BEQUALM / NATIONAL MARINE BIOLOGICAL ANALYTICAL QUALITY CONTROL SCHEME

Proceedings of the NMBAQC's 'PSA for Supporting Biological Analysis' Workshop

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1 Introduction

Over the 15 years of the NMBAQC's Particle Size component, some anomalies in participant's results have raised questions about the methods that are used by different laboratories to conduct Particle Size Analysis. To assess the extent of these suspected methodological differences between laboratories, the NMBAQC sent out a questionnaire to all of its Yr 15 participants (6 Competent Monitoring Authorities (CMAs) and 6 private laboratories) in June 2008. The results of this questionnaire highlighted substantial variation in the methods of sediment sample collection, analysis and reporting between the laboratories who are involved in national level marine monitoring in the UK (e.g. CSEMP and WFD programmes).

Following the results of the questionnaire, the NMBAQC decided that a UK wide Standard Operating Procedure (SOP) should be written to make recommendations of 'best practice' methods which should be followed by all laboratories involved in PSA for supporting biological analysis in the CSEMP and WFD marine monitoring programmes. Before a SOP is written the NMBAQC decided that a workshop should be held to discuss current methodological differences between laboratories and discuss options of new or modified methods which all labs could follow.

1.1 Aims

The aim of this workshop was to initiate discussions about creating a SOP which will recommend 'best practice' methods for laboratories involved in PSA for supporting biological analysis. It was emphasised that any 'best practice' methods recommended in the SOP must have supporting experimental evidence which proves the importance of standardisation across laboratories. It was also stressed that any methodological differences that had no significant impact on the analysis (again through experimental evidence) should also be indicated in the SOP, so laboratories can continue using their own preferred method.

This workshop involved 28 representatives (biologists and laboratory analysts) from each of the CMAs and private laboratories who are involved in PSA for supporting biological analysis for national marine monitoring. Participants were introduced to the current methodological differences in PSA which were highlighted in the results of the NMBAQC Questionnaire. Experimental evidence was presented by CEFAS and NIEA which demonstrated the effect of some existing methodological differences on PSA results. Presentations were also given about the current use of PSA in biological analysis.

The key objectives of the workshop were:

- To assess the needs/willingness of laboratories taking on standardised PSA methods.
- To allow open workshop discussions about:
  - Participants experience of current differences in PSA methods.
  - 'Best practice' methods which participants consider should be recommended in a UK wide SOP to ensure PSA data collected is consistent and comparable across the UK.

- To consider further research/experimentation which is needed before evidence based 'best practice' methods can be recommended.
- To produce a structure of an SOP.

The following sections of the Proceedings capture the main points of discussion at the workshop.

2 Welcome to the workshop

Colin Allchin (CEFAS, outgoing NMCAG Chair) and Tim Mackie (NIEA, NMBAQC Chair) both gave a brief welcome to the workshop and re-iterated the aims and key objectives of the workshop.

3 Sample Collection

3.1 Introduction to what methods CMAs currently use – Prue Addison

The different methods used by CMAs in field collection of PSA samples were presented (refer to NMBAQC PSA Workshop.ppt, NMBAQC PSA Questionnaire Summary Report.doc and Summary of NMBAQCs PSA questionnaire results.xls). In summary CMAs still vary in:

- The source of PSA sample (biology vs. separate grab).
- The depth of core sample taken from a grab.
- The volume of sample taken.

The current text relating to PSA sample collection in the Green Book was also presented (refer to NMBAQC PSA Workshop.ppt and PSA for biological analysis and the Green Book.doc). In summary, the Green book states samples should be:

- Taken from a separate grab (to the biology grab sample).
- Taken from the surface and to a minimum depth of 5 cm.
- Kept cool and frozen as soon as possible.

3.2 Environment Agency (EA) method of PSA collection – Prue Addison

The PSA sample collection methods used in the field were discussed (refer to Sample Collection EA.ppt). The main points raised include:

- PSA samples taken from separate (or chemistry) grab.
- Depth integrated core to 5cm.
- Samples are not frozen (which goes against the Green Book).

3.3 Northern Ireland Environment Agency (NIEA) method of PSA collection – Mike Allen

The PSA sample collection methods used in the field were discussed (refer to PSA Methodology NIEA.ppt). The main points raised include:

- PSA samples taken from a separate grab.
- Samples collected vary greatly in sediment type.

- Depth integrated core to 15cm taken for biology. A separate 2cm scrape is taken for PSA for supporting chemistry.
- Inspection of grab and rejection of samples suffering from washout, inequal bite or insufficient penetration is a first QA step in the field.
- The value of photographic record acknowledged, especially at multiple process steps, i.e. undisturbed surface in grab, on field sieve and material retained on the field sieve.

3.4 Scottish Environment Protection Agency (SEPA) method of PSA collection – Myles O'Reilly

The PSA sample collection methods used in the field were discussed (refer to PSA Methodology SEPA.ppt). The main points raised include:

- PSA samples taken from a separate grab, so as not to compromise the biology. Sediment in the PSA sample must represent the biology grab.
- Digitised photo taken of each sample for QA.
- Depth integrated core to depth of grab (minimum 5cm depth, 6cm diameter).
- Core is bagged and frozen immediately.
- Heterogeneous samples – problems encountered include questions of where in the grab should the sub-sample be taken. Also PSA samples taken have a bias to exclude large shells.

3.5 Centre for Environment, Fisheries & Aquaculture Science (CEFAS) method of PSA collection – Keith Cooper

The PSA sample collection methods used in the field were discussed (refer to Sample Collection CEFAS.ppt). The main points raised include:

- Day grab or Hammon grab used (unlike other CMAs).
- PSA samples take from the biology grab (unlike other CMAs).
- Depth integrated core taken to the full depth of the grab with a 3cm diameter syringe. But if a coarse sediment is collected (where a Hammon grab is used), then a plastic scoop is used to collect 500mL of material (made up by taking 6 sub-samples) – Cobbles are also left out of PSA samples and are recorded (this is particularly for habitat mapping), and then integrated into analysis once sieve and laser analysis have been done.
- Samples are frozen.
- Advantage of PSA from biology – PSA is directly comparable with biology in analysis.
- A separate PSA sample for supporting chemistry (2cm scrape) also taken.

3.6 Fisheries Research Services (FRS) Inshore Fisheries Group method of PSA collection – Lynda Allen

The PSA sample collection methods used in the field (nb this is not for CSEMP monitoring programme) were discussed (refer to Sample Collection FRS.ppt). The main points raised include:

- Van Veen grab on a video sledge is generally used – this is seen to be more efficient than using a day grab (and less time consuming).

- PSA samples taken at the end of a video transect, therefore directly comparable to biology.
- Sample is frozen.

3.7 Workshop Discussion – PSA sample collection in the field

3.7.1 Source of PSA sub-sample (biology, chemistry or separate grab)

- By taking PSA from biology it halves the number of grabs (if not doing chemistry).
- The size of the vessel impacts on accuracy of dropping down a separate grab for PSA and chemistry (compared to where biology grab was taken). E.g. NIEA use quite small boats and are confident they drop down separate grabs within a 5m radius of where biology grab was taken.
- There are concerns over the comparability of PSA sample to the biology if PSA is collected from a separate grab (to the biology). This means that replicate biology and PSA samples can not be directly linked when analysing the data (as they are not from the same grab).
- An alternative to the above point was also raised - PSA from a sample (regardless of its origin – biology vs. separate grab) is only a 'representative' of the biology, as sediment is so spatially variable even within a biology grab, therefore it does not represent the exact sediment that the biology was found in.
- PSA could also be done once biology has been sieved off at 1mm – use the residual for PSA, and have biologists send >1mm fraction to PSA laboratory once finished sorting. Only problem is how to contain the <1mm fraction (with all of the water used to sieve) and also relate that to the proportion of the >1mm fraction collected (because no weights or volumes taken at the beginning).
- Maybe the current method of taking PSA from a separate grab should be continued for CSEMP, but for new programmes (e.g. WFD) a new method (e.g. taking PSA from biology grab) should be recommended.
- Or both methods could be run in parallel for a while, and assess how much difference it makes.
- Perhaps another level could be added to the MERMAN database to indicate old and new methods being used.

Conclusion of this discussion:

- There are very different views in relation to this topic – discussions continued in the 'PSA for Biology' session.
- It depends on what statistical analysis is done on the data (what are these programmes designed for) – more consultation with CMAs needed through the NMBAQC.
- Evidence needed for the effect of using biology vs. separate grab for PSA sample representing the biology. NIEA will assess the level of variation in PSA between biology vs. separate grabs.

3.7.2 Method of sub-sample collection from grab (depth integrated core/mixed sample/surface sample)

- All in agreement that a depth integrated core should be taken for PSA for supporting biology, but do not think this can be used for supporting chemistry as well.
- NMBAQC needs official chemist's involvement in decision about whether or not PSA sample for biology should or should not be used for supporting contaminants data.
- CMAs are generally happy with the Green Book's recommendation that PSA samples are to be collected using a depth integrated core to >5cm.
- Generally happy with the Green Book's recommendation of >5cm depth taken. However due to the nature of day grabs, it is likely that the top 5cm of a grab does not necessarily represent the top 5cm of the seabed.
- Suggestion for PSA to be taken >10cm, as this represents the Redox Discontinuity Layer, which is more relevant to the biology.

Conclusion of this discussion:

- PSA for supporting biology should be a depth integrated core to >5cm.
- NMBAQC needs to consult NMCAG re their agreement with PSA still taken for biology and chemistry separately.

3.7.3 Sample volume collected:

- There should be a tiered approach to volume of sample collected, which is dependent on sediment characteristics (e.g. much bigger volumes need to be collected for cobble sediments compared to sand/silt sediments). Rob Nunny has a curve which represents the relationship between sediment type and volume that should be collected.
- Volume of sample and the sediment type will ultimately effect whether PSA samples can be taken from biology grab.

Conclusion of this discussion:

- NMBAQC to investigate a more simple tiered approach with regard to sample volume to be collected for PSA from varying sediment types.

4 Sample Analysis

4.1 Introduction to what methods CMAs currently use – Prue Addison

The different methods used by CMAs in the sample analysis were presented (refer to NMBAQC PSA Workshop.ppt, NMBAQC PSA Questionnaire Summary Report.doc and Summary of NMBAQCs PSA questionnaire results.xls). In summary CMAs still vary in:

- Sample preservation (Freezing/not freezing/oven drying).
- Removal of organic material with hydrogen peroxide.
- Removal of conspicuous fauna (i.e. snail shells, urchins, etc.).
- Volume of sub-sample used for laser and sieve analysis.
- Obscuration range of laser analysis.
- The use of a dispersant in laser analysis.

- Wet/dry sieving (to what size) to separate laser and sieve fraction.

The current text relating to PSA sample collection in the Green Book was also presented (refer to NMBAQC PSA Workshop.ppt and PSA for biological analysis and the Green Book.doc). In summary, the Green book states samples should be:

- Samples should be frozen as soon as possible.
- Procedural Guidelines for analysis of sediment – to be added by the NMBAQC group.

4.2 EA method of Sample Analysis – David Johns

PSA sample analysis methods were discussed (refer to Sample Analysis EA.ppt). The main points raised include:

- Sample is not freeze dried, it is just wet sieved at 2mm to separate sieve and laser fractions.
- Homogenising laser sample is difficult, as Hydro G unit struggles with coarse sand.
- Sieve and laser fraction merged with the Emulation function in the Malvern Mastersizer 2000.

4.3 NIEA method of Sample Analysis – Mike Allen

PSA sample analysis methods were discussed (refer to PSA Methodology NIEA.ppt). The main points raised include:

- Samples are freeze dried, and kept dry for separating sieve and laser fraction (at 1mm) and sieving.
- Sieving stack is put on a sieve shaker for 20 minutes.
- Hydro G used to homogenise sample.
- Sieve and Laser fractions are merged in NIEA's own spreadsheet.

4.4 SEPA method of Sample Analysis – Myles O'Reilly

PSA sample analysis methods were discussed (refer to PSA Methodology SEPA.ppt). The main points raised include:

- Frozen sample is thawed and homogenised, and then sub-samples taken for analysis.
- 30g sub-sample of the entire sample (i.e. not <1mm) is used for laser analysis.
- 100g sub-sample is taken for sieve analysis which is freeze dried for dry sieving.
- Laser and sieve fractions combined in SEPA's own spreadsheet (the origin of this is unknown).

4.5 CEFAS method of Sample Analysis – Claire Mason

PSA sample analysis methods were discussed (refer to Sample Analysis CEFAS.ppt). The main points raised include:

- Sieve and laser fractions currently split at 63um, however CEFAS are investigating the option of splitting at 1mm (as this is quicker and sieve fractions then only need to be oven dried).

- Trace metal/ organic carbon and nitrogen sediment prepared within the PSA sample analysis process.
- <63um samples are freeze dried which then have to be re-disaggregated with an autosampler attached to the lasersizer– CEFAS currently investigating the possibility of running wet sediment through lasersizer (i.e. not freeze drying).

4.6 FRS Inshore Fisheries Group method of Sample Analysis – Lynda Allen

PSA sample analysis methods (nb this is not for CSEMP monitoring programme) were discussed (refer to Sample Analysis FRS.ppt). The main points raised include:

- Laser analysis involves sub-samples being passed through until an Obscuration range of approx 15% is achieved.
- Clear protocols followed for laser and sieve analysis.

4.7 AFBI method of Sample Analysis - Richard Hartley, Plymouth University

PSA sample analysis methods were discussed. The main points raised include:

- Size of sample used for analysis is dependant on sediment type – e.g. coarse sediment – all of the sample is used, muddy sediment – subsample of 2-3 teaspoons is used (as per BS1377).
- Hydrogen peroxide is used.
- Sieve and laser fractions separated at 1mm by wet sieving.
- Laser analysis conducted as per ISO13320.
- Dispersant also used (approximately 1 %), and samples subject to 90 seconds of ultrasonic dispersion.

4.8 Experimental evidence for different sample analysis methods:

4.8.1 CEFAS experiment results – Claire Mason

- Laser and sieve analysis of samples which were frozen vs. refrigerated and run through the lasersizer with dispersant vs. no dispersant.
- Results indicated that dispersant had little effect.
- Frozen samples resulted in more fines (5.5 – 6.5phi) compared to refrigerated samples – possibly indicating that freezing breaks up particles.
- Statistics still yet to be done on this experiment.

4.8.2 NIEA experiment results – Mike Allen

- See presentation: PSA experiments_NIEA.ppt
- Sample analysis conducted on freeze dried vs. oven dried, using hydrogen peroxide vs. none, dispersant vs. none. 80 samples were used for this experiment which meant 10 samples for each treatment.
- Freeze dried samples resulted in little difference between no dispersant and dispersant, and using hydrogen peroxide vs. none.
- Oven drying samples resulted in little difference between no dispersant and dispersant, but there was a difference in using hydrogen peroxide vs. none. This suggests that if oven drying PSA samples, hydrogen peroxide should also be used.

4.9 Workshop Discussion – PSA sample analysis

4.9.1 Sample preservation (Freezing/not freezing)?

- Freezing is done by most CMAs and is considered more practical for sample preservation. Freezing is definitely needed for contaminants samples.
- If samples are not frozen, then they should be at least kept in a cool dark place.

Conclusion of this discussion:

- CEFAS to complete analysis of experiments (assessing the effect of freezing vs. not freezing samples prior to analysis), and communicate results to the PSA group.
- If CEFAS find no difference between freezing and refrigerating samples, then samples can be stored either by freezing or refrigerating.

4.9.2 Freeze drying/Oven Drying

- Oven drying can break down fine sediments, as mortar and pestle used to break up sample – this does not represent the sediment that infauna live in.
- NIEA experiment indicated that freeze drying is considered better than oven drying.
- It was also discussed that the best option (for consideration of the biology) is not to dry at all.

Conclusion of this discussion:

- Labs doing PSA for assessing biology should not oven dry their samples.
- NIEA to complete analysis of experiments (assessing the effect of freeze drying vs. oven drying samples prior to analysis), and communicate results to the PSA group.

4.9.3 Removal of organic material with hydrogen peroxide?

- It is critical to accurately represent the %clay in sediments – therefore samples should not be treated with hydrogen peroxide.
- NIEA experiment suggests that only hydrogen peroxide should be used is samples are oven dried.

Conclusion of this discussion:

- NIEA to complete analysis of experiments (assessing the effect of the use of hydrogen peroxide), and communicate results to the PSA group.
- NMBAQC need to assess whether we are interested in describing the sediment and also organics. Or can organics be just represented by the measure of organic carbon?

4.9.4 Removal of conspicuous fauna (i.e. snail shells, urchins, etc.) - weigh and id?

- Conspicuous fauna are removed, measured and identified by CEFAS. These notes are included with the stored PSA data.
- How do you know it is already dead when removing a frozen or refrigerated sample?

- Any live animals in a sample should be removed at time of sampling.
- As PSA is for supporting biology, the feeling is that particles that influence the presence of infauna (e.g. shell debris or maerl) should be recognised in PSA.
- What about plant matter? This is not removed by hydrogen peroxide, but can be removed from samples in water if samples are shaken, and then algae decanted off.

Conclusion of this discussion:

- NMBAQC needs to more clearly define 'conspicuous' fauna/shells that should/should not be removed form a PSA sample and also write a procedure for dealing with plant matter in PSA samples.

5 PSA in biological analysis (Biologists discussion)

5.1 PSA - A Sedimentologist Perspective, Ken Pye, Kenneth Pye Associates

The contents of this presentation can be found in the draft paper by Ken Pye (TBA). Main points discussed by Ken include:

- There are many uses of PSA by many different professions. There are also many different methods of conducting PSA.
- The accuracy of lasersizers can be variable, especially with complex samples (e.g. polymodal samples). This is because lasersizers will fit a sample to a normal distribution.
- There is evidence of differences in PSA results due to different pre-treatment of samples (e.g. dispersed in calgon, using hydrogen peroxide, and freezing samples).
- Merging sieve and laser data is not good practice, as these methods measure different things – sieving measures particles by weight and laser sizing measures particles by volume. Laser fractions are commonly over-estimated by 10-15% compared to sieve fractions, therefore the curves from these two methods will not match up. Also the proportions of coarse sand and clay are frequently underestimated by laser analysis. As a last resort, laser and sieve fractions could be merged at either 63um or 2mm – definitely do not merge at 1mm (which is in the sand fraction).
- Summary statistics should not be used to describe marine sediments (e.g. Folk and Ward (Inclusive) statistics). This is because these statistics assume a normal distribution of a sample, and are therefore meaningless for samples with multi-modal distributions (which marine sediments often are).
- Alternative measures to summarise marine sediment include: %sand, gravel and mud fractions, modal sizes, %<20um, d50, d90-d10.
- Raw data should be archived for post processing and QC.

5.2 The Application of Particle Size Analysis in Biology for Marine Consultancy, Ken Neal, CMACS

Main points discussed by Ken include (see PSA and biology_Ken Neal CMACS.ppt):

- CMACS are involved in a range of biological work which involves PSA (e.g. Environmental Impact Assessments and post construction monitoring for offshore wind farms, marinas, ferry terminals and marine current turbines).
- Ken provided examples of long term monitoring of intertidal sediments using biotope classification which included PSA. In these projects PSA summary statistics were used to help inform biotope classification (which included different sediment types, e.g. mud/sandy mud, muddy sand, sand, and shingle biotopes).
- Ken also provided an example of subtidal sampling of biota and PSA. In this project PSA is mapped at sampling locations over time to show the spatial changes in the sediment characteristics (average size in Phi) in relation to the location of anthropogenic pressures.

5.3 Uses of PSA to inform Biology, Keith Cooper, CEFAS

Main points discussed by Keith include (see Uses of PSA data to inform Biology_Keith Cooper CEFAS.ppt):

- Keith provided examples of long term monitoring aggregate extraction sites, where cumulative direct and indirect impacts have been demonstrated.
- The % of sediment groups (gravel, coarse sand, medium sand, fine sand, silt/clay) are used as covariates in MDS ordination of infaunal samples and in a Dendogram using Euclidian distance.
- In general PSA has a low correlation with biology (other variables such as sediment mobility and OCN are more correlated with biology).
- PSA does however help explain some individual species distributions.
- It is more important to know how the sediment behaves on the seabed, which is not represented from PSA. However, Ken Pye commented on this point and said that a sediment core could actually be used to make inferences about the energy of the environment through conducting PSA on that sample.

5.4 PSA for Habitat Mapping, Markus Diesing, CEFAS

Main points discussed by Markus include (see PSA for habitat mapping_Markus Diesing CEFAS.ppt):

- Markus provided an introduction to habitat mapping done by CEFAS.
- Markus explained the EUNIS classifications that define marine sediment. These include four different types of sediment: Coarse sediment, Sand, Mud and Mixed Sediment – currently there is no precise definition for these groups.
- PSA can be re-classified through the Ternary (Folk Triangle) Diagram to fit into the four EUNIS sediment classes (however this does have some problems where sediment is classified into the same class, but sediment actually has very different grain-sizes and backscatter).
- There is a different form of re-classification of PSA called Entropy Analysis (based on a chemistry algorithm) which is more commonly used in Australia and New Zealand.
- Markus provided evidence (MDS plots) from his research of infauna samples separating out into Fine Sand and Coarse Sediment classes.

- There is further research underway which is investigating linking backscatter histograms, particle-size distributions and infaunal species abundance using Primer.

5.5 The use of PSA data in biological studies, Roger Coggan, CEFAS

Main points discussed by Roger include (see Use of PSA data in biological studies_ Roger Coggan CEFAS.ppt):

- There are two options for classifying sediments – Top Down, where existing classification (e.g. EUNIS) are imposed on data; or Bottom up, where data is analysed and associations used to create classes.
- The method of PSA should only be to a resolution that is fit for purpose.
- Sediment can be classed into EUNIS based on video information.
- There are questions about using a Hammon Grab for collecting PSA samples, as Roger suggested that this method is not representative of the seabed structure.

5.6 Workshop Discussion – PSA in Biological Analysis

The following were key points raised in the workshop discussion between Biologists:

5.6.1 EUNIS sediment classification

- If CMAs only need 4 categories of sediment (for WFD and CSEMP), the PSA samples could be just wet sieved on board.
- SOP recommendations should be for more detailed analysis than this, so it is applicable to a wider range of studies.

Conclusion of this discussion:

- NMBAQC SOP needs to highlight what methods are being recommended for the level of resolution the data is going to. It should go down to a more detailed resolution than just the EUNIS sediment classifications.

5.6.2 What PSA data should be used for biology?

- Folk and Ward (Inclusive) statistics (which are currently reported into MERMAN) should be used with caution as they assume a normal distribution of a sample, therefore they are meaningless for samples with multi-modal distributions (which marine sediments often are).
- Suggested measures to summarise marine sediment include: %sand, gravel and mud fractions, modal sizes, %<20um, d50, d90-d10.

Conclusion of this discussion:

- NMBAQC SOP and website should point people to Ken Pye's paper, which details the best measures of PSA for supporting biological analysis.

5.6.3 What data should be requested in databases (e.g. MERMAN for CSEMP monitoring)?

- MERMAN currently requests data at 1 phi intervals between 63um and 8mm, and inclusive statistics.

- Data reported to MERMAN should change to be in ½ phi intervals across the entire sediment distribution (this would be 35 determinands).
- Inclusive statistics should not be reported in MERMAN. If they are, then a description needs to go with them to explain the sediment distribution (e.g. normal distribution, or multi-modal distribution) to alert users when they should/shouldn't be using these statistics in data analysis.
- Sieve and laser data should be reported separately, as Ken Pye discussed – these two methods should not be merged.
- Raw data should be extracted from Malvern lasersizer and archived in an accessible format (e.g. .xls), which would be another form of internal QC (to validate data). At the moment you have to manually select the fields that you want to extract from Malvern. Is there a way to have a mass download of all data generated by Malvern lasersizers into a spreadsheet?

Conclusion of this discussion:

- NMBAQC to request new determinands in MERMAN: sediment fractions which are in ½ phi intervals across the entire sediment distribution. Also inclusive statistics should no longer be reported.
- NMBAQC to investigate how separate sieve and laser fractions can be reported into MERMAN.
- NMBAQC to follow up on suggestion of extracting all raw data from Malvern lasersizers. Recommendations to include: variables that should be extracted, and how an internal QC check of this could be run.

5.6.4 Sample collection – Biology vs. Separate grab?

- Views on collecting PSA samples from biology vs. separate grabs were re-iterated (from previous discussions in Sample Collection section – *see section 3.7.1*).
- NIEA stated that they are happy to use PSA from a separate grab, as PSA is only 'representative' of the biology (as it still would be if it came from the biology grab).
- PSA is the strongest determinand for biology compared to chemistry, which provides argument for collecting PSA samples directly from a biology grab.
- A decision matrix could be developed to help inform whether or not to collect PSA from a biology grab. E.g. if a muddy sample, then take 'x' amount, but if a gravely sample then take a larger amount. This however has inherent problems as you are changing the volume of sediment remaining for the biology sample (therefore the original volume of each biology replicate will be different), which would not be acceptable for biological analysis.

Conclusion of this discussion:

- NMBAQC needs to further investigate a tiered approach to the volume of samples collected, and whether this can always come from a biology grab. If not, then will continue to suggest collecting PSA from a separate sample.
- Evidence is needed to assess NIEA's claim that PSA samples are only 'representative' of biology regardless of whether they are taken from a biology vs. separate grab. This will be done by assessing the level of variation between PSA samples taken from within a grab compared to separate grabs.

The NMBAQC can then make a recommendation about the whether PSA samples should be collected from biology or separate grabs.

6 Laboratory session: Analysts discussion of PSA focusing on use of Malvern lasersizer for measurement of PSA

6.1 Comparison of Malvern SOPs

All looked at a CEFAS Malvern SOP and discussed the different inputs required by the user, such as optical properties of sediment, defining type of optical model required in terms of whether the sample is expected to be unimodal or have different peaks within the distribution; length of time of measurement; use of the autodilute facility. Background measurements and indication of proof that optical model works were also discussed.

The main points coming from this part of the session:

- Using autodilute is not advised for mixed sediment samples as this may preferentially lose fine/suspended material and bias results.
- An obscuration of 15-20% is best for the broad distributions expected from sediment samples. Users should test samples and determine when multiscattering effects to best determine the maximum obscuration acceptable but all agreed that 15-20% was expected to be the optimum obscuration range for these types of samples.
- Simon Blott advised that different SOPs are needed for different sediment types – this requires the user to assess the sediment before analysing it and determining the best sop to use for each sample and not use a generic SOP for every sediment sample being analysed.

Conclusion of this discussion:

- All laboratory analysts will send through SOPs to Claire Mason at CEFAS. Once collected Claire will forward to Richard Hartley to test these different SOPs, and report results.
- All agreed that in principle it may be possible to produce a range of bespoke Malvern SOP for different sediment types which can be made available to members of the NMBAQC, and be recommended to CMAs for completion of monitoring samples with specified guidance as to how to use these.
- These should include any pre-treatment, such as ultrasound is needed and whether the samples have been dried before analysis or kept wet.
- Anne Virden wants to produce a Malvern technical note on best methods to use for sediment analysis for all Malvern users. We plan to share all the information we use with her and assist in the development of this technical note.

6.2 Use of the emulation facility to add sieve data to laser data within the Malvern software

Anne Virden (Malvern) showed users how to use the emulation facility to add sieve data to laser data for a sample. Sedimentological statistical parameters can then be calculated using a Sediment Report 'Soil Reportv3' page. The Report page is easily

added to the current reports offered by Malvern and is available from Malvern. The calculations presented in the Report page can be copied into a custom calculation field and then placed in the main results page for all the samples measured in a file. These can then be added to other fields and exported into Excel.

6.3 Sediment user group

All agreed that a sediment user group forum was required to enable sharing of good practise and allow discussion of differences in methodology used, as well as highlight any development of new instrumentation/techniques tested. This should be available for all NMBAQC participants.

Conclusion of this discussion:

- All of the group would like to start a 'PSA user group' where they could continue discussion, post links and documents on a PSA forum on the NMBAQC website.

7 Data Interpretation and Reporting

7.1 Introduction to data interpretation and reporting currently done by CMAs – Prue Addison

The different fractions and statistics reported by CMAs were presented (refer to NMBAQC PSA Workshop.ppt, NMBAQC PSA Questionnaire Summary Report.doc and Summary of NMBAQCs PSA questionnaire results.xls). In summary CMAs still vary in:

- How sieve and laser data are merged (Malvern Software vs. Own Spreadsheets).
- What derived statistics are reported (Inclusive vs. Moments).

The current text relating to data interpretation and reporting in the Green Book was also presented (refer to NMBAQC PSA Workshop.ppt and PSA for biological analysis and the Green Book.doc). In summary, the Green book states:

- The following statistics must be reported: Inclusive Mean, Median, Kurtosis, Sorting and Skewness.
- The following fractions must be reported: <20um, <63um, 63-125um, 125-250um, 250-500um, 500-1000um, 1000-2000um, 2000-4000um, 4000-8000um, >8000um.

7.2 EA data interpretation and reporting – David Johns

The EA's method of data interpretation and reporting was discussed (refer to Data Interpretation and Reporting EA.ppt). The main points raised include:

- Sieve and laser data split at 2mm, and merged in an excel spreadsheet and then put through Malvern software (using Malvern's Emulation function) to calculate fractions and stats.
- Advantages: quick and easy; Disadvantages: fractions don't always add up (e.g. sometimes there are negative fractions).

7.3 NIEA data interpretation and reporting – Mike Allen

NIEA's method of data interpretation and reporting was discussed (refer to PSA Methodology NIEA.ppt). The main points raised include:

- Sieve and laser data split at 1mm, and merged in an excel spreadsheet which normalises different fractions.
- A cumulative distribution is plotted by hand, and statistics are calculated based on percentiles.

7.4 SEPA data interpretation and reporting – Myles O'Reilly

SEPA's method of data interpretation and reporting was discussed (refer to PSA Methodology SEPA.ppt). The main points raised include:

- Sieve and laser data combined at 1mm in a customised spreadsheet (whose origins are unknown).
- The mean is calculated differently to other Inclusive means (based on Buller and McMannus, 1979).
- Disadvantages: Data export from Malvern is by hand copying of printed export sheets, and data has to again be re-exported by hand copying from spreadsheet for archiving in NEMS database – prone to errors.

7.5 CEFAS data interpretation and reporting – Claire Mason

CEFAS's method of data interpretation and reporting was discussed (refer to Data Interpretation and Reporting CEFAS.ppt). The main points raised include:

- Sieve and laser data combined at 63um in a customised spreadsheet. There is an overlap between sieving and laser included.
- Whole sample is normalised by taking into account the amount of sample which fell through the 63um sieve pan (added to total amount of fine sediment from the lasersizer). All of the volumes estimated by the lasersizer are then converted into weight. The percentage weight in each fraction is then calculated, and following this the statistics can be calculated.

7.6 Introduction to GRADISTAT, Simon Blott, Ken Pye Associates Ltd.

The following are the key points covered in Simon's presentation:

- GRADISTAT (Grain-size Distribution Statistics) is a programme run through Microsoft Excel, which automatically calculates particle size statistics (Method of Moments and Graphical (Folk and Ward) Methods). This is available on http://www.kpal.co.uk/gradistat_abstract.htm. NB: a new version is being written in late 2009.
- Sieve and laser data should not be merged. This is because laser fractions are commonly over-estimated by 10-15%, therefore the two methods never join up properly.
- There are differences between Method of Moments and Folk and Ward (Graphical) statistics. In general the mean of each method are equal, but no other statistical parameters (Sorting, Skewness or Kurtosis) are comparable between the two methods.
- When a sediment sample has a poly-modal distribution, both Method of Moments and Folk and Ward statistics are meaningless. Much better

estimates are %sand, gravel and mud fractions, the location of modes and the proportion between modes.
- Gradistat still calculates all statistics for you, regardless of your sediment sample's distribution, but it provides a warning box explaining that your sample is not unimodal, therefore the Sorting, Skewness and Kurtosis statistics are unreliable.
- When entering sediment fractions, you must remember to enter a '0' for the fraction which is bigger than your largest fractions measured with a sieve. You also need to enter one smaller fraction than your samples smallest fraction, and put a value in this cell (e.g. ½ of your samples smallest fraction).
- Gradistat creates a table with all statistics, as well as a cumulative distribution curve of your sediment sample, and plots your sample onto the Ternary diagram.

7.7 Workshop Discussion – PSA data interpretation and reporting

7.7.1 Calculating fractions and statistics via Malvern Software vs. Own Spreadsheets?

- This was discussed more in the laboratory session. Mainly, some people like the Malvern software but some don't. The main thing is occasionally you should be checking your results against Gradistat.

Conclusion of this discussion:
- Either Malvern Software or Own Spreadsheets can be used, but these should be occasionally checked against Gradistat.

7.7.2 What data should be requested in databases?

- The <20um fraction currently requested by MERMAN is considered a mistake, as this is in the middle of the silt fraction. It is more likely to be <2um which is the clay fraction.
- Comments about knowing what you want to use your data for were re-iterated, as this will inform what level of detail is required. But CMAs could report to a more detailed level than just the 4 EUNIS classes which need to be defined.
- All agreed that stat's should not be reported into databases like MERMAN. Instead, just sediment fractions which are in ½ phi intervals across the entire sediment distribution.
- The technique used to conduct PSA needs to be entered into a field within MERMAN, for end users to assess how they can use the data.
- It was stressed that sieve and laser data really should not be merged.

Conclusion of this discussion:
- NMBAQC to request new determinands in MERMAN: sediment fractions which are in ½ phi intervals across the entire sediment distribution.
- NMBAQC to suggest removing statistics from MERMAN, this will eliminate the potential for future miss-use of these (as they are meaningless).
- NMBAQC to suggest adding a filed into MERMAN which will allow CMAs describe the technique used to conduct PSA.

- NMBAQC will need to consider whether past data in CSEMP should be fixed up to the new standards prescribed in the SOP.
- NMBAQC to investigate if merging sieve and laser data could be avoided, and how this could be entered into MERMAN.

8 Quality Assurance and Quality Control

8.1 Introduction to QA/QA currently followed by CMAs – Prue Addison

The different QA/QC that CMAs follow was presented (refer to NMBAQC PSA Workshop.ppt, NMBAQC PSA Questionnaire Summary Report.doc and Summary of NMBAQCs PSA questionnaire results.xls). In summary:

- All 6 CMAs and 6 private labs are signed up to the NMBAQC's PS component (considered external QA).
- CMAs vary in the level of internal QC practiced in their labs.

The Green Book states nothing about QA/QC in relation to PSA (refer to NMBAQC PSA Workshop.ppt and PSA for biological analysis and the Green Book.doc).

8.2 EA QA/QC – David Johns

The QA/QC practiced by the EA was discussed (refer to QA QC EA.ppt). The main points raised include:

- Laboratory method is UKAS accredited to IOS17025.
- Certified reference material (although not a great representation of marine sediment) used in lasersizer, Mastersizer serviced annually, and three samples run through the lasersizer.
- Sieves are certified, balances calibrated annually and checked daily.

8.3 NIEA QA/QC – Mike Allen

The QA/QC practiced by the NIEA was discussed (refer to PSA Methodology NIEA.ppt). The main points raised include:

- Certified Reference Material used in lasersizer, all samples run through laser meet the obscuration check (13-18%).
- Sieve's checked annually.

8.4 SEPA QA/QC – Myles O'Reilly

The QA/QC practiced by the SEPA was discussed (refer to PSA Methodology SEPA.ppt). The main points raised include:

- Certified Reference Material used in lasersizer run at beginning and end of batch of test samples.
- Sieve – 1 of each batch of 10 test samples is re-analysed (QC ranges to be within 90% original) – but often not enough sediment for re-analysis.

8.5 CEFAS QA/QC – Claire Mason

The QA/QC practiced by the CEFAS was discussed (refer to QA QC CEFAS.ppt). The main points raised include:

- Natural reference sediment sample (created in-house) used to check sieves and lasersizer. Certified reference material also used to check lasersizer.
- Laser obscuration is monitored for each sample.
- Balances calibrated annually.
- Sample weighed before, during, and after - if doesn't match up: re-sieve.
- Samples are re-sieved if deemed necessary (see above). One sample in 10 is routinely repeated for laser analysis, and more repeats are completed for some samples.
- The only QC for analysis of the whole sample (before splitting for sieve and laser analysis) is the NMBAQC ring test. There are only 2 of these a year, one muddy and one sandy.

8.6 AFBI QA/QC – Richard Hartley, Plymouth University

The QA/QC practiced by the Plymouth University was discussed:

- No certified reference material for sieves – just use an ultrasound sieve cleaner regularly to ensure sieves are clean.
- Balances are not checked on a regular basis.
- Lasersizer is serviced on an annual basis, and certified reference material run through.

8.7 NMCAG AQC Approaches – Colin Allchin, CEFAS

The following are the key points covered in Colin's presentation (see NMCAG AQC Approaches_Colin Allchin CEFAS.ppt):

- QUASIMEME:
  - Aimed to develop a holistic quality assurance programme for marine monitoring.
  - Transferred to Alterra in 2005 (not for profit organisation).
  - Two Performance Test (PT) rounds per year for chemistry measures for sediment, biota and water samples.
- QUASH:
  - One off two year programme which covered chemical and physical co-factors for biota sampling (including dissection).
- NMCAG Data Filter:
  - "Independent" assessment of an individual CMAs data and it's fitness for purpose for NMMP/UKMMAS.
  - Determinand/matrix combinations that fail to meet the required standard are flagged on MERMAN. This is based on 22 criteria.
  - * A similar filter should be provided from the results of the NMBAQC's PS component to flag data in MERMAN.
- MERMAN:
  - AQC returns are submitted on two spreadsheets, one for PT performance another for laboratory AQC.

  - Data filter scores determined automatically, proportion still checked for anomalies.
  - There is recognition that some determinands are more difficult to measure.
- The Green Book:
  - Problems include: it is out of date, there is a lack of editorial control, inconsistencies between sections, adherence is not enforced, and it is not on the SEPA website anymore. NB: Since the workshop it has been put on the CEFAS website: (http://www.cefas.co.uk/publications/scientific-series/green-book.aspx)
- Protocols Database:
  - Communication about the Protocols DB has not been effective, as most people are unaware of it (see WRC website: http://www.wrcplc.co.uk/marineprotocols/)
  - It is unclear what the relationship between the Protocols DB and Green Book is. *Comment from Tim Mackie suggests that the Protocols DB sits above the Green Book, and does not include the technical detail which is in the Green Book.
- Summary of AQC:
  - Requires a degree of commitment and co-ordination, and is costly and time consuming.
  - CMAs Responsible Officers should manage their own data if it is 'failing' a data filter.

8.8 Workshop Discussion – QA/QC

8.8.1 Internal QC recommendations?

- There is a call for the NMBAQC to include recommendations of internal QC which is considered best practice.

Conclusion of this discussion:

- NMBAQC to put together a list of internal QC recommendations based on what is thought to be some of the best practice QC currently done by CMAs.

8.8.2 External QA recommendations?

- Colin Allchin suggested the QUASIMEME approach could be adopted by the NMBAQC:
  - The QUASIMEME approach is considered true external QA, with a larger group of participants.
  - QUASIMEME provides reference material to 50 labs who conduct PSA on the sediment (which is not certified).
  - This sediment could then be used (after the QUASIMEME participants) by the NMBAQC to conduct more ring tests for their Particle Size component.
  - NMBAQC PS component should also follow the QUASIMEME approach and flag labs which have failed the PS ring tests in MERMAN. Although this is quite crude, since it is based on only two tests per year.

Conclusion of this discussion:

- NMBAQC could look into using QUASIMEME as another form of external QA to create a measure for labs that is then flagged up in databases.

9 Workshop wrap up

The following points need to be considered by the NMBAQC when producing a UK wide SOP for 'PSA for Supporting Biological Analysis':

- NMBAQC's SOP needs to be evidence based 'best practice', therefore more experiments needed before recommendations made.
- The SOP should also highlight what methods don't have significant differences, therefore doesn't matter what people do.
- Consideration needs to be given to the cost involved in different methods, and what methods are fit for purpose.
- NMBAQC must explore options of implications if organisations don't comply (some sort of feedback loop).
- All of the group would like to start a 'PSA user group' where they could continue discussion, post links and documents on a PSA forum on the NMBAQC website.

10 Appendix 1: Workshop Programme

Tuesday 10 February (9.30am – 5.00pm)

- Introduction and Welcome
- Sample Collection
  - Summary of NMBAQC Questionnaire Results
  - CMAs presentations of field methods
  - Workshop discussion on key methods to be recommended for UK wide SOP
- Sample Analysis
  - Summary of NMBAQC Questionnaire Results
  - CMAs presentations of laboratory methods
  - Results of PSA experiments testing different laboratory methods
  - Workshop discussion on key methods to be recommended for UK wide SOP

Concurrent Sessions:

- Sample Analysis Laboratory Session (Laboratory Analysts)
  - Discussion and demonstration of sieve and laser analysis
  - Malvern SOPs
- How is PSA data currently being used (Biologists)
  - Presentations from Ken Pye, Tim Mackie, Keith Cooper, Ken Neall, and Markus Diesing
  - Discussion group on the use of PSA data for biology

Wednesday 11 February (9.30am – 1.00pm)

- Laboratory Analysts and Biologists reconvene
  - Share key discussion points from each group
  - Workshop discussion on key methods to be recommended for UK wide SOP
- Data Interpretation and Reporting
  - Summary of NMBAQC Questionnaire Results
  - CMAs presentations of data interpretation
  - Presentation from Simon Blott: An introduction to Gradistat - a freeware particle size analysis programme
  - Results of data experiments
  - Workshop discussion on fractions and/or statistics to be recommended for UK wide SOP
- QA/QC
  - Summary of NMBAQC Questionnaire Results
  - CMAs presentations of QA/QC
  - Presentation by Colin Allchin: QA/QC completed under NMCAQC
  - Workshop discussion on key methods to be recommended for UK wide SOP
- Workshop wrap up and finish by 1.00pm!

11 Appendix 2: Attendees List

Organisers: Prue Addison, Environment Agency and Claire Mason, CEFAS.

Name	Organisation
Rob Nunny	AMBIOS
Ian Wilson	Benthic Solutions
Keith Cooper	CEFAS
Clare Jackson	CEFAS
Paul Whomersley	CEFAS
Thomas Maes	CEFAS
Ken Neal	CMACS
David Johns	EA
Ella Cheng	ERT
Lynda Allan	FRS
Samantha Lines	Gardline
Jake Ganther	Gardline
Isabelle Rundle	Gardline
Ken Pye	Kenneth Pye Associates Ltd
Simon Blott	Kenneth Pye Associates Ltd
Anne Virden	Malvern Instruments
Adrian Patterson	Martin Ryan Marine Science Institute, Ireland
Bob Kennedy	Martin Ryan Marine Science Institute, Ireland
Tim Mackie	NIEA
Mike Allen	NIEA
Richard Hartley	Plymouth University
Jim Allen	Precision Marine
Myles O'Reilly	SEPA
Nigel Grist	Unicomarine