component). This year's ninety-three Own Samples resulted in a pass rate of 81% (the highest being 100% achieved in exercise OS01 that involved just fourteen samples; the lowest being 67% recorded in Year 7 from forty-five samples). The number of non-returned results, 'Deemed Fails', have been significantly reduced in recent years of the Scheme. This can be attributed to the 'deadline reminders' dispatched throughout the Scheme year. Table 7 shows the trend of OS results for each participating laboratory over the past fifteen years. There appears to be a fairly high level of consistency within each laboratory with an overall increase in data quality, *i.e.* generally fewer failing samples and a higher average Bray-Curtis similarity score. The commitment of participants to address 'failing' samples is also increasingly evident; these remedial actions are highly commendable and reflect the value of quality assured data (see 2.4.4.3 Remedial Action). Monitoring the situation over a longer period is required before a firm statement about changes in laboratory standards could be made. However, the introduction of 'blind' audits in Scheme year eight have not caused an increase in the number of failures, as initially expected.

2.5.4.3 Remedial Action

It is imperative that failing CSEMP (formerly UK NMMP) samples, audited through the Own Sample exercise, are addressed. Remedial action should be conducted upon the associated CSEMP replicates to improve upon the flagged data. The revised NMBAQC Scheme OS standards, introduced in Scheme year eight, 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 the achievement of a Bray-Curtis similarity indices of <90%. The performance indicators used to determine the level of remedial action required are %taxa in residue, %taxonomic errors, %individuals

in residue (see Table 5, columns 7, 10 and 16 in Own Sample Module Summary Report – OS41, 42 & 43) 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 and notify Unicomarine or the NMBAQC Scheme Contract Manager when this has been completed. Any remedial action undertaken should be audited externally where required. The NMBAQC Contract Manager and Scheme's contractor, Unicomarine 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 16 (see 2.5.4.3.6). Also 'failing' samples with outstanding remedial action from the previous five Scheme years are listed.

2.5.4.3.1 Scheme Year 11 (OS26, 27 & 28) – 2004/05

Three samples 'failed' in Scheme year 11 (including two UK NMMP samples). Remedial action, outlined below, is still outstanding for the associated replicates of the following Own Samples:

NMMP samples

LB1110 OS26- Review *Fabricia stellaris / Manayunkia aestuarina* identifications;
Re-sort residue for remaining replicates and re-audit.
Remedial Action - status unknown.

LB1110 OS28- Review *Tubificoides* cf. *galiciensis* identifications.
Remedial Action - status unknown.

Non-NMMP samples

LB1120 OS28- Review policy for recording *in-situ* records;
Review identification of live versus dead *Hydrobia ulvae*.
Remedial Action - status unknown.

2.5.4.3.2 Scheme Year 12 (OS29, 30 & 31) – 2005/06

Seven samples 'failed' in Scheme year 12 (including five UK NMMP samples). Remedial action, outlined below, is still outstanding for the associated replicates of the following Own Samples:

NMMP samples

LB1226 OS31- Review *Bathyporeia elegans* / *B. pelagica* identifications;
Review methods for estimation of taxa and abundance.
Remedial Action - status unknown.

Non-NMMP samples

LB1201 OS29- Reprocess residues for remaining replicate samples;
Review identifications of *Pholoe inornata*, *Monocorophium sextonae*, *Eumida sanguinea* and *Malmgreniella arenicolae*.
Remedial Action - status unknown.

2.5.4.3.3 Scheme Year 13 (OS32, 33 & 34) – 2006/07

Six samples 'failed' in Scheme year 12 (including three UK NMMP samples). All recommended remedial actions for Year 13 have been successfully completed. All Own Samples and associated data are deemed to have fulfilled the Schemes quality assurance standards.

2.5.4.3.4 Scheme Year 14 (OS35, 36 & 37) – 2007/08

Twelve samples 'failed' in Scheme year 14 (including five CSEMP samples). Remedial actions for nine of these samples has been successfully completed (including the five CSEMP samples); remedial action is still outstanding for the associated replicates of the following three Own Samples:

Non-CSEMP samples

LB1429 OS35- Reprocess residues for associated replicate samples. Submit revised data for random selection of additional sample for audit.
Review faunal extraction policy.
Remedial Action - status unknown.

LB1429 OS36- Review *Spisula elliptica / S. solida* identifications.
Reprocess residues for associated replicate samples. Submit revised data for random selection of additional sample for audit.
Review faunal extraction policy.
Remedial Action - status unknown.

LB1431 OS37- Reprocess taxonomic errors for any associated samples; *Dosinia lupinus* / *Lucinoma borealis*, *Modiolus modiolus* / *Mytilus edulis* juv., Isaedae / *Autonoe longipes* and *Photis longicaudata* / Aoridae (female) identifications.
Review faunal extraction methods and reprocess residues for any associated samples. Submit revised data for random selection of additional sample for audit.
Remedial Action - status unknown.

2.5.4.3.5 Scheme Year 15 (OS38, 39 & 40) – 2008/09

Twenty-four samples 'failed' in Scheme year 15 (including five CSEMP samples). Remedial actions for twenty-one of these samples has been successfully completed (including all five CSEMP samples); remedial action is still outstanding for the associated replicates of the following three Own Samples:

Non-CSEMP samples

LB1505 OS40- Reprocess residues for associated samples.
Submit revised data (associated samples) for the selection of an additional sample for audit (residue resort audit only) via an external auditor. This remedial action effectiveness audit is available free of charge from Unicomarine.
Remedial Action - status unknown.

LB1508 OS40- Review the taxonomic error (*Liljeborgia kinahani*).
Reprocess residues for associated samples.
Submit revised data (associated samples) for the selection of an additional sample for audit (residue resort audit only) via an external auditor. Remedial action effectiveness audit is available free of charge from Unicomarine.
Remedial Action - status unknown.

LB1525 OS39- Reprocess sample residues for associated samples.
Supply remedial action revised data matrix for the random selection of one sample for external audit (faunal extraction efficiency only.
Remedial Action - status unknown.

2.5.4.3.6 Scheme Year 16 (OS41, 42 & 43) – 2009/10

Eighteen samples 'failed' in Scheme year 16 (including four CSEMP samples). Remedial action, outlined below, was required for associated replicates of the following Own Samples:

CSEMP samples

LB1604 OS41- Review taxonomic errors and transcription error for associated samples.
Remedial Action - completed (02/08/2010).

LB1605 OS41- Reprocess taxonomic errors for associated samples.
Remedial Action - completed (17/07/2010).

LB1605 OS42- Review taxonomic errors for associated samples.
Remedial Action - completed (31/08/2010).

LB1617 OS43- Review taxonomic errors for associated samples.
Remedial Action - status unknown.

Non-CSEMP samples

LB1607 OS42- Review taxonomic errors for associated samples.

Remedial Action - completed (01/06/2010).

LB1608 OS42- Reprocess the taxonomic errors for associated samples.
Review abundance for associated samples.
Remedial Action - completed (18/08/2010).

LB1609 OS43- Conduct an internal review of faunal extraction methods.
Remedial Action – completed (12/10/2009).

LB1625 OS41- Reprocess taxonomic errors for associated samples.
Remedial Action - status unknown.

LB1625 OS42- Reprocess associated sample residues.
Reprocess taxonomic errors for associated samples.
Submit revised data matrix for selection of one sample for full audit.
Remedial Action - status unknown.

LB1625 OS43- Remedial action as LB1625 OS42 (see above).
Remedial Action - status unknown.

LB1626 OS41- Review taxonomic errors for associated samples.
Remedial Action – completed (09/06/2010).

LB1627 OS41- Reprocess associated sample residues.
Reprocess taxonomic errors for associated samples.
Submit revised data matrix for selection of one sample for full audit.
Remedial Action - status unknown.

LB1627 OS43- Remedial action as LB1627 OS41 (see above).
Remedial Action - status unknown.

LB1629 OS41- Review taxonomic errors for any associated samples.
Remedial Action – completed (04/03/2010).

LB1632 OS41- Review taxonomic errors for any associated samples.
Remedial Action – completed (11/10/2010).
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LB1632 OS42- Review taxonomic errors for any associated samples.
Remedial Action – completed (11/10/2010).

LB1633 OS41- Reprocess associated sample residues.
Reprocess taxonomic errors for associated samples.
Submit revised data matrix for selection of one sample for full audit.
Remedial Action - status unknown.

LB1633 OS43- Remedial action as LB1633 OS41 (see above).
Remedial Action - status unknown.

3. Conclusions and Recommendations

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

1. The majority of participating laboratories submit data / samples in accordance with the Scheme's timetable, however late submissions 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; this would greatly facilitate the analysis of results and effective feedback.

2. Several samples submitted as Own Sample comprised very small volumes of sorted residues and no faunal fragments. Participants are reminded that Own Sample must include all sorted residues, including all extracted materials deemed 'unrecordable' during the initial processing.

3. All Scheme participants use e-mail as their primary means of communication. All interim results / reports are now provided as secure PDF documents via direct email or posted on the Scheme's website. Electronic methods of communication, data transfer and reporting are to continue and expand wherever possible; hard copies of data sheets will be provided only where appropriate or specifically requested.

4. Laboratories involved in CSEMP data submission should endeavour to return data on all necessary components of the Scheme in the format requested. This will be required to allow the setting of performance "flags". Non-return of data will result in assignment of a "Fail" flag. For CSEMP laboratories this deemed "Fail" for no submitted data is to be perceived as far worse than a participatory "Fail" flag. Participating laboratories are assigned 'deemed fail' flags as a result of not informing Unicomarine Ltd. of their intentions to abstain from particular exercises. Participating laboratories should ensure that any changes to the level of their subscription / participation in the Scheme's modules are communicated to Unicomarine Ltd as soon as possible.

5. There were continued problems associated with the measurement of biomass for individual species. In this and previous Scheme years several laboratories, despite using blotted wet weight biomass techniques, rendered some of their specimens too damaged to be re-identified. Some laboratories are still presenting data to five or three decimal. This produces spurious errors due to nominal weights one hundred times smaller than those reported at four decimal places. The initial processing of a CSEMP sample should in no way compromise the effectiveness of an audit. Biomass procedures should not render the specimens unidentifiable; trials should be commissioned to derive the best protocol for the blotted weighing technique. Biomass must be reported to four decimal places with nominal weights recorded as 0.0001g. A standardised protocol and reporting format for CSEMP analysis is to be developed via the NMBAQC Scheme.

6. 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 Laboratory Reference exercise (LR) can be used as a means of verifying reference specimens. Laboratories are strongly recommended to implement and expand in-house reference collections of fauna. The inclusion of growth series material is extremely useful for certain faunal groups, *e.g.* identifying certain molluscs. All surveys should have an associated reference collection to enable ease of cross-checking or adopting future taxonomic developments.

7. Differences in the literature used for identification of invertebrates have been highlighted by the RT, MB and OS exercises. Unpublished keys from Scheme workshops, *etc.* will continue to be posted on the Scheme's website. The Scheme has produced a UK Standard Taxonomic Literature database. Laboratories are encouraged to review the content and give details of additions wherever possible.

8. All MB and OS 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.

9. The Own Sample component has shown repeated taxonomic errors for some laboratories from the same UK NMMP / CSEMP sites over several years. Participating laboratories are encouraged to redress or resolve disagreements for taxonomic errors reported in their Own Samples even if their 'whole samples' achieve a 'pass' flag.

10. 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.

11. In Scheme year seven a NMBAQCS Sorting Methods Questionnaire was circulated to all laboratories participating in macrobenthic analysis components (OS & MB). The responses showed that little or no consistency in extraction or identification protocols existed between participating

laboratories. The results of this questionnaire have been reported separately to the participating laboratories (Worsfold & Hall, 2001). The report concluded that there is a need for standardisation of extraction protocols, in terms of which fauna are extracted/not extracted. Also a consensus needs to be reached for what constitutes 'countable' individuals and at which taxonomic level specific taxa should be identified. Protocols are to be developed to standardise the approach towards headless and partial specimens. This also has implications for comparing biomass estimations; certain laboratories pick headless portions of specimens from residues and assign them to the relevant taxa for combined biomass measurements. In Scheme year eight RT19 targeted 'Oligochaeta and similar fauna' and was complimented by a questionnaire regarding oligochaete identification. The ring test and accompanying questionnaire were reported to the participating laboratories (Hall & Worsfold, 2002) and reiterated the need for a standard identification protocol for UK NMMP samples. A proposal for a standard NMMP approach to oligochaete identification was included in the report. Artificial macrobenthic samples circulated as MB11 (Yr10) and MB17 (Yr16) showed that identical samples processed by differing laboratories can result in sample data that are interpreted as having little similarity due to inconsistency of extraction, enumeration and identification policy. Standard statutory monitoring protocols are being developed through the NMBAQC Scheme, to standardise the faunal groups to be extracted from CSEMP / WFD samples and reasonable levels of identification for all taxa likely to be encountered. MB samples are currently audited according to policy and details sheets submitted by the individual participants; however NMBAQC standard processing methods will be applied and tested in the MB training module.

12. An improved learning structure to the Scheme through detailed individual exercise reports has been successfully implemented and was continued in this Scheme year. For the LR, OS and MB exercises, detailed results have been forwarded to each participating laboratory as soon after the exercise deadlines as practicable. After each RT exercise a bulletin was circulated, reviewing the literature used and detailing the correct identification of the taxa circulated. Participants are encouraged to review their exercise reports and provide feedback concerning content and format wherever appropriate.

13. Positive, constructive feedback has been received from participants during Scheme Year 16. As in previous years participants have expressed the benefits of the modules, especially RT and OS. The primary aim of this 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. Ten of the eighteen 'failing' Own Samples in Scheme Year 16 have already been rectified via the recommended remedial action.

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