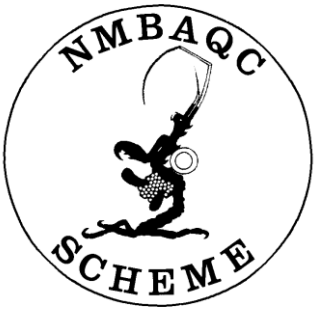

BEQUALM

NATIONAL MARINE BIOLOGICAL

ANALYTICAL QUALITY CONTROL SCHEME

Benthic Invertebrate Component Report

Scheme Operation Year 18 - 2011/2012

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BENTHIC INVERTEBRATE COMPONENT REPORT FROM THE CONTRACTOR

SCHEME OPERATION – YEAR 18 – 2011/12

1. Introduction

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

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

Year 18 of the Scheme (2011/12) followed the format of Year 17. 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).

Forty laboratories participated in the benthic invertebrate component of the NMBAQC Scheme in Year 18. Fifteen participants were Competent Monitoring Authorities (CMAs) and twenty-five were private consultancies. One of the participants was a consortium of sole traders. Four of the CMA participants were responsible for CSEMP (Clean Seas Environment Monitoring Programme) sample analysis. To 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 Thomson 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. These flags are indicated in Table 5 of the Own Sample Module Summary Report – OS47, 48 & 49 presenting the comparison of laboratory results with the standards.

1.1 Summary of Performance

This report presents the findings of the Benthic Invertebrates Component for Year 18 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 fully marine macrobenthic sample (MB, Macrobenthic Sample module).
- Re-analysis by Thomson Unicomarine of three own samples supplied by each of the participating laboratories (OS, Own Sample module).
- Identification of two sets of twenty-five invertebrate specimens (RT, Invertebrate Ring Test module).
- Re-identification of a set of twenty-five specimens supplied by each of the participating laboratories (LR, Laboratory Reference module).

The analytical procedures of the various modules were the same as for Year 17 of the Scheme, which includes the specification that the Macrobenthic Sample module and CSEMP samples within the Own Sample Module should be conducted using the NMBAQC guidance for macrobenthic invertebrate sample analysis (Worsfold, Hall & O'Reilly (Ed.) 2010). The results for each of the Scheme exercises are presented and discussed. Comments are provided on the performance for each of the participating laboratories in each of the exercises.

Two **Ring Tests (RT)** of 25 specimens were distributed. One set contained 25 general invertebrate specimens (RT41) and a second set consisted of 'targeted' specimens from taxa that occur in Scottish waters (RT42). For the general set of fauna (RT41), there was fairly good agreement between the identifications made by the participating laboratories and those made by Thomson Unicomarine On average each participating laboratory recorded 2.1 generic differences and 6.3 specific differences. Eight taxa (two molluscs, four polychaetes and two crustaceans) were responsible for two thirds of the specific differences. The 'targeted' ring test (RT42, taxa from Scottish waters) produced much better results than the standard exercise. On average each participating laboratory recorded 2.9 generic differences and 3.6 specific differences. Four taxa (three molluscs and one polychaete) were responsible for more than half of the differences.

Laboratory Reference (LR): Eleven laboratories submitted their specimens for confirmation. Six of these 11 laboratories were responsible for one third or more differences to the

identifications made by Thomson Unicomarine. The taxa involved were mainly bivalves, amphipods and polychaete families which are either speciose or for which keys are inadequate.

Analysis of the **Macrobenthic Sample (MB)** by the participating laboratories and subsequent re-analysis by Thomson Unicomarine provided information on the efficiency of extraction of the fauna, accuracy of enumeration and identification and the reproducibility of biomass estimations. For MB19 fully marine samples from the southern North Sea were distributed. This was the second MB exercise with strict extraction and processing instructions and, in contrast to the previous year, results for MB19 showed a high degree of agreement to the re-analysis by Thomson Unicomarine. Extraction efficiency (of individuals) was on average 95.84% with only one laboratory extracting less than 90 % of the individuals. Comparison of the results from the laboratories with those from analysis by Thomson Unicomarine (following the NMBAQC macrobenthic analysis guidelines) was made using the Bray-Curtis similarity index (untransformed). The value of the index varied between 76.02% and 99.06%. It was better than 90% in 71% of the comparisons and less than 85% in only one laboratory.

The revised protocols of Scheme Year 10 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 2010; formerly NMMP) samples or similar alternative sampling programmes (if not responsible for CSEMP samples). The OS 'Pass/Fail' flagging system, introduced in Scheme Year 8, was continued (see Description of the Scheme Standards for the Benthic Invertebrate Component). The results for the Own Sample Module were slightly better than those from the Macrobenthic Sample Module. Agreement between the laboratories and Thomson Unicomarine was generally very good. Extraction efficiency was better than 90% in 96% of the comparisons and better than 95% in 87% of all comparisons. All countable faunal specimens were extracted from the sample residues in 58% of the samples. The Bray-Curtis similarity index ranged from 86% to 100% with an average figure of 97%. The Bray-Curtis similarity index was greater than 95% in 82% of comparisons and in most cases (96%) the value of the index was greater than 90%. These samples all achieved 'Pass' flags. Twenty-seven samples (27%) achieved 'Pass-Excellent' flags with Bray-Curtis similarity scores of 100%.

1.1.1 Statement of Performance

Each participating laboratory was supplied with a 'Statement of Performance', which included a summary of results for each of the Scheme modules and details the resulting flags where

appropriate. These statements were first circulated with the Year 5 annual report (1998/1999) for the purpose of providing evidence of Scheme participation and for ease of comparing year on year progress.

2. Summary of Benthic Invertebrate Component

2.1 Introduction

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

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

2.1.1 Logistics

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

2.1.2 Data Returns

Return of data to Thomson Unicomarine followed the same process as in previous years. Spreadsheet based forms (tailored to the receiving laboratory) were distributed to each laboratory via email, with additional hard copies where appropriate. All returned data have been converted to Excel 2003 format for storage and analysis. In this, and previous, Scheme years slow or missing returns for exercises lead to delays in processing the data and resulted in difficulties with reporting and rapid feedback of results to laboratories. Reminders were distributed shortly before each exercise deadline.

2.1.3 Confidentiality

To preserve the confidentiality of participating laboratories, each are identified by a four-digit Laboratory Code. In September 2011 each participant was given a confidential, randomly assigned Scheme Year 18 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 18 will be recorded as LB1804.

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

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

2.2.1 Description

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

Two sets of 25 benthic invertebrate specimens were distributed in 2011/12. The first of the year's RT circulations (RT41) was a general invertebrate ring test. The specimens included representatives of the major phyla; 32% of the taxa were molluscs, 28% were crustaceans, 28% were annelids, 8% were echinoderms and 4% from minor phyla. The second circulation (RT42) comprised 'targeted' specimens of taxa found in Scottish waters; participants were not aware of the theme of this exercise. The specimens included representatives of the major phyla; 48% of the taxa were molluscs, 12% were crustaceans, 36% were annelids, and 4% from minor phyla. 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 (RT41) and the 'targeted' RT (RT42), 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 level. If a laboratory would not routinely have identified the specimen to the level of species then they were asked to state this 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 Thomson Unicomarine for verification, resolution of any disputed identifications and potential reuse in future Scheme exercises. The implementation of this part of the Scheme was the same as previous years. Participating laboratories were permitted to supply multiple data entries (*i.e*. different sets of results from different analysts) for each exercise to enhance the training value of this module. The protocols followed for the two circulations, in particular the method of scoring results, were the same as for previous circulations. Approximately eight weeks were allowed for the analysis of both RT exercises (RT41 and RT42).

2.2.2 *Results*

2.2.2.1 *General Comments*

A number of laboratories use the ring tests for training purposes and have selected them preferentially over other modules. CSEMP laboratories are required to participate in this exercise though the results are not used to assign 'Pass' or 'Fail' flags. In total 23 laboratories subscribed to RT41 and RT42. For RT41, all 23 laboratories returned data (28 individual data sets). For RT42, 20 laboratories returned data (24 individual data sets).

2.2.2.2 *Returns from Participating Laboratories*

Identifications made by the participating laboratories were compared with those made by the AQC to determine the number of differences. In the case of an identification being different from the AQC identification through the use of synonyms or mis-spelling of names, these differences were ignored for the purpose of calculating the total number of differences.

Tables 1 and 2 of the Ring Test Bulletins RTB41 and RTB42 show identifications made by each of the participating laboratories for the twenty-five specimens arranged by specimen and by laboratory respectively. For clarity the name is given only in those instances where the generic or specific name given by the laboratory differed from the AQC identification. Where it was considered that the name referred to the same species as the AQC identification, but differed for one of the reasons indicated above, then the name is presented in brackets "[name]". Errors of spelling or the use of a synonym are not bracketed in this way if the

species to which the laboratory was referring was not the same as the AQC identification. A dash, "-", in the Tables indicates that the name of the genus (and / or species) given by the laboratory was considered to be the same as the AQC identification. A pair of zeros, "0 0", in the Tables indicates that the subscribing laboratory did not return data.

2.2.2.2.1 Scoring of RT Results

The laboratory's score was increased by one for each difference between their identification and the AQC identification, *i.e*. for each instance where text other than a dash or a bracketed name appears in the appropriate column in the tables (Tables 1 and 2 in RTB41 and RTB42). Two separate scores were maintained for differences at genus and species level.

2.2.2.3 Ring Test Results

The intention of this training module is to discover where particular difficulties lie within specific common taxa. Results were presented in the Ring Test Bulletins (RTB) outlining the reasons for each individual identification discrepancy. These bulletins contained images of the test material and the alternative, incorrectly recorded taxa, where available. Participating laboratories were advised to retain their ring test specimens, for another couple of weeks after the arrival of their results, in order to review their identifications where necessary. On completion of each exercise, specimens were required to be returned to Thomson Unicomarine for potential future circulation.

2.2.2.3.1 Ring test 41 (Type: General)

The results discussed below are given in Table 1 of RTB 41 which displays the data arranged by species to enable quick reference to the range of answers received and in Table 2 which presents the results arranged by laboratory (see Ring Test Bulletin – RTB41).

Eight of the 25 specimens circulated were molluscs; seven were crustaceans; seven were polychaetes; two were echinoderms; and one was from a minor phylum. The agreement at generic level was generally good; 60 differences (from a potential 700) were recorded in the 28 data sets received from 23 participating laboratories. Agreement at specific level was less good; 175 differences were recorded, equal to one quarter of all specific identifications.

Eight of the specimens circulated were incorrectly identified at species level by two-third (67%) of the participants. These were the molluscs *Musculus subpictus* and *Nucula nucleus*, the polychaetes *Ditrupa arietina*, *Hesiospina similis*, *Marenzelleria neglecta* and *Ophelia rathkei*, and the crustaceans *Gammarus oceanicus* and *Gammarus pulex.*

One of the 25 specimens circulated (i.e. the polychaete *Scalibregma inflatum*) was correctly identified by all participants.

Further details and analysis of results can be found in the Ring Test Bulletin RTB41 which was circulated to each laboratory that supplied results for this exercise and was also posted on the Scheme's website (www.nmbaqcs.org).

2.2.2.3.2 Ring test 42 (Type: Targeted, Scottish Fauna)

The results discussed below are given in Table 1 of RTB42 which displays the data arranged by species to enable quick reference to the range of answers received and in Table 2 which presents the results arranged by laboratory (see Ring Test Bulletin – RTB42).

Twelve of the 25 specimens circulated were molluscs, nine were polychaetes, three were crustaceans and one was from a minor phylum. The agreement at generic level was generally good; 72 errors (from a potential 600) were recorded in the 24 data sets received from 20 participating laboratories. Agreement at specific level was much better than in RT41 with 90 differences recorded, equal to 15% of all specific identifications.

Four of the specimens circulated were incorrectly identified at species level by more than half of the participants (54%). These were the molluscs *Ecrobia ventrosa, Onoba aculeus* and *Turtonia minuta* and the polychaete *Polyphysia crassa.*

Five of the twenty-five specimens circulated (i.e. the polychaete *Fabricia stellaris* and the molluscs *Omalogyra atomus, Skeneopis planorbis, Timoclea ovata* and *Bittium reticulatum*) were correctly identified by all participants.

Further details and analysis of results can be found in the Ring Test Bulletin RTB42 which was circulated to each laboratory that supplied results for this exercise and was also posted on the Scheme's website (www.nmbaqcs.org).

2.2.2.4 Differences between Participating Laboratories

Differences recorded at genus and species level for each of the participating laboratories are shown in Figure 1 in RTB41 and RTB42 respectively. The laboratories are ordered by increasing number of differences at species level. The division of laboratories into three bands (Low, Medium and High) on the basis of the number of differences at the level of species is also shown.

2.2.2.5 Differences by Taxonomic Group

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

Taxon	**Nbr. species**	**Generic differences**		**Specific differences**	
Polychaeta	16	62	47%	106	40%
Mollusca	20	45	34%	94	35%
Crustacea	10	11	8%	51	19%
Minor Phyla	2	9	7%	9	3%
Echinodermata	2	5	4%	5	2%
TOT.	50	132	100%	265	100% (adjusted)

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

2.2.3 Discussion

The results were in general comparable with those from previous exercises, with a high level of agreement between participating laboratories for the majority of distributed species. The RT component is considered to provide a valuable training mechanism and to be an indicator of problem groups and possible areas for further 'targeted' exercises for inclusion at taxonomic workshops. Multiple data entries from each laboratory and the inclusion of images in the Ring Test Bulletins have enhanced the training value of this component. All participating laboratories have been made aware of the variety of problems encountered during these ring tests via the ring test bulletins RTB41 & 42, which include also a list of useful literature.

The best results were obtained by LB1809 and LB1814 for RT41 with zero differences at genus level and only one difference at species level. In RT42 the best participants were LB1809b, LB1814 and LB1820. LB1814 had two generic and one specific difference, while LB1809b and LB1820 had one generic and two specific differences.

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

2.3.1 Description

The Laboratory Reference Module is a training module which encourages laboratories to build extensive, verified reference collections to improve identification consistency. The value of reference material in assisting the process of identification cannot be over-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 3 (1996/97). This module can help assess the ability of participating laboratories to identify material from their own area, or with which they are familiar. Laboratories are also able to use this exercise to verify identifications of difficult or problematic taxa about which they are unsure. Specimens wherever possible were to be representatives from CSEMP reference collections. This was the sixteenth Laboratory Reference exercise (LR16). The participants were able to submit up to 25 specimens for re-examination by Thomson Unicomarine.

2.3.1.1 Preparation of samples

A prepared results sheet was distributed with the exercise's instructions and attached labels for the laboratories to identify each of the specimens. Participating laboratories were asked to prepare and submit their reference specimens within 12 weeks. All specimens were re-identified and the identification made by Thomson Unicomarine compared with that made by the participating laboratories. All specimens were returned to the laboratories after analysis.

2.3.2 Results

In total, 11 laboratories participated in this exercise (LR16). Detailed results have been reported to each of the participating laboratories separately. Six of these 11 laboratories were responsible for one third or more differences to the identifications made by Thomson Unicomarine. The taxa involved were mainly bivalves, amphipods and polychaete families which are either speciose or for which keys are inadequate.

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. Most laboratories sent well known species while others elected to obtain a 'second opinion' on more difficult species.

2.4 Macrobenthic Sample (**MB**) Module

2.4.1 Description

The Macrobenthic Sample Module is a training module which assesses the participants' ability to process macrobenthic samples from the same habitat. In the case of MB19, fully marine samples from the southern North Sea were distributed in order to examine differences in sample processing efficiency and identification plus their combined influence on the results of multivariate analysis. In addition, an examination of the estimates of biomass made by each of the participating laboratories was undertaken.

2.4.1.1 *Analysis Required*

Each participating laboratory was required to carry out sorting, identification, enumeration and biomass estimations of the macrobenthic fauna contained in the sample. Precise protocols were provided (see Appendix 1 of Macrobenthic Sample Results – MB19 and Worsfold, Hall & O'Reilly (Ed.) 2010). The participating laboratories were required to complete a Macrobenthic Sample Details Form, which specified their processing methodology. The extracted fauna were to be separated, identified and stored in individually labelled vials. Labels were provided and cross-referenced to the recording sheets.

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

Participants were asked to complete sample analysis within 12 weeks. All sorted and unsorted sediments and extracted fauna were to be returned to Thomson Unicomarine, together with the data on counts and biomass determinations.

2.4.1.2 *Post-return Analysis*

Upon return to Thomson Unicomarine, 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 Thomson Unicomarine staff using a standard technique.

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

2.4.2 *Results*

2.4.2.1 *General Comments*

The distributed macrobenthic sample (MB19) was a fully marine sample from the southern North Sea. Seven laboratories returned fauna and data for re-analysis. Three participating

laboratories did not supply biomass data. A report for this exercise was distributed to the participating laboratories (Macrobenthic Sample Results – MB19) and was also posted on the Scheme's website (www.nmbaqcs.org).

2.4.2.2 *Efficiency of Sample Sorting*

Table 1 of the MB19 Report presents a summary of the numbers of taxa and individuals counted by each of the participating laboratories for sample MB19, together with the corresponding count made by Thomson Unicomarine after re-analysis. Comparison of the respective counts is given as a percentage. Table 2 shows the composition of fauna missed by each participating laboratory.

2.4.2.3 *Number of Taxa*

Column 5 in Table 1 shows the variation between laboratories in the percentage of taxa identified in the samples. Compared to the number of taxa found by Thomson Unicomarine three of the seven laboratories (LB1806, LB1808 and LB1810) extracted the same number of taxa. Two other laboratories (LB1802 and 1807) extracted fewer taxa, the re-analyses showing that additional taxa were either present in the taxon pots or new taxa present in the residue. Only one laboratory (LB1822) recorded more taxa, the main reason for this being mis-identification by the laboratory; the re-analysis showed that a number of taxa found by the laboratory were repeat taxa which reduced the total number of taxa compared to the number found by Thomson Unicomarine.

The values presented for the number of taxa not extracted (column 10) refer to taxa not recorded or extracted (even if misidentified) elsewhere in the results. Four of the seven laboratories (LB1804, LB1808, LB1810 and LB1822) extracted representatives of all the taxa present in their samples. Two laboratories (LB1806 and LB1807) missed only one taxon each and in the worst case one laboratory (LB1802) missed five taxa.

2.4.2.4 *Number of Individuals*

Re-analysis of the sample residues showed that all seven participants missed individuals (see columns 11 and 12 of Table 1). The proportion of missed individuals in all of the samples was rather low; less than 5% of the total number found by Thomson Unicomarine. For comparison, the same figure for the previous year (MB18) was 18%.

In the worst case, 16 out of 379 individuals (4.2%) were not extracted during the initial sample processing, and in the best case, only one out of 265 individuals (0.4%) was not extracted.

A breakdown of the missed individuals by taxonomic group is presented in Table 2. Of the three major taxa Polychaeta, Crustacea and Mollusca, Crustacea was the best 'picked' faunal group with six participants picking all individuals and only one participant missing four (11.8%) overall. The worst extracted faunal group was Mollusca. Only one laboratory (LB1808) picked all molluscs from their sample.

2.4.2.5 Uniformity of Identification

Only one of the participating laboratories (LB1807) correctly identified all taxa (Table 1, column 15). In the worst case, 23 taxonomic errors (55%) were recorded. On average 6.4% taxonomic errors were encountered per sample.

2.4.2.6 Comparison of Similarity Indices (Bray-Curtis)

The faunal list for each sample analysed by the participating laboratory was compared with the list of fauna after re-analysis by Thomson Unicomarine. The comparison was made by calculating the Bray-Curtis similarity index for the pair of samples using non-transformed data. The results of this calculation are presented in column 14 of Table 1. There was variation among laboratories in the values calculated for the index, from 76.02% to 99.06%, with an average value of 92%. The index for the majority of laboratories (5 of 7) was better than 90%; these five laboratories would have achieved 'Pass' sample flags if the NMBAQC / CSEMP standards were applied. Further details of each participating laboratory's performance are given in the MB19 report.

2.4.2.7 Biomass Determinations

A comparison of the biomass estimates made by the participating laboratories and Thomson Unicomarine broken down by major taxonomic group for the MB19 sample is presented in Table 3. Three laboratories did not supply biomass data. The average difference between the two total weight values was +11.9% (*i.e.* heavier than the weight values made by Thomson Unicomarine), with variable measurements by major faunal groups. The range of overall biomass percentage differences between participating laboratories and Thomson Unicomarine was from -0.9% to +31.5%. The average difference varied greatly between faunal groups, ranging from -2.7% to +53.6% (from Mollusca to Nemertea, respectively).

2.4.3 Discussion

MB19 was the second sample with strict extraction and processing instructions. In contrast to the previous year, results showed a high degree of agreement to the re-analysis by Thomson

Unicomarine. Extraction efficiency (of individuals) was on average 95.84% with only one laboratory extracting less than 90% of the individuals. Comparison of the results from the laboratories with those from analysis by Thomson Unicomarine (following the NMBAQC macrobenthic analysis guidelines) was made using the Bray-Curtis similarity index (untransformed). The value of the index varied between 76.02% (LB1822) and 99.06% (LB1806). and was better than 90% in 71% of the comparisons and less than 85% in only one laboratory, i.e. in 14% of comparisons.

The average BCI of 92% for this fully marine sample is much better than the one achieved for the previous artificially created sample (MB18) with 78%. However the MB18 BCI was rather low if compared with the range and average BCI results over the last 16 years (see below). The mean BCI result is 89.3%. It is not clear how participating labs approach this training exercise. Some labs may use it to train their junior staff while other may use it to demonstrate on-going competence of more senior analysts.

Summary of average Bray Curtis similarity indices achieved overall:

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

The ‘blot-drying’ procedure employed by Thomson Unicomarine for the determination of biomass was as specified in the Green Book, *i.e.* avoiding excessive pressure when blotting specimens dry. The estimates of total biomass made by the four participating laboratories and Thomson Unicomarine show considerable variation. Estimates of one laboratory (LB 1807) are 0.9% lighter than Thomson Unicomarine’s, those of the other three laboratories are all

heavier, with an extreme of 31.5% recorded by LB1810. Overall the average difference of +11.9% is lower than in the previous exercise with + 30.3% (MB18), but much higher than in the years before (-2.6% in MB17, -3.4% in MB16, +2.4% in MB15, -2.3% in MB14 and +9.9% in MB13). It is difficult to see a pattern in this variance of biomass estimations. The main reason for the observed differences between the measurements is probably due to variable drying by laboratories prior to weighing. As stated in the last MB report for Year 17, further studies are necessary to find the best methods for accurate, consistent and comparable 'blotted' dry weight figures.

2.5 Own Sample (**OS**) Module

2.5.1 Description

The Own Sample Module examines laboratory analytical performance on material from each participating laboratory's 'home' area. Following a review of the Own Sample Module (Unicomarine 2001), several changes to sample selection and scoring were implemented in Scheme Year 8 (2001/02). All participants must meet these new Own Sample requirements. Own Sample participants must supply their previous year's CSEMP data matrices, where relevant, for Own Sample selection, *i.e.* 2010/2011 CSEMP data. This is to ensure that all processing is completed (prior to selection of samples for audit), preventing reworking of the selected Own Samples and enabling samples to be audited earlier in the Scheme year. Each participating laboratory was requested to send data from which three samples were selected. The selection was in turn notified to the laboratories. Laboratories responsible for CSEMP 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 Thomson Unicomarine 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), except for CSEMP samples where the NMBAQC guidelines for macrobenthic sample analysis were to be followed (Worsfold, Hall & O'Reilly (Ed.) 2010). The names and counts of specimens were to be recorded on a matrix and linked to the vials through a specimen code number. Biomass analysis was to be carried out in the same manner as for the MB exercise.

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

2.5.2 Results

2.5.2.1 General Comments

Following the request to participating laboratories to submit data of suitable samples for re-analysis, ninety-nine selected Own Samples were received from thirty-three laboratories, together with descriptions of their origin and the collection and analysis procedures employed. Samples were identified as OS47, OS48 and OS49 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 supplied for resorting varied from mud to gravel in various volumes of residue. The number of taxa per sample ranged from 1 to 180, with the number of countable individuals from 1 to 13497. Of the ninety-nine submitted Own Samples twenty-one had to be audited externally by Aquatic Environments due to Thomson Unicomarine being responsible for the initial sample processing. Detailed results have been reported to the participating laboratories. A summary of results from this module is presented in the Own Sample Module Summary Report – OS47, 48 & 49.

2.5.2.2 Efficiency of Sample Sorting

Table 1 of the OS Summary Report displays a summary of the data obtained from the analysis of the Own Samples. 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 58 samples out of the total 99 the number of taxa recorded by the participating laboratories was identical to that obtained by Thomson Unicomarine (column 4). In the 41 cases, the difference was at most six taxa and the average difference was

less than two taxa. Data for the numbers of individuals recorded (columns 6 and 7, Table 1) shows a range of differences from re-analysis of 0% to 25%. The average difference was 2.8% with 25 samples exceeding this average.

Fifty seven of the 99 samples reported showed 100% extraction of fauna from the residue (column 12), and in 18 samples various numbers of individuals (but no new taxa) were missed during sorting (column 11). The remaining 24 samples contained taxa in the residue that were not previously extracted, the worst example being four new taxa found in the residue (column 10). In the worst instance residue was found to contain 114 individuals. A breakdown of the missed individuals by taxonomic group is presented in Table 2 of the OS Summary Report. The average number of missed individuals found upon re-sorting the residue was approximately four, and the average number of missed taxa was half a taxon.

2.5.2.3 Uniformity of Identification

Taxonomic differences between Thomson Unicomarine and participating laboratories' results were found in forty-two (42%) of the ninety-nine samples re-analysed. A summary of mis-identified taxa is presented in Table 3 of the OS Summary Report. An average of 1.04 taxonomic errors per laboratory was recorded; in the worst instance eleven identification errors occurred. A great variety of samples (and fauna) was received. Polychaetes accounted for 48% and Mollusa for 40% of the taxonomic errors with a variety of species responsible for these errors.

2.5.2.4 Comparison of Similarity Indices (Bray-Curtis)

The procedure for the calculation of the similarity index was as used for the MB exercise. The Bray-Curtis similarity index figures (Table 1, column 14) ranged from 86% to 100% with an average figure of 97%. Only four samples from three laboratories achieved a similarity figure of less than 90%. Twenty-seven samples produced a similarity figure of 100%; these were submitted by 18 different laboratories (LB1802, LB1805, LB1807, LB1809, LB1814, LB1816, LB1817, LB1818, LB1819, LB1821, LB1827, LB1835, LB1837, LB1838, LB1839, LB1840, LB1841, LB1842). The best overall results were achieved by LB1840 with 100% similarity across all three Own Samples. The lowest overall results were achieved by LB1829 and LB1830 with an average similarity index of less than 90% over all three samples.

2.5.2.5 Biomass Determinations

It was not possible to make an accurate comparison of the biomass determination in all cases; sixty-two samples were not supplied with species blotted wet weight biomass data.

Consequently, only thirty-seven of the ninety-nine samples received have been used for comparative analysis. Table 4 of the OS Summary Report shows the comparison of the participating laboratory and Thomson Unicomarine biomass figures by major taxonomic groups. The total biomass values obtained by the participating laboratories varied greatly compared to those obtained by Thomson Unicomarine. The average was a +11.6% difference between the two sets of results (*i.e.* heavier than Thomson Unicomarine); the range was from –47.0% to +84.8%. 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) in addition to transcription errors. Further analysis of biomass results by major taxonomic groups indicated an average difference of +7.5% for polychaetes, +18.5% for oligochaetes, +8.8% for nemerteans, +9.2% for crustaceans, +4.6% for echinoderms, -80.0% for molluscs (extreme figure mainly due to transcription error by a participating laboratory) and +5.9% for all remaining faunal groups. These figures are not comparable to those produced by the same module in each of the previous years due to the variability in the duration and method of drying and the consistency of results within each major taxonomic group. The Thomson Unicomarine biomass data were achieved using a non-pressure drying procedure as specified in the Green Book.

2.5.3 Discussion

The average Bray-Curtis similarity index of 97% achieved for this Own Sample Module shows that the agreement between the participating laboratories and Thomson Unicomarine was generally very good. The results obtained were slightly better than for the Macrobenthic Sample Module with an average index of 92%.

There were 99 samples submitted for the Own Sample Module, including the 21 processed by the Scheme's external auditor. Of the 99 samples, 95 (96%) exceeded the 90% Bray-Curtis Pass mark and approximately 82% of the samples exceeded 95% Bray-Curtis similarity. These results are the second best achieved since the beginning of this module in Year 02 of the scheme (see Table 6 of the OS Summary Report).

Since the beginning of the OS module 1010 admissible samples have been received (OS01-49). Of these, 173 samples (17%) have fallen below the 90% Pass mark and 148 samples have achieved a similarity figure of 100% (15% of all returns). Overall these results are fairly good and show the efficacy of the OS module. Some participating laboratories could improve their results by reviewing their extraction methods and their use of taxonomic literature and identification keys.

2.5.4 *Application of NMBAQC Scheme Standards*

One of the key roles of the 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 3 (1996/97). These standards were the subject of a review in 2001 (Unicomarine, 2001) and were altered in Scheme Year 8; each performance standard is described in detail in the Description of the Scheme Standards for the Benthic Invertebrate Component document. Laboratories meeting or exceeding the required standard for a given exercise would be considered to have performed satisfactorily for that particular exercise. A flag indicating a 'Pass' or 'Fail' would be assigned to each laboratory for each of the exercises concerned. It should be noted that, as in previous years, only the OS and PS Modules 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.

2.5.4.1 *Laboratory Performance*

The target values for each Own Sample and the corresponding laboratory results are presented in Table 5 of the OS Summary Report. The assigned flags for each sample are also given. An assessment is performed separately for each of the three Own Samples. Comparisons between 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 module the majority of laboratories are considered to have met or exceeded the required standard for three of the OS targets - the enumeration of taxa, enumeration of individuals and the Bray-Curtis comparison. Overall 97% of the comparisons were considered to have passed the enumeration of taxa standard, 98% exceeded the enumeration of individuals standard and 96% 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 08 (Table 5, column 23); 4 samples are flagged as 'Fail - Poor', 14 as 'Pass - Acceptable', 55 as 'Pass - Good' and 26 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 56% of the eligible samples meeting the required standard.

2.5.4.2 Comparison with Results from Previous Years

A comparison of the overall results for recent years is presented in Table 6 of the OS Summary Report. The Table shows the number of laboratories assigned 'Pass' and 'Fail' flags for the OS exercises over the past seventeen years based upon the current NMBAQC Scheme standards (see Description of the Scheme Standards for the Benthic Invertebrate Component). This year's ninety-nine Own Samples resulted in a pass rate of 96% (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 due to the sending of 'deadline reminders' dispatched throughout the Scheme year. Table 7 shows the trend of OS results for each participating laboratory over the past seventeen 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 2.5.4.3 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 8 has 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 Module, are addressed. Remedial action should be conducted upon the associated CSEMP replicates to improve upon the flagged data. For a CSEMP sample, the associated samples are the five sample replicates or the five dispersed samples in the same water body. For a Water Framework Directive (WFD) sample, the associated samples would normally be the samples (5-10 in number) collected from the same water body. The revised NMBAQC Scheme OS standards, introduced in Scheme Year 08, give clear methods for discerning the level of remedial action required (see Description of the Scheme Standards for the Benthic

Invertebrate Component). A failing Own Sample is categorised by 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 Thomson 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, Thomson Unicomarine, 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 18. (For a list of 'failing' samples with outstanding remedial action from the previous seven Scheme years please refer to the Year 17 Benthic Invertebrate Component Report).
Four samples 'failed' in Scheme Year 18 (none of them was a CSEMP sample). Remedial action, outlined below, was required for associated replicates of the following Own Samples:

Non-CSEMP samples

LB1802	Review the taxonomic errors for associated samples
OS47	Remedial Action - completed (09/05/2013).
LB1829	Review the taxonomic errors and enumeration for associated
OS48	samples.
	Remedial Action - completed (24/10/2013).
LB1830	Review the taxonomic errors and enumeration for associated
OS48 & 49	samples.
	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 Samples comprised very small volumes of sorted residues and no faunal fragments. Participants are reminded that Own Samples must include all sorted residues, including all extracted materials deemed 'unrecordable' during the initial processing.

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 not 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 Thomson Unicomarine 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 Thomson Unicomarine Ltd as soon as possible.

4. There were continued problems associated with the measurement of biomass for individual species. In this and previous Scheme years several laboratories, despite using blotted wet weight biomass techniques, rendered some of their specimens too damaged to be re-identified. The initial processing of a CSEMP sample should in no way compromise the effectiveness of an audit. Biomass procedures should not render the specimens unidentifiable; trials would help to derive the best protocol for the blotted weighing technique. Biomass must be reported to four decimal places with nominal weights recorded as 0.0001g. A standardised protocol is available and must be followed for CSEMP analysis.

5. The maintenance of a comprehensive reference collection has numerous benefits for improving identification ability, maintaining consistency of identification between surveys and access to growth series material. The 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.

6. Participants submitting data for the ring test exercises should complete the 'confidence level' section of their datasheets to enable additional information to be gathered regarding the difficulty of ring test specimens.

7. Differences in the literature used for identification of invertebrates have been highlighted by the RT, MB and OS Modules. 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 Own Sample submissions must be accompanied with a 'processing details sheet' to ensure that the re-analysis (audit) matches that of the initial processing. Laboratories should also ensure that these sheets are completed accurately. Own Samples processed for CSEMP/WFD must be processed according to the NMBAQC guidelines (Worsfold, Hall & O'Reilly (Ed.) 2010).

9. The Own Sample Module 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. The NMBAQC guidelines for processing macrobenthic samples (Worsfold, Hall & O'Reilly (Ed.) 2010) issued with MB18 in Scheme Year 17 have improved the consistency of analysis, *i.e.* all analysts extracting and recording all biota. A detailed taxonomic discrimination policy (TDP) needs to be developed and added to the processing requirement protocol (PRP) to ensure that macrobenthic data from multiple analysts are as consistent and intercomparable as possible.

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 Modules, detailed results have been forwarded to each participating laboratory as soon after the exercise deadlines as practicable. After each RT exercise a bulletin was circulated, reviewing the literature used and detailing the correct

identification of the taxa circulated. Participants are encouraged to review their exercise reports and provide feedback concerning content and format wherever appropriate.

13. Positive, constructive feedback has been received from participants during Scheme Year 18. As in previous years participants have expressed the benefits of the modules, especially RT and OS. The primary aim of the Benthic Invertebrate Component of the Scheme is to improve the quality of biological data via training and audit modules. An informal constructive reporting system exists to assist in the overall improvement of data quality. For example, laboratories struggling with particular faunal groups in their Own Samples often receive additional support as well as receiving their returned OS faunal material separated according to the AQC identifications for future reference. Two of the four 'failing' Own Samples in Scheme Year 18 have already been rectified via the recommended remedial action.

4. References

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