The National Marine Biological Analytical Quality Control Scheme

Benthic Invertebrate Component Report from the Contractor Scheme Operation – Year 14 2007/08

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BENTHIC INVERTEBRATE COMPONENT REPORT FROM THE CONTRACTOR

SCHEME OPERATION – YEAR 14 – 2007/08

Linked Documents (hyperlinked in this report)

Ring Test Bulletin – RTB#32

Ring Test Bulletin – RTB#34

Macrobenthic Exercise Results – MB15

Own Sample Module Summary Report – OS35, 36 & 37

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 fourteenth year of the Scheme (2007/08) followed the format of the thirteenth year. A series of 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-two laboratories participated in the benthic invertebrate component of the NMBAQC Scheme. Sixteen participants were government laboratories; sixteen were private consultancies. Fourteen 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 – OS35, 36 & 37).

1.1 Summary of Performance

This report presents the findings of the Benthic Invertebrates component for the fourteenth 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 thirteenth 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 (RT32) and a second set consisted of 'targeted' bivalve specimens (RT34). For the general set of fauna (RT32) 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 2.4 generic errors and 5.1 specific errors. The majority of the generic errors can be

attributed to three mollusc, one chelicerate and one polychaete taxa. The 'targeted' ring test (RT34 – 'Bivalves'), also posed few problems for species identification. On average each participating laboratory recorded just 3.8 generic errors and 5.7 specific errors. Six specimens were responsible for 56% of all generic and 57% 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. Agreement between the laboratories and Unicomarine Ltd. was variable with generally good results, which were similar to those achieved in previous MB exercises. The samples posed some problems associated with faunal extraction and identification of the taxa. Extraction efficiency, irrespective of sorting, was on average 97.1%; eight laboratories extracted greater than 95% of the individuals from the residue; none of the laboratories extracted all fauna from the residue. Comparison of the results from the laboratories with those from analysis by Unicomarine Ltd. was made using the Bray-Curtis similarity index (untransformed). The value of the index varied between approximately 84.6% and 97.6% and was better than 90% in 80% of comparisons and greater than 95% in 20% of comparisons. As observed in all previous MB exercises, a variety of sample processing methodologies were followed by the participants (*e.g.* some excluded nematodes from their 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 2006; 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 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 95% of comparisons and better than 95% in 85% of all comparisons. All countable faunal specimens were extracted from the sample residues in 42% (34) of the samples. The Bray-Curtis similarity index ranged from 16.7% to 100% with an average figure of 94.9%. The Bray-Curtis similarity index was greater than 95% in 77% of comparisons and in most cases (85%) the value of the index was greater than 90%, these samples all achieved 'pass' flags. Twelve samples (15%) achieved 'excellent' pass flags with Bray-Curtis similarity scores of 100%.

1.1.1 Statement of Performance

Each participating laboratory has received a 'Statement of Performance', which includes 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. Each Scheme year fourteen participant was given a confidential LabCode in September 2007, these codes were randomly assigned. These 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 fourteen will be recorded as LB1404.

In this report all references to Laboratory Codes are the post-August 2007 codes (Scheme year fourteen), 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 invertebrates, 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 2007/08. The first of the year's RT circulations (RT32) was a general invertebrate ring test. The specimens included representatives of the major phyla and approximately 40% of the taxa were annelids, 36% were crustaceans, 20% were molluscs and 4% were chelicerates. The second circulation (RT34) comprised 'targeted' bivalve specimens. 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 (RT32) and the 'targeted' RT (RT34), 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). After a successful trial in the last Scheme year, participating laboratories were permitted to supply multiple data entries for each exercise to maximise results and enhance the training aspect of this module. Other aspects of the two circulations, in particular the method of scoring results, were the same as for previous circulations. **Eight weeks** were allowed for the analysis of the first RT exercise (RT32) and **seven weeks** were allowed for the second RT exercise (RT34 – bivalve taxa).

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-three laboratories were distributed with RT32 and RT34 specimens. For RT32, nineteen laboratories returned data (twenty-three individual data sets); two laboratories specified non-participation for this exercise; two did not supply data or indicate non-participation. For RT34, twenty laboratories returned data (twenty-five individual data sets); two laboratories specified non-participation for this exercise; one did not supply data or indicate non-participation.

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. Nucula turgida* for *Nucula nitidosa*.
- Simple mis-spelling of a name, *e.g. Modicolarca tumida* for *Modiolarca tumida*.

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.

Table 1 (Ring Test Bulletin – RTB#32) presents the identifications made by each of the participating laboratories for the twenty-five specimens in circulations RT32. Tables 1 and 2 (Ring Test Bulletin – RTB#34) present the identifications made by each of the participating laboratories for the twenty-five specimens in circulations RT34, 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 (Table 1 in RTB32 and Tables 1 and 2 in RTB34). 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 (RTB32 and RTB34), 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'.

2.2.2.3.1 Thirty-second distribution – RT32

Table 1 (Ring Test Bulletin – RTB#32) presents the results for the RT32. One of the specimens was donated by Lin Baldock (independent marine consultant) and one was donated by Myles O'Reilly (SEPA, East Kilbride). Nine of the twenty-five specimens circulated were polychaetes; nine were crustaceans; five were molluscs; one was an oligochaete; and one was a chelicerate. The agreement at the generic level was generally very good; fifty-six errors (from a potential five hundred and seventy-five) were recorded in the twenty-three data sets received from nineteen participating laboratories. Agreement at the specific level was also generally very good; one hundred and seventeen errors were recorded. Seven of the specimens circulated were incorrectly identified at species level by approximately two thirds 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 seven specimens. *Ammothea hilgendorfi* (large, good specimen), *Mya truncata* (juvenile, good specimen), *Praxillella affinis* (medium, fair specimen), *Tubificoides* cf. *galiciensis* (medium, fair specimen), *Gammaropsis lobata* (medium, poor specimen), *Modiolarca tumida* (small, good specimen) and *Eurydice truncata* (medium, good specimen) accounted for a total of 73% of all generic and 69% of all the specific differences recorded. Five of the twenty-five circulated specimens were correctly identified by all participating laboratories (*Odontosyllis gibba, Terebellides stroemi, Turritella communis, Sabellaria alveolata* and *Chelura terebrans*). Further details and analysis of results can be found in the Ring Test Bulletin (Ring Test Bulletin – RTB#32) 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-fourth distribution – RT34

RT34 contained twenty-five bivalves. The results from the circulation are presented in Table 2 (Ring Test Bulletin – RTB#34) 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. The agreement at the generic level was relatively good; ninety-five errors (from a potential six hundred and twenty-five) were recorded in the twenty-five data sets received from twenty participating laboratories. Agreement at the specific level was also generally good; one hundred and forty-three errors were recorded. Seven of the specimens circulated were incorrectly identified at species level by approximately two thirds 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 seven specimens. *Nucula nucleus* (juvenile, good specimen), *Goodallia triangularis* (juvenile, good specimen), *Fabulina fabula* (juvenile, poor/fair specimen), *Mytilus edulis* (juvenile, good specimen), *Parvicardium scabrum* (medium, good/fair specimen), *Adontorhina similis* (medium, good specimen) and *Thyasira sarsi* (large/medium, fair/good specimen) accounted for a total of 64% of all generic and 63% of all the specific differences recorded. One of these specimens, *Adontorhina similis*, was incorrectly identified by all participating laboratories. One of the twenty-five circulated specimens was correctly identified by all participating laboratories (*Glycymeris glycymeris* (medium, good specimen)). Further details and analysis of results can be found in the ring test bulletin (Ring Test Bulletin - RTB#34) 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 RTB32 and Figure 1 in RTB34) present the number of differences recorded at the level of genus and species for each of the participating laboratories, for RT circulations RT32 and RT34. 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 RT32 were of crustaceans and molluscs. Crustacean specimens (nine specimens in total) were responsible for 16% of generic differences and 39% of the total number of specific differences. Five of the total twenty-five specimens circulated were molluscs and these produced 54% of the generic and 28% of the specific differences recorded. Ten annelid specimens were responsible for 16% of generic differences and 26% of the total number of specific differences. One chelicerate (sea spider) specimen completed the ring test circulation and was responsible for 14% of generic differences and 7% 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.

RT32 identified discrepancies with literature used by some participating laboratories for their identification of the *Ammothea hilgendorfi*, *Eurydice affinis*, *E. truncata*, *Pholoe inornata*, *Gammaropsis lobata* specimens. One Laboratory (LB1401) identified all twenty-five RT32 specimens correctly. One taxon circulated in this ring test is likely to an undescribed species; specimen circulated in vial number 4 did not fully fit the description of *Chaetozone vivipara*; these have been identified as *C.* cf. *vivipara*. All participating laboratories have been made aware of the variety of problems encountered for these ring tests via the RT32 ring test bulletin (Ring Test Bulletin - RTB#32).

RT34 identified discrepancies with literature used by all participating laboratories for their identification of the *Adontorhina similis* specimens. One Laboratory (LB1419) identified twenty-four out of the twenty-five RT34 specimens correctly. All participating laboratories have been made aware of the variety of problems encountered for these ring tests via the RT34 ring test bulletin (Ring Test Bulletin - RTB#34).

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) component of the Scheme was introduced in Scheme year three (1996/97). This component assesses the ability of participating laboratories to identify material from their own area, or with which they are familiar. This was the twelfth Laboratory Reference exercise (LR12). 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 taxa including 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 **five 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 (LR12), ten laboratories supplied specimens for verification; one laboratory decided not to participate; five 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.

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. A single unsorted grab sample from coastal waters was 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

Sample MB15 was collected at a depth of forty-one metres from Filey Bay, Scarborough; in an area of moderately rich, marine, muddy sand sediment. A set of samples was collected using a 0.1m² Day Grab. Sampling was carried out while at anchor and samples for distribution were collected within a five hour period. All grabs taken were equal in size. Sieving was carried out on-board using a mesh of 0.5mm, followed by fixing in buffered formaldehyde solution. Samples were mixed after a week in the fixative. Prior to distribution to the participating laboratories the samples were washed over a 0.5mm sieve and transferred to 70% IMS (Industrial Methylated Spirits).

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 1 mm 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.

Nine 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 (MB15) was from a marine location in Filey Bay, Scarborough. The distributed samples comprised approximately 1.5 litres of muddy sand sediment, collected from a depth of approximately forty-one metres. The samples contained on average sixty-seven taxa and three-hundred and forty-seven individuals, covering a variety of phyla. The composite list from all samples was one-hundred and twenty-nine taxa. None of the participating laboratories subsampled their residues. Four participating laboratories did not supply biomass data. Ten of the fifteen laboratories subscribing to this module returned samples and data; three laboratories communicated their intention to abstain; two laboratories did not supply data or communicate their abstention. Detailed results have been reported to the participating laboratories (Macrobenthic Exercise Results – MB15) and were also posted on the Scheme's website (www.nmbaqcs.org); additional comments are added below.

2.4.2.1.1 Efficiency of sample sorting

Table 1 (MB15 Report) presents a summary of the estimate of numbers of taxa and individuals made by each of the participating laboratories for sample MB15, together with the corresponding count made by Unicomarine Ltd upon reanalysis. 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.

2.4.2.1.2 Number of Taxa

Column 5 in Table 1 shows variation between laboratories in the percentage of taxa identified in the samples. At most fourteen taxa (and 22% of the total taxa in the sample) were either not extracted or not recognised within the picked material. Unicomarine Ltd. recorded the more taxa than the participating laboratory in all ten of the returned samples.

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 by the laboratory. None of the laboratories extracted representatives of all the taxa present in their samples. On

average laboratories missed approximately seven taxa in their residues and in the worst instance ten new taxa were missed during the picking stage of this exercise.

2.4.2.1.3 Number of Individuals

Re-sorting of the sample residues by Unicomarine Ltd. retrieved additional individuals from all samples; these data are presented in columns 11 and 12 of Table 1. 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 80% of the samples was less than 5% of the true total number in the sample. In the worst instances thirty-seven individuals and 9.1% 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 approximately ten. A breakdown of the missed individuals by taxonomic group is presented in Table 2.

2.4.2.1.4 Uniformity of identification

Most of the species in the distributed sample were identified correctly by the participating laboratories. All of the participating laboratories produced taxonomic differences, *i.e.* disagreement with the AQC identification (Table 1, column 15 in the MB15 Report). In the worst instances eleven taxonomic differences were recorded. On average over six taxonomic differences were encountered per sample. These showed no obvious correlation across the data set, however *Tharyx killariensis*, *Abra nitida* and *Anobothrus gracilis* produce the highest number of taxonomic error instances.

2.4.2.2 Comparison of Similarity Indices (Bray-Curtis)

The fauna list for each sample obtained by the participating laboratory was compared with the list obtained for the same sample following its re-examination by Unicomarine Ltd. 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 (MB15 Report). There was variation among laboratories in the values calculated for the index, from 84.6% to 97.6%, with an average value of 92.4%. The index for the majority of laboratories (8 of 10) was below 95% and 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 (MB15).

2.4.2.3 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 MB15 circulation is presented in Table 3. Four laboratories did not supply biomass data. The average difference between the two weight values was 2.4% (*i.e.* heavier than that made by Unicomarine Ltd.), however the measurements by major faunal groups made by Unicomarine Ltd. were typically less (*i.e.* lighter) than that made by the participating laboratory. There was great variation in biomass estimations between participating laboratories and between taxonomic groups. The range of overall biomass percentage difference results, between participating laboratories and Unicomarine Ltd., was from –20.6% (measurements by laboratory were lighter than those made by Unicomarine Ltd.) to +23.8% (measurements by laboratory were greater than those made by Unicomarine Ltd.). The average difference between estimations varied greatly between faunal groups, ranging from –3.1% to +24.6% (from Echinodermata to Nemertea, respectively).

2.4.2.4 Uniformity of samples

The faunal content of the samples distributed as MB15 is shown in Table 4. Data received from the participating laboratories were very similar showing only very slight natural variation. The samples can be assigned to the *Amphiura filiformis*, *Mysella bidentata* and *Abra nitida* in circalittoral sandy mud biotope (SS.SMu.CSaMu.AfilMysAnit) (Connor *et al.* 2004).

2.4.3 Discussion

The sample distributed as MB15 comprised a diverse and relatively well populated marine muddy sand sample. The extraction of fauna from the sediment was difficult, due to the volume of sediment and

quantities of infaunal and epifauna taxa and individuals present. The dominant taxa present in the majority of samples were *Amphiura filiformis, Euclymene oerstedii, Mysella bidentata, Owenia fusiformis* and *Galathowenia oculata*. 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 (MB15 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. None of the participating laboratories extracted all the countable material from the residue (according to their specified processing requirements); in the best instance LB1404 missed just one individual. In the worst instance thirty-seven individuals, 9.1% of the total individuals, were not extracted from the residue. Identification of the extracted fauna also caused several problems for participants. None of the laboratories correctly identified all their extracted fauna. There were a total of sixty-three taxonomic mistakes from all ten participants, these included misidentifications of *Tharyx killariensis, Ennucula tenuis, Abra nitida, Chamelea striatula, Anobothrus gracilis, Modiolus* sp. juv., *Vitreolina philippi, Retusa umbilicata, Abra prismatica, Chaetoderma nitidulum, Nephtys hombergii* and *Goniada maculata*. Eight of the ten returning laboratories attained a Bray-Curtis similarity higher than 90%. The highest Bray-Curtis similarity index achieved was 97.6% (LB1411). The average Bray-Curtis figure achieved was 92.4%. This figure is relatively consistent for a coastal sample in the MB module; 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 those samples circulated for the macrobenthic exercise (MB15). The area sampled was uniformed in its faunal composition. The samples were typical of the area and showed only slight natural variation. All samples were of relatively equal volume and sediment characteristics.

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; four provided data that was lighter in total than Unicomarine Ltd.; two supplied data that was heavier than Unicomarine Ltd. estimations. The extremes recorded were 20.6% lighter (LB1403) and 23.8% heavier (LB1414) than the Unicomarine Ltd. estimations. Overall the average difference between the values determined by the participating laboratories and Unicomarine Ltd. was 2.4% (*i.e.* laboratory measurements were heavier than those made by Unicomarine Ltd.). Previous Scheme years have not shown any particular pattern of variance for biomass estimations; the last three year's average biomass difference figures were 2.3% lighter (MB14), 9.9% heavier (MB13) and 2.2% heavier (MB12). 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 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.* 2006 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 **seven 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, eighty-one selected samples were received from twenty-seven laboratories, together with descriptions of their origin and the collection and analysis procedures employed. Samples were identified as OS35, OS36 and OS37 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 20 ml to 8 L of residue. The associated fauna of the samples was also very varied; the number of taxa recorded ranged from 2 to 198, with the number of countable individuals from 3 to 9193. Twenty-seven of the thirty laboratories that subscribed to the OS module returned three Own Samples; eighteen of these Own Samples have been audited externally by Aquatic Environments due to Unicomarine Ltd. being responsible for the initial sample processing; two laboratories decided not to participate in this module; one laboratory (LB1432) failed to supply their three selected Own Samples, these samples have been excluded from the summary statistics in this report and have been assigned 'deemed fail' sample flags. Detailed results have been reported to the participating laboratories. A summary of results from the Own Sample module is presented in the Own Sample Module Summary Report (OS35, 36 & 37).

2.5.2.2 *Efficiency of sample sorting*

Table 1 (Own Sample Module Summary Report – OS35, 36 & 37) 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-nine samples (60% of all samples) the number of taxa recorded by the participating laboratories was identical to that obtained by Unicomarine Ltd. (column 4). In the thirty-two exceptions, the difference was at most nine taxa and the average difference was less than one taxon.

Data for the numbers of individuals recorded (columns 6 and 7) shows a range of differences from re-analysis of between 0% and 85.7%. The average difference was 4.1% (fifteen samples exceeded this average). Thirty-four of the eighty-one 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 twenty-eight samples contained taxa in the residue which were not previously extracted, the worst example being nine new taxa found in the residue (column 10). In the worst instance residue was found to contain seventy-one 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 (0.79).

2.5.2.3 *Uniformity of identification*

Taxonomic differences between Unicomarine Ltd. and participating laboratories' results were found in thirty-six (44%) of the eighty-one samples re-analysed. A summary of mis-identified taxa is presented in Table 3 (OS Summary Report). An average of 1.3 taxonomic differences per laboratory were recorded; in the worst instance fifteen 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, maldanids and *Abra* spp. 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 16.7% to 100%, with an average figure of 94.8%. Twelve samples from nine laboratories achieved a similarity figure of less than 90%. Twelve samples produced a similarity figure of 100%; these were submitted by nine different laboratories (LB1401, LB1407, LB1409, LB1410 x2, LB1416, LB1419, LB1423 x2, LB1426 x2 and LB1427). The best overall results were achieved by laboratory LB1426 (results comprised 100%, 99.45% and 100%), which averaged 99.82% similarity. The worst overall results were achieved by laboratory LB1429, whose results comprised 84.34%, 16.67% and 96.69%. 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; thirty-four samples were not supplied with species blotted wet weight biomass data; five samples were reported to five decimal places and three to three decimal places (4 decimal places is required). Consequently, only forty-seven of the eight-one 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 +8.0% difference between the two sets of results (*i.e.* heavier than Unicomarine Ltd.); the range was from –10.0% to +33-1%. 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 +12.2% for polychaetes, -32.0% for oligochaetes, -71.8% for nemerteans, +6.5% for Chelicerata, -165.6% for crustaceans, -202.4% for echinoderms, +2.1% for molluscs and -19.7% 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 better in the OS exercise compared to the MB15 exercise. The average value of the index was 94.8% for the OS, compared with 92.4% for MB15. Both modules have produced several good results and some instances of excellent sample processing.

There were eighty-one samples submitted for this module, including eighteen samples that have been processed by the Scheme's external auditor. One laboratory (LB1432) did not supply their three selected Own Samples (due to 'gross processing errors by a subcontractor'); these samples are deemed to have failed the Scheme's standards. They are not included in the summary statistics within this report, however remedial action is still recommended. The recommended remedial action in this instance is to ensure in the future that all selected Own Samples are supplied for audit. The Own Sample exercises can quantify failing samples, suggest remedial actions and provide a framework for evaluating any subsequent remedial action.

Approximately 85% of the eighty-one samples reported exceeded the 90% Bray-Curtis pass mark and approximately 77% of the samples exceeded 95% Bray-Curtis similarity. The average Bray-Curtis similarity index achieved was 94.8%. These figures are consistent with the high quality results from previous OS exercises. In the 2006/07 Scheme year thirteen (OS32, 33 and 34) the average Bray-Curtis figure was 96%, and 91% (of the sixty-nine comparable samples) achieved more than 90% Bray-Curtis results. In the 2005/06 Scheme year twelve (OS29, 30 and 31) the average Bray-Curtis figure was 96%, and 93% (of the fifty-four comparable samples received) achieved more than 90% Bray-Curtis results. In the 2004/05 Scheme year eleven (OS26, 27 and 28) the average Bray-Curtis figure was 96%, and 94% (of the fifty-four samples received) achieved more than 90% Bray-Curtis results. In the 2003/04 Scheme year ten (OS 23, 24 and 25) the average Bray-Curtis figure was 94%, and 84% (of the fifty-one samples received) achieved more than 90% Bray-Curtis results. In the 2002/03 Scheme year nine (OS 20, 21 and 22) the average Bray-Curtis figure was 92%, and 75% (of the forty-four samples received) achieved more than 90% Bray-Curtis results. In the 2001/02 Scheme year eight (OS 17, 18 and 19) the average Bray-Curtis figure was 90.5% and 78% (of the forty-five samples received) achieved more than 90% Bray-Curtis results. In the 2000/01 Scheme year seven (OS 14, 15 and 16) the average Bray-Curtis figure was 90.8% and 67% (of the forty-five samples received) achieved more than 90% Bray-Curtis results. In the 1999/2000 Scheme year six (OS 11, 12 and 13) the average Bray-Curtis figure was 91.4% and 73% (of the fifty-one samples received) achieved more than 90% Bray-Curtis results. In the 1998/99 Scheme year five (OS 08, 09 and 10) the average Bray-Curtis figure was 89.3% and 71% (of the forty-two samples received) achieved more than 90%. In the 1997/98 Scheme year four (OS 05, 06 and 07) the average Bray-Curtis figure was 93.6% and 83% (of the forty samples received) achieved more than 90%.

Since the beginning of the OS component six hundred and twenty-eight admissible samples have been received (OS01-37), with an average Bray-Curtis similarity figure of 93.53%. One hundred and thirteen samples (18%) have fallen below the 90% pass mark. Eighty 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 – OS35, 36 & 37). 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 89% of the comparisons were considered to have passed the enumeration of taxa standard; 91% exceeded the enumeration of individuals standard and 85% 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); six of the eighty-one applicable samples are flagged as 'Fail - Bad'; six are flagged as 'Fail - Poor'; seven are flagged as 'Pass - Acceptable'; fifty are flagged as 'Pass - Good'; and twelve 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 slightly poorer (Table 5, column 19) with 79% 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 (OS Summary Report). The Table shows the number of laboratories assigned 'Pass' and 'Fail' flags for the OS exercises over the past thirteen years based upon the current NMBAQC Scheme standards (see Description of the Scheme Standards for the Benthic Invertebrate Component). This year's eighty-one Own Samples resulted in the fifth highest percentage pass rate, 85% (the highest being 100% achieved in exercise OS01 that involved just fourteen samples), since the beginning of the Own Sample module. 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 thirteen 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. 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 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 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 14. Also 'failing' samples with outstanding remedial action from the previous three 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 action, outlined below, was required for associated replicates of the following Own Samples:

CSEMP samples

LB1402 OS36- Review estimation of taxa / methods for checking *Turritella communis* specimens for attached Actiniaria.
Remedial Action - completed (02/09/2009).

LB1402 OS37- Reprocess taxonomic errors for any associated samples; primarily *Abra alba* / *A. nitida* identifications.
Remedial Action - completed (02/09/2009).

LB1405 OS36- Review *Capitella* sp. / *Mediomastus fragilis* identifications for any associated samples.
Remedial Action - completed (30/04/2009).

LB1417 OS35- Reprocess residues for associated replicate samples. Submit revised data for random selection of additional sample residue for audit.
Remedial Action – completed (17/06/2009).

LB1420 OS36- Review *Paranais litoralis / Manayunkia aestuarina* identifications.
Review enumeration of Oligochaeta taxa.
Remedial Action – completed (17/08/2009).

Non-CSEMP samples

LB1409 OS36- Review *Bathyporeia gracilis / B. elegans* identifications.
Remedial Action – completed (21/05/2009).

LB1411 OS36- Reprocess taxonomic errors for any associated samples; *Abra alba / A. nitida, Circomphalus casina* juv? / *Chamelea striatula* and *Leptosynapta* sp. juv. / Cucumariidae juv.
Remedial Action – completed (08/12/2009).

LB1421 OS35- Reprocess taxonomic errors for any associated samples; *Chaetozone christiei* / *Tharyx* sp. / *Chaetozone* sp. D, *Amphiura chiajei* / *Amphipholis squamata, Chone filicaudata* / *Jasmineira caudata, Thysanocardia procera* / *Golfingia elongata, Glycera tridactyla* / *G. alba, Amphicteis gunneri* / *Ampharete lindstroemi / Sosane sulcata, Paraonides* sp. / *Paradoneis* sp. #2, *Raphitoma* sp. / *Mangelia brachystoma, Rhodine* sp. / *Clymenura* sp., *Eucylmene* sp. / *Praxillella affinis* / *Clymenura* sp. / *Eucylmene oerstedii, Streptosyllis* sp. / *Syllides* sp. and *Praxillella praetermissa* / *Praxillella affinis* #1 identifications.
Review faunal extraction methodology for future sample processing.
Ensure that all samples (fauna and residues) associated with Own Samples are retained until successful completion of the audit, as these may be required for remedial action.
Remedial Action – completed (09/02/2010).

LB1421 OS37- Reprocess taxonomic errors for any associated samples; *Nucula sulcata* / *N. nitidosa, Gouldia minima* / *Mysia undata, Chamelea gallina* / *C. striatula*

and *Chaetozone* sp. / *C. setosa* / *C. zetlandica* / *Monticellina* sp. / *Tharyx killariensis* identifications.
Reprocess residues for associated replicate samples. Submit revised data for random selection of additional sample for full audit.
Ensure that all samples (fauna and residues) associated with Own Samples are retained until successful completion of the audit, as these may be required for remedial action.
Remedial Action – completed (09/02/2010).

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.

One participating laboratory, LB1432, did not supply their three selected Own Samples. These samples are deemed to have failed the NMBAQC Scheme standards. The recommended remedial action is to review in-house laboratory procedures to ensure that all subsequent requested Own Sample are provided for audit.

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. All Scheme participants now use e-mail as their primary means of communication. Many of the interim results are now provided as secure PDF documents via 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.
3. 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.
4. A minority of participating laboratories have received '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 List database. Laboratories are encouraged to review the content and give details of additions wherever possible.

8. 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.

9. 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.

10. 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. In Scheme year ten MB11 (artificial macrobenthic sample) 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, once devised, will be applied and tested in the MB training module.

11. 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.
12. Positive, constructive feedback has been received from participants during Scheme year 14. Some new Scheme participants detailed their 'enjoyment' of the RT module and realised the benefits of standardised external Own Sample audits. 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.

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