Chlorophyll a Round Robin SOP

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DWQ began conducting chlorophyll a round robins in 2007 as a response to questions concerning the quality of the chlorophyll a analyses being performed by NC DWQ certified laboratories. The chlorophyll a round robins have been used by participating laboratories to evaluate and improve their analysis, improving the quality of the chlorophyll a data being used in North Carolina.

The round robins have historically been run by ESS personnel under the direction of the Laboratory Certification Unit.

Definitions

Sample: water collected from area waterbodies; prior to splitting

Subsample: a subset of sample that has been split from the whole

Replicate Samples: Samples collected at the same waterbody during the same time period; two samples collected at the same time period are referred to as "duplicate samples"

Equipment

Churn Splitter (Two 14L churn splitters are available in ESS)

Coolers (One for each out-of-state laboratory to keep and one for each in-state-lab that will be returned to ESS)

Zip-loc Bags (One bag for every three subsample bottles)

Packing Tape

Garbage bags (Two for each cooler being shipped out of state)

Ice

Temperature Blanks (One for each cooler)

Containers (One 20L container for each sample collected)

Subsample Bottles (500 mL brown HDPE bottles – same ones used by monitors for chlorophyll a samples)

Sample Tags (One for each subsample bottle)

Field Meter with Chlorophyll a Fluorescence Probe

Buckets with Rope (One for each waterbody sampled)

Field Meters (One for each waterbody sampled)

Personnel

Organizer (typically someone from ESS who organizes the round robin with input from the Laboratory Certification Unit)

Certification Staff Contact (works directly with Organizer; is responsible for contacting the certified laboratories, sending out the final report, and insuring that Laboratory Certification Unit tasks are completed)

Two Person Teams to Collect Water Samples (one team per waterbody sampled)

Two or Three Person Team for Splitting Samples into Subsamples

Two People to Pack Bags and Coolers (this has not been used in the past, but is strongly recommended)

Drivers (one for each route; typically six routes)

Timing

Round robins have typically been held in the summer months when large amounts of algae are present in area waterbodies. The 2008 round robin was held in June and resulted in lower chlorophyll values than desired. It is recommended that future studies be held in the late summer months of August and September.

Participants

Due to the size of the ESS current churn splitters (14L), each sample can only be split into twenty subsamples. One of these subsamples has been analyzed by the DWQ phycologists for algal species which limits the number of possible participants to nineteen. The primary participants are the NC certified laboratories and the DWQ laboratory. Remaining slots are filled by academic and governmental (both state and federal) laboratories. A list of 2008 participants with contact information is located in Appendix A of this document.

Two months before the round robin, laboratories are invited to participate. Prior to this, decisions have been made regarding the date of the study, the number of subsamples, and the delivery methods. Historically, the Laboratory Certification Unit has contacted the certified laboratories while the Organizer contacts the other laboratories. All laboratories are contacted by approximately three to four weeks prior to the round robin to confirm participation and delivery methods.

Throughout the round robin the laboratories are referred to by letter IDs that are assigned to each laboratory by the Organizer. Each laboratory is informed of their ID prior to receiving the subsamples and the drivers are informed of the IDs of the laboratories on their routes. However, in an attempt to control bias, the full list of IDs is known only to the Organizer and the Certification Staff Contact until after the delivery of all subsamples. The letters have been assigned in previous round robins by picking letters out of a cup and by a random sequence generator. (To choose letters by random sequence, a sequence of numbers 1 through 26 is produced by random sequence generator (have used the generator at random.org in the past). Each number is represents a letter in the English alphabet (A=1, B=2, etc.) and is assigned to the list of participants in the order in which the participants are listed.)

Questionnaire

At least a week prior to the round robin, participants are sent electronically a questionnaire concerning details of their chlorophyll analyses. Appendix B a list of questions included in the 2008 questionnaire. Participants are asked to electronically return the completed questionnaires with the chlorophyll results to the Certification Staff contact. Answers are included in the round robin report.

Samples

Historically, the number of subsamples each laboratory has been asked to analyze has been capped at nine. This is thought to be enough to ensure differences can be discerned without burdening the laboratories with the cost of the analyses. Typically three to four waterbodies are chosen for sampling with at least duplicates at each waterbody. If there are questions regarding how many locations and samples are required, it is recommended that the ESS statistician (currently Joe Olinger) be consulted.

The goal is to have samples with a range of chlorophyll concentrations from low (< 20 µg/L) to high (> 80 µg/L). This goal was not met with the 2007 and 2008 round robins. Samples are collected from area waterbodies that are an hour or less drive from ESS. Waterbodies are scouted two to three days prior to the round robin for chlorophyll concentration using a field meter with a chlorophyll fluorescence probe.

Samples are collected by teams on the day of the round robin. Historically, the Organizer has cleaned and prepared the equipment (buckets, ropes, 20L sample containers) for the team and provided directions to the waterbodies. (Buckets and sample containers are cleaned with non-phosphate detergent and rinsed with DI water. After being completely dried, they are covered with aluminum foil until used at the sampling location. Non-opaque sampling containers are covered with aluminum foil.) Samples are transported on ice to ESS. The teams also measure the waterbody's temperature, DO, pH, and conductivity at the time of sampling. In the past, the sampling teams have been asked not to discuss the waterbodies sampled to prevent the perception of any bias that would aid the DWQ Laboratory.

Splitting

The splitter is rinsed with each sample prior to splitting. Between samples, the splitter is rinsed with DI water. All rinses are drained through the spigot.

A sample are shaken while in the 20L sampling containers and poured into the churn splitter. The sample is churned for two minutes prior to splitting and continues to be churned while subsamples are drawn from the splitter using the spigot. Only ten liters of the fourteen liters of the sample can be used for splitting. While churning, care is taken not to break the sample's surface. The churning rate is increased as the volume in the splitter is decreased.

Subsamples are drawn from the splitter using the spigot into 500mL brown HDPE bottles. A sample tag is placed on each subsample which included the subsample ID (Subsample ID format is CRRXY where CRR stands for chlorophyll a round robin, X is the three digit sample ID chosen by random number generator, and Y is the laboratory ID). The tag order for each sample is determined prior to splitting in an order generated by random sequence generator using the laboratory ID letters. (For instance, with the sequence A,B,C, the subsample that was drawn first would go to lab A while the second subsample would go to lab B.)

Subsamples are placed on ice immediately after splitting.

Packing Coolers

Samples are placed in zip-loc bags (three to a bag) prior to packing in coolers. For shipments to out-of-state laboratories, the coolers are lined with two garbage bags. Ice is placed in the garbage bags and the bagged subsamples are placed in the ice along with a temperature blank. The garbage bags are taped shut. Paperwork is placed in waterproof bags and taped to the cooler's lid. The coolers are taped closed. For in-state-laboratories, the bagged subsamples and a temperature blank are placed in ice in coolers. Since the coolers are delivered by DWQ staff, other precautions are not made.

Delivery

For out-of-state laboratories, the coolers containing the subsamples are shipped overnight. The shipper is contacted the day before the round robin to schedule the pick-up which is usually around 13:00. All samples must be split and coolers packed prior to this time. A letter, describing the round robin and providing contact information if there are any problems, and copies of the DWQ sample submittal sheets are placed in each cooler.

Subsamples for in-state-laboratories are delivered to the laboratories the afternoon of the round robin by DWQ staff. Historically, there have been six routes – the Triangle, Morehead City/Beaufort,

Reidsville/Kernersville, Charlotte, Greenville, and Wilmington. Drivers are asked to note who accepted the subsamples for each laboratory and to hand over copies of the DWQ sample submittal sheets.

All laboratories are given the same information. (In the past all laboratories have been provided copies of the completed DWQ sample submittal forms.) No laboratories (including the DWQ laboratory) are provided with sample locations or even the counties where sampling occurred. Arrangements have been made in the past with the laboratory QA/QC officer to minimize problems with sample acceptance by the DWQ laboratory.

Results

Laboratories are asked to report their results to the Certification Staff Contact within one month. These results are complied and analyzed by ESS staff.

Analysis

The goal of the analysis is to differentiate the performance of the participating laboratories. Variation caused by the lab is separated from other sources of variation through regression and partitioning. For example, samples are expected to vary from waterbody to waterbody. The average result for a given waterbody can be compared to the individual lab's result for that waterbody, which yields a residual value. The residuals of a single lab for the entire series of waterbodies provide an assessment of how close each lab is to the average results of all the participating labs. The distribution of these residuals is displayed as a box plot, to demonstrate the accuracy and stability of the lab's results compared to the rest of the labs. They are also displayed on a graph of standard deviation of residuals vs. average distance of residuals from the average.

Report

A report of the round robin describing the process and stating the results is provided to the participants. The participating laboratories are indentified but laboratory IDs are used in reporting the questionnaire answers and the results. A key relating the laboratories with the laboratory IDs have not been included in past reports. The report is reviewed by ESS and Laboratory Supervisors prior to being made public.

Appendix A – Participants in the 2008 DWQ Chlorophyll a Round Robin

Laboratory	Contact Person	Phone Number
Central Environmental Laboratory, Department of Biology, East Carolina University	Rebecca Cooper	252-328-6287
Charlotte Mecklenburg Utilities Lab Services Division	Eric W. Newell	704 336-3701
City of Durham	James Blake	919-560-4386
Columbia Analytical Services, Inc	Kathryn Brungard, QAPM	904.739.2277 or 904.394.4414 (direct)
Environment 1, Inc.	John Melvin	252-756-6208
EPA	Bob Quinn	706-355-8723
Florida Department of Environmental Protection Bureau of Labs Biology Section	Lori Wolfe	850-245-8185
Meritech, Inc.	Kris Pawlak	336-342-4748
NCDENR/DWQ	James Staley	919 733 3908
NOAA Beaufort	Chris Holland	252-728-8799
North Carolina State University - Center for Applied Aquatic Ecology	Jenny James	919-515-3421
REI Consultants, Inc	Brenda Barnett	304-255-2500
Research & Analytical Laboratories, Inc.	Sidney L. Champion	336-996-2841
Tennessee Dept of Health Laboratory Services-Env. Labs	Pat Alicea or Tim McCollum	(615) 262-6327/(615) 262-6329
Tritest	Karen Ferrand	919-834-4984

Laboratory	Contact	Phone Number
UNC Institute of Marine Sciences	Nathan Hall	252-726-6841 ext. 228
UNCW Aquatic Ecology Laboratory	Matthew McIver	910-962-2357
USGS National Water Quality Laboratory, P.O. Box 25046, MS 407 Bldg 95, Denver, Co 80225-0046	Angie White (USGS NWQL) or Mary Giorgino (Raleigh contact)	303.236.3173 or 919.571.4087

Appendix B – Questions from the 2008 DWQ Chlorophyll a Round Robin Questionnaire

1. Laboratory ID
2. Laboratory
3. Contact Person
4. Phone Number
5. Email Address
6. Method Used
7. Date Samples were Received
8. Time Samples were Received
9. Temperature of Samples at Time Received
10. Temperature at which Samples were Stored Prior to Filtering
11. Describe technique for homogenization of samples prior to filtering
12. Date Samples were Filtered
13. Time Samples were Filtered
14. Type of Filters Used
15. Pressure at which Samples were Filtered (if not measured, please state that)
16. Volume of Sample Filtered
17. Describe Filtering Technique (how were sample volumes measured, were sides rinsed)
18. How long were samples filtered? (if not same for all samples, please state range)
19. Describe light conditions during filtering
20. Temperature at which Samples were Stored after Filtering
21. Time Samples were Stored after Filtering
22. Extraction solvent used? Volume used?
23. Steeping Time?
24. Grinding Used? (yes or no)
25. If grinding was used, describe grinding setup (ie. Type of tip used, Was the temperature controled)
26. Make and Model of Instrument
27. Spectral Bandwidth of Instrument (spectrophotometric)
28. Spectral Bandwidth of Instrument (fluorometric excitation)
29. Spectral Bandwidth of Instrument (fluorometric emission)

30. Excitation Wavelength
31. Emission Wavelength
32. Samples Acidified? If so, please state type, concentration, and volume used
33. If samples were acidified, how long did samples sit between acidification and analysis by instrument?
34. Type of calibration standard used and source
35. Any noteable differences between samples?