National Marine Biological Analytical Quality Control Scheme
Own Sample Format and Standards Review

Current Problems and Proposed Solutions

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Summary

Problems

There is no standardisation of recording criteria for NMMP data.

There are no clear procedures for auditing subsamples.

The pass / fail criteria for samples produce certain anomalies.

There is no standard procedure for remedial action.

Samples requested for auditing are treated differently by participating laboratories.

It is not clear whether a representative selection of NMMP laboratories, subcontractors or NMMP sites are currently being audited.

Proposed solutions

Create an NMMP Sample Processing Policy.

Measures for subsample auditing must be devised.

Revised pass / fail criteria are proposed.

Remedial action must be standardised – a proposal is included.

Sample selection procedure must be randomised.

Auditor must have access to full macrobenthic NMMP database.

1. Introduction

The NMBAQC Scheme Own Sample (OS) component was introduced by Unicomarine Ltd. in 1995 as a response to participant comments and perceived requirements. It is the only component of the Scheme that assigns pass / fail flags to biological data and is generally considered to be one of the most important aspects of the Scheme. It addresses three main areas relating to NMMP benthic biological data collection:

- The processing of NMMP samples.
- The identification of NMMP macrofauna.
- The application of defined standards of quality control to validate NMMP data.

As the component has great significance to NMMP data and to participating laboratories, it is important to identify and resolve all problems concerned with this component and NMMP data as efficiently as possible. Several problems have been identified through the Scheme to date and they are discussed in Section 3. Our proposals for solutions are introduced in Section 4. The current procedures of the OS component are described below (Section 2) for reference.

2. Description of the current Own Sample component (Scheme years 1995/96-2000/01)

The NMBAQC Scheme is currently in its seventh year (2000/01). NMMP laboratories are required to participate in all components and standards are applied to agreed components (PS and OS). The Own Sample (OS) component was introduced in 1995/96 and performance targets, devised by the NMBAQC Committee, were applied for this in 1996/97. These targets have been applied to the results from laboratories and "Pass" or "Fail" flags assigned accordingly. As the resulting flags have been deemed the basis for quality target assessment, where laboratories failed to fulfil these components through not returning the data, a "Deemed Fail" flag has been assigned.

The OS exercise examines laboratory analytical performance on material from each laboratory's 'home' area. Each laboratory is requested to send a list of samples from which three samples are selected. The selection is in turn notified to the laboratories. NMMP laboratories are advised to use NMMP samples if possible, otherwise there is free choice.

2.1 Analysis required

Participating laboratories are instructed to carry out macrobenthic analysis of the samples using their normal procedures. Samples requiring sub-sampling are to be avoided where possible. All procedures are required to be documented and details returned with each sample (Own Sample Details Form). All material from the sample is required to be sent to Unicomarine Ltd. broken down as follows:

- Sorted residue - material from which all animals have been removed and counted.
- Separated taxa - individually labelled vials containing the identified fauna.
- Other fractions - e.g. material containing fauna which have been counted *in situ*.

Identification is required to be to the normal taxonomic level employed by the laboratory (usually species). The names and counts of specimens are to be recorded on a spreadsheet matrix and linked to the vials through a specimen code number. In addition, measurements of the biomass of the recorded taxa are requested. Detailed instructions are provided for this component; measurements are to be blotted wet weights to 0.0001g and to be made for each of the taxa recorded during the enumeration.

Upon receipt at Unicomarine Ltd., all OS samples are re-analysed by the same operator. The sorted residue is re-examined and any countable material extracted. The identified fauna is checked for the accuracy of enumeration and identification and, if biomass data was submitted, all specimens are re-weighed using the same specified procedure.

2.2 Application of current NMBAQC Scheme Own Sample standards

A primary purpose of the NMBAQC Scheme is to assess the reliability of data collected as part of the National Marine Monitoring Plan. With this aim, the NMBAQC Committee defined target standards for the Own Sample component. These standards have remained unchanged since their introduction. The targets compare the participating laboratories results for each Own Sample against the results of re-analysis by Unicomarine Ltd. Three areas are examined for each sample: total number

of taxa, total abundance and the Bray-Curtis similarity index. The applied standards are as follows:

Total number of Taxa	**± 10% of the reanalysis**
Total Abundance	**± 10% of the reanalysis**
Bray-Curtis similarity index	**≥ 90%**

Each of the three Own Samples is awarded three flags according to these standards. In order to achieve an overall 'Pass' for this component, the participating laboratory must achieve six passes out of the nine flags applied.

3. Problems with the current Own Sample component

Since the introduction of the OS component, there have been several recurring concerns raised concerning four aspects: standard recording procedures, sample randomisation, the pass/fail criteria and remedial action.

3.1 Standardisation of processing procedures for NMMP samples

It is imperative that all NMMP samples are processed and stored by laboratories in the same manner.

NMMP data must be available for a variety of possible uses, including comparisons between sites. The "horses for courses" approach of tailoring data to the supposed objectives of particular laboratories must be phased out.

At present, there appears to be little standardisation of sample sorting / recording criteria between laboratories carrying out NMMP work.

3.2 Sample selection for auditing

3.2.1 Specialised treatment of samples for auditing

It is widely suspected that, in some instances, the samples selected for submission in the OS component are either reworked prior to dispatch or are processed in an exceptional manner. Therefore, the resultant numbers of overall passes achieved in this exercise may not truly reflect the standards of 'normal' sample processing.

The requirement for separating the fauna into individual taxon vials is, in some cases, only applied to the NMMP samples selected by Unicomarine Ltd. for this component. This has implications for biomass estimations. Currently the NMMP requires that total biomass be reported. However, total biomass weighed *en masse* will usually produce figures that are higher than the sum of the biomass of each individual taxon vial. Hence, a variance within the biomass data is potentially introduced by treating these Own Samples differently to the remaining unselected NMMP samples. It is imperative that all NMMP samples are processed and stored in the same manner. For example, in extreme cases, the residues of all unselected NMMP samples have been disposed of before feedback or potential remedial action could be undertaken.

In order to address these issues and create a truly random auditing procedure major changes need to be made to the operation of this component.

3.3 Pass/Fail criteria

As some participating laboratories are still failing this component, in spite of probable preferential treatment of samples, it raises the question of whether or not the standards are currently set at a reasonable level. Alternatively, in the absence of a workable quality control / accreditation system and regular taxonomic workshops, sample processing may have been carried out by non-specialists who have not had adequate training or experience. Efforts should be made wherever possible to 'raise the game' rather than 'lower the standards'.

The current standards have given rise to ninety-six 'fail' flags (up to the end of Scheme Year six and excluding deemed failures), but just nine overall 'fail' flags. Unfortunately, due to the current scoring system, a failing laboratory has raised questions not of the reanalysis of their sample but of the pass/fail criteria. The following statement appeared in the 1999/2000 NMBAQCS Annual Report:

> *The current OS 'flagging' system can result in anomalies. The use of taxa, individual and Bray-Curtis scores combined with a 'six from nine' pass threshold could theoretically pass a laboratory which picks and counts all the individuals perfectly but identifies all the species incorrectly. The flagging should reflect the importance of achieving potentially truly representative data (i.e. completely picked residues) and also accurately identified taxa .... there is little point having an excellently identified sample which was poorly picked and is consequently unrepresentative of the true sample.*

Similar observations and recommendations have been made in previous Annual Reports. It is possible for a participating laboratory to fail all three standards for one of their Own Samples, pass all three in each of the remaining two samples and receive an overall 'Pass' flag. The consequences of this are that data from an extremely poorly processed NMMP site could reside in the NMMP database unchallenged. Overall flags should be applied on a sample by sample basis to remove this possibility. In order to address this problem the entire flagging process is in need of review.

3.4 Remedial action

Currently, if a participating laboratory receives an overall 'Fail' flag no formal procedure for implementing remedial action is applicable and no facility for checking the effectiveness of such remedial action is in place. This should be urgently addressed to prevent similar errors occurring for year upon year. There must be an improvement to the learning aspect of the Own Sample component.

4. Solutions to the above problems - Proposed modifications

The changes required to this component are extensive in order to meet the following aims:

- Create an NMMP Sample Processing Policy (Section 4.1).
- Implement an accessible framework for NMMP QC/QA.
- Prevent Own Sample reworking.

- Provide a framework for proposing and assessing remedial action.
- Improve / validate the NMMP data set more effectively.

There is a need to keep costs at a minimum in order to maintain a feasible auditing framework. The primary duty of the Scheme is to validate and provide a means of quality control for NMMP data. Therefore, to keep OS cost at the same level the following proposed procedures are tailored towards NMMP laboratories.

Participating laboratories which do not undertake NMMP analysis that wish to participate in this component must process their samples according to the NMMP procedures in order to avoid further cost implications (see Section 5).

4.1 NMMP Sample Processing Policy

The 1999/2000 NMBAQCS Annual Report stated:

> *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.*

NMBAQCS Sample Sorting Questionnaires were circulated in October of 2000. The returns have not yet been fully analysed (see forthcoming Annual Report Year seven). However, it is apparent that there is wide variation between laboratories in terms of recording criteria. There is a need for a standard approach towards the processing of NMMP samples. This is a wide topic for debate and all participating laboratories must be consulted before formulating levels of specific identification and deciding upon which data filters to apply. The matter must, however, be given urgent consideration.

Recording protocols must be the same for all laboratories. This cannot be achieved immediately. However, the following requirements are proposed for immediate effect to meet the needs of the NMBAQC Scheme.

- Residues must be retained until Own Sample results are received.
- Fauna must be stored in individual taxon vials.
- All NMMP laboratories must be aware of the schedule, structure and purpose of the Own Sample component.
- Processing must be completed by NMMP deadlines.
- All NMMP sample processing laboratories must participate in the NMBAQC Scheme Own Sample component.
- NMMP Laboratories to address their failing Own Samples by performing remedial action upon the remaining site replicates.

The separation of a sample's fauna into individual taxon vials provides the participating laboratory with an 'instant reference collection' and facilitates efficient auditing and back referencing if necessary. Taxon separation can be time and space consuming but the effects of this can be minimised by separating taxa at the time of

identification and making use of a range of storage vials. The 1999/2000 NMBAQCS Annual Report states that:

> *Laboratories are strongly recommended to implement an in-house reference collection of fauna. The maintenance of a comprehensive collection has numerous benefits for improving identification ability, maintaining consistency of identification between surveys and access to growth series material.*

4.2 Sample selection for auditing

In order to facilitate a change in the Own Sample selection procedure, it is proposed that Scheme Year eight (2001/2002) should not include an Own Sample component. This will have no cost implications because Unicomarine propose to bill this component on a *pro-rata* basis. However NMMP laboratories will continue to process their 2001 samples according to an NMMP Sample Processing Policy (see Section 4.1). At the beginning of Scheme Year nine (May 2002) participating laboratories will submit their entire NMMP data matrices from which three samples from different NMMP sites will be selected for audit (OS17, 18 and 19). These selected Own Samples are to be provided for audit within one calendar month.

Each Scheme year Own Samples will be selected from different NMMP sites wherever possible. This will enable a gradual examination of processing of samples from all NMMP sites.

4.3 Pass/Fail criteria

The 1999/2000 NMBAQCS Annual Report included the following comment:

> *The limitations of the Bray-Curtis similarity index should be recognised when interpreting the results from the OS and MB exercises. Of particular importance is the potential for a relatively large effect on the index of few differences in identification and the associated danger of misinterpreting a low index in terms of quality of service.*

This was derived largely from comments by Rees (2000), who illustrated various scenarios of sample sorting error and their affect upon the Bray-Curtis similarity index. He suggested various transformations upon the data prior to calculating the Bray-Curtis Index to limit the effects of mistakes involving the dominant taxa.

While the above concerns have been noted, careful consideration of implications of the changes suggest that the data should remain untransformed. This is because errors involving the dominant taxa, although easily rectifiable in some cases, constitute a near catastrophic error in terms of community studies or inter-site comparisons. Own Samples should be flagged purely upon the untransformed Bray-Curtis scores achieved. The current targets for taxa and abundance will continue to be calculated and presented to identify areas of weakness.

All participating laboratories are encouraged to study their Own Sample results and consider possible areas for improvement.

"You don't have to be ill to get better."

A five tier system of classifying individual Own Samples is proposed:

100% BCSI	**Excellent**
95 - <100	**Good**
90 – 95	**Acceptable**
85 – 90	**Poor – Remedial Action Suggested**
<85	**Fail – Remedial Action Required**

4.4 Remedial action

After Own Sample re-analysis, each laboratory will receive its results. Samples which do not meet the required standards will be flagged, along with all the other replicates from the same NMMP site. Feedback will be provided which specifies the remedial action required to reach a pass standard and improve the NMMP site's flag. Each failing sample will be logged to track the remedial action required. The laboratory with a failing Own Sample must undertake the remedial action (*e.g.* residue re-sorting) and provide an updated data matrix for the flagged NMMP site. This should occur within an agreed reasonable time scale.

A further sample will be selected for auditing the effectiveness of the remedial action (*e.g.* OS17RA). This sample will be processed via the Extra Own Sample facility and the cost will be met by the participating laboratory. In the event of a subsequent failure, the laboratory concerned must address the issue by obtaining specific staff training in the areas required. Also the quality of all NMMP samples processed by the laboratory in question (*i.e.* NMMP sites that have not been audited) must be flagged.

5. Cost implications

NMMP laboratories conducting their analysis according to the NMMP Sample Processing Policy will not incur any further cost for the auditing of their three Own Samples. However, if any of these Own Samples fail the applied standards, then the additional cost of in-house remedial action and subsequent auditing will be met by the participating laboratory.

Those participating laboratories (non-NMMP) that submit Own Samples which are not divided into individual taxon vials will be charged the following figures according to their faunal storage:

All taxa combined in a single vial	Standard Own Sample Rate + 75%
Taxa split into major groups (phyla)	Standard Own Sample Rate + 50%
Taxa split into Family groups	Standard Own Sample Rate + 25%

Laboratories submitting samples that are not split into taxon vials must acknowledge that the detail of feedback which can be provided will also be reduced.

6. References

Howson, C.M. & Picton, B.E. (eds), 1997. The species directory of the marine fauna and flora of the British Isles and surrounding seas. Ulster Museum and the Marine Conservation Society, Belfast and Ross-on-Wye. Ulster Museum Publication No. 276.

Rees, E. I. S., 2000. Review of the standards for the National Marine Biological Analytical Quality Control Scheme. Commissioned by the NMB AQC Committee.

Turner, G. S., 1999. A practical application of the National Marine Biological AQC Standards.

Unicomarine Ltd., 2000. National Marine Biological AQC Report 1999/2000, Annual Report (Year Six).