Michael F. Easley, Governor

William G. Ross Jr., Secretary
North Carolina Department of Environment and Natural Resources

Coleen H. Sullins, Director
Division of Water Quality

DATE: October 5, 2007

TO: Participating Certified, Academic and Government Laboratories

FROM: The State of North Carolina Wastewater/Groundwater Laboratory Certification Program

SUBJECT: 2007 Chlorophyll a round robin results

Attached are the results for the North Carolina Division of Water Quality (DWQ) Chlorophyll a round robin interlaboratory comparison study. The purpose of this study was to determine the analytical conditions and the level of interlaboratory agreement for the determination of chlorophyll *a* in surface water samples.

The Division of Water Quality would like to take this opportunity to thank the laboratories for their participation in the study. With one hundred percent cooperation, we were able to obtain a sizable data pool and generate meaningful results.

From the data received, it is obvious that the quality of the participating laboratories is not consistent in terms of the precision and the accuracy of the reported results. It is recommended that each laboratory in the study examine their results with respect to the data pool and the meta-data provided and initiate a thorough review of the methodology and procedures where appropriate. The North Carolina Wastewater/Groundwater Laboratory Certification (NC WW/GW LC) program staff will be available to assist those labs pursuing corrective action.

As a result of the success of this round robin and due to the fact that there are no commercially-prepared performance evaluation samples, the DWQ intends to use the round robin split sample approach to continue to assess laboratory performance. We will use the data generated in this study to evaluate a system for assessing acceptable performance for future split sample studies.

Contact us at (919) 733-3908 if you have any questions.

Sincerely,

Pat Donnelly

Pat Donnelly
Certification Branch Manager
Laboratory Section

Attachment

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Jennie Atkins
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August 2007 Chlorophyll a Round Robin

Currently there is no performance evaluation sample to assess an entire chlorophyll a method from pigment extraction to instrumental analysis. Therefore, the NC Division of Water Quality (DWQ) is conducting split sample studies to evaluate the condition of analysis by laboratories across the state. In August 2007, fifteen commercial, academic, and government laboratories participated in a chlorophyll a round robin organized by the division.

Methods

Sampling

Several days prior to sampling, DWQ staff scouted Triangle area waters in order to locate waters within the desired chlorophyll a concentration range. On August 16th, 2007, DWQ staff collected nine 19 L grab samples from five area lakes. The locations are presented on page 2. At four locations duplicate samples were taken; at the fifth, only one sample was collected. Staff measured field parameters (water temperature, dissolved oxygen (DO), conductivity, and pH) at each location.

Samples were placed in light protected containers and transported on ice to DWQ. At DWQ, samples were split into 500 mL subsamples using a churn mixer. Five of the samples (CRR016, CRR234, CRR326, CRR652, and CRR937) were each split into eighteen subsamples -- seventeen subsamples were sent to participating laboratories while the eighteenth was provided to DWQ phycologists for community analysis of algae. The other four samples were each split into seventeen subsamples. (Two of the laboratories requested an extra subsample from each sample in order to conduct additional analysis.) Every sample was churned for two minutes prior to splitting and was continually churned while split. The order in which the subsamples were split from the samples was randomized in an effort to control bias. Subsamples were collected in brown HDPE bottles, placed on ice, and delivered to laboratories either by DWQ staff (in-state laboratories) or shipped overnight (out-of-state laboratories).

Analysis

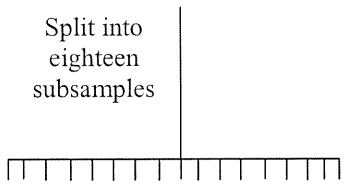
Laboratories were asked to filter samples on the morning of August 17th and then analyze the samples according to their SOPs. Each laboratory was also asked to complete a questionnaire regarding analysis details. The answers of the questionnaires are found on page 3. The analysis results are located on page 6. A graph on page 7 contains a statistical analysis of the data which looks at the residual standard deviation of the laboratories versus the residual mean of the data after normalization for the differences in chlorophyll a concentrations at the various stations. This graph represents one way of viewing the data.

Sampling Chart

Lake Benson at Buffalo Road
35.67268, -78.63174

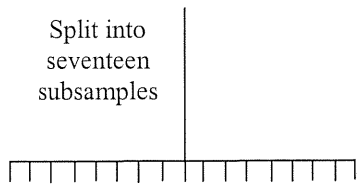
Sample CRR 016

Split into eighteen subsamples



Sample CRR 643

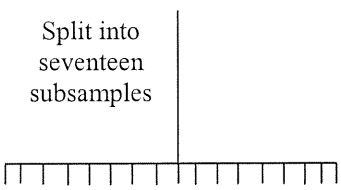
Split into seventeen subsamples



Raleigh Area Pond
35.79710, -78.68620

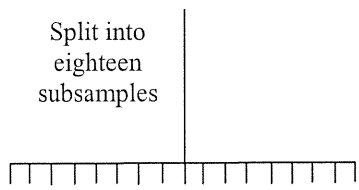
Sample CRR 206

Split into seventeen subsamples



Sample CRR 234

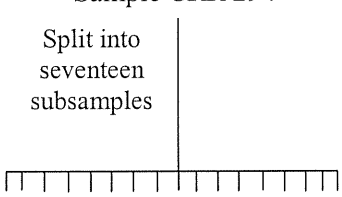
Split into eighteen subsamples



Robeson Creek (Jordan Lake) Boat Access
35.70271, -79.09975

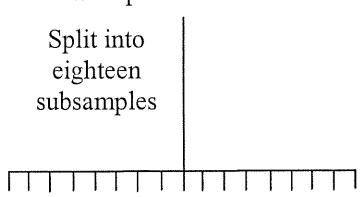
Sample CRR 294

Split into seventeen subsamples



Sample CRR 937

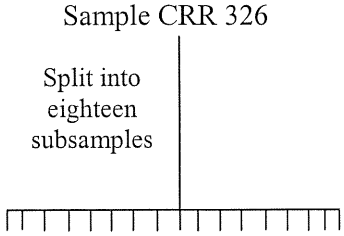
Split into eighteen subsamples



Yates Mill Pond at walkway
35.72067, -78.68777

Sample CRR 326

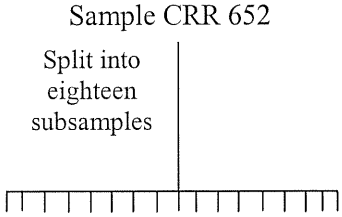
Split into eighteen subsamples



Jordan Lake Bells Church Public Fishing Area
35.75145, -79.00721

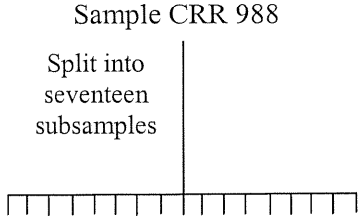
Sample CRR 652

Split into eighteen subsamples



Sample CRR 988

Split into seventeen subsamples



Chlorophyll a Round Robin Analysis Details
Answers from Participants' Questionnaires

Lab ID	Method Used	Temperature Samples Stored Prior to Filtering	Volume of Sample Filtered	Type of Filters Used	Pressure at which Samples were Filtered
A	EPA 445	10° C	50 mL	Glass Fiber	<6 in Hg
B	SM 10200H	4.0° C	100 mL	934-AH Glass Fiber	not measured
C	Welschmeyer 1994	2.2° C	47mL	GFF 25mm circles	3 in Hg
D	EPA 445.0 standard	Samples were re-iced on receipt and stored on ice overnight. Temperatures were recorded immediately prior to filtering and ranged from 1.6 to 2.4° C	100 mL	Glass fiber (GF75; 47mm)	Not measured; used low-vacuum hand-pump filtration apparatus
F	EPA Method 445.0	4° C	150 mL	GF/F 47mm	< 20kpa (< 6 in Hg)
G	SM 10200 H 19th Edition	2° C	20 mL	0.45 um Metricel	not measured
I	EPA Method 445.0 modified	6.5° C	50 mL	glass fiber filter	5 in Hg
K	EPA 445.0 (Welschmeyer, non-acidification)	4° C	44 - 80 mL	GF/C	<5 in Hg
L	445.0	0.4° C	250 mL	GF/F glass fiber	5 Torr (0.2 in Hg)
M 1	EPA 445.0 - Modified	N/A	25 or 50 mL	GF/F	5-6 in Hg
M 2	EPA 445.0 - Corrected	N/A	25 or 50 mL	GF/F	5-6 in Hg
N	Standard method 10200 H	3° C	250 mL	Glass Microfibre	not measured
O	spectrophotometric	14.5° C	dependant upon sample, typically 200mL	Whatman 934-AH (1.5 uM)	aimed for 10 psi (20 in Hg); fluctuated between 5 and 10 psi (10 and 20 in Hg)
P	EPA 445.0	2.5° C	150 - 300 mL	47 mm	< 20 Kpa (< 6 in Hg)
U	SM 10200-H.c; 18th Ed.	Room Temp	515-547 mL	934-AH	not measured
Y	Standard Methods 18th edition10200H Spectrophotometric Determination	3.8° C	500 mL	A/E Glass Fiber 47 mm	not measured

Chlorophyll a Round Robin Analysis Details
(continued)

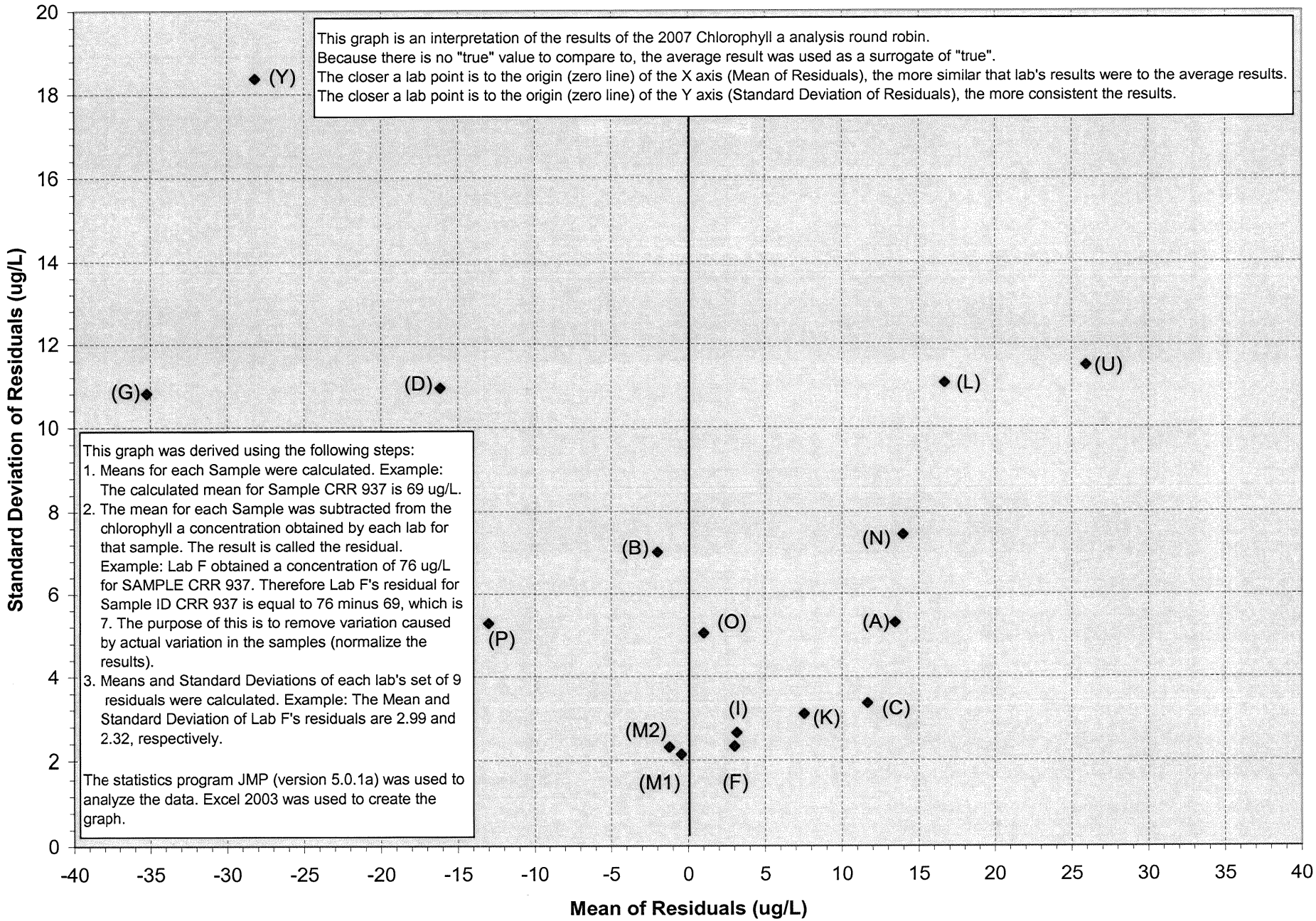
Lab ID	Temperature Samples were Stored after Filtering	Time Samples were Stored after Filtering	Steeping Time?	Grinding Used?	Description of Grinding Setup
A	-21° C	11 days	3 hours	yes	Tissue grinder with a teflon tip attached to a mechanical stirrer, no temperature control
B	-15° C	12 days	16 hours	yes	Teflon Tissue Grinder in glass Pestle , Temp not controlled but not allowed to get too warm
C	-80° C	immediately analyzed	10 minutes	yes	Teflon tissue grinder was used with a drill to grind the filter and 7.5mL of acetone completely (30seconds) Temperature was not controlled
D	-20° C	10 days	N/A (sonication method does not require steeping)	no	Filters undergo sonication rather than grinding
F	-19° C	1 day	19 hours	yes	Teflon tip attached to a drill press
G	0° C	5 days	4 hours	no	
I	-20° C	6 days	24 hours	yes	Teflon (PTFE) tissue grinder, temperature was not controlled however grinding time was very short ~ 15 seconds per sample to prevent heating of the acetone/ filter slurry
K	-20° C	Extracted on 9/3/07 (17 days after filtered)	20 hours	yes	stainless steel tip homogenizer, not temperature controlled
L	-16° C	4 days	24 hours	yes	Teflon pestle with radial serrations on lower part of pestle. Glass tube, powered by electric drill. Temperature controled by touch.
M 1	-20° C	6 days	Overnight (18 hours)	yes	10 mL Glass grinding tube with teflon tip on grinder
M 2	-20° C	6 days	Overnight (18 hours)	yes	10 mL Glass grinding tube with teflon tip on grinder
N	-20° C	7 days	4 hours	yes	Round bottom glass grinding tube with matching glass pestle
O	-20° C	3 days	1.5 hours in cold room (14.5° C) followed by 30 minutes in centrifuge (4°C at 2800 rpm) = 2 hours total	yes	a heavy duty drill (Milwaukee; 120 v, 5.5 amp) fitted with a PTFE pestle from a Wheaton Potter-Elvehjem tissue grinder set. No temperature control - samples were in a box with ice packs, removed, ground for ~30 seconds, then returned to the box.
P	< -5° C	13 days	16 hours	yes	Samples are ground in the steeping vessel using slotted teflon grinder. Tubes are montired to be sure that they do not heat up.
U	-7° C	11 days	2 hours	yes	Maceration by spatula and vortex mixer. No temperature control used.
Y	-1.3° C	6 dys	2.25 hours	yes	Arrow 850 motor 1/10 hp; Kontes tissue grind pestle SZ 24 and matching tube. No temperature control

Chlorophyll a Round Robin Analysis Details
(continued)

Lab ID	Make and Model of Instrument	Date and Time Instrument was Calibrated	Excitation Wavelength	Emission Wavelength	Samples Acidified?
A	Turner 10-AU	7/9/2007 12:00	436 nm	680 nm	no
B	Turner Designs fluorometer Model 10	05/23/2007 @ 14:30	340 - 500 nm	> 665 nm	Yes - 8 to 10 drops of 2N HCL / 25 mL of extract
C	Turner 10 AU	8/16/07 by Rance Hardison	436nm	680nm	no
D	Turner Designs, Model TD- 700	8/27/2007 approximately 13:30	340 - 500 nm	> 665 nm	0.1 N HCl solution, 137 uL to 4.5 ml of sample
F	Turner 10-AU	8/22/2007 9:30	436 nm	680 nm	no
G	Turner Model 450 Digital fluorometer	8/22/2007 13:00	430 nm	663 nm	yes used 20 ul of 2N HCl
I	Turner Designs TD700 bench top fluorometer	8/24/2007 9:30	436 nm	680 nm	no
K	Turner Designs 10-AU	9/4/2007	436 nm	680 nm	no
L	Turner Trilogy fluorometer	8/22/2007, 20:00	480 nm	685 nm	no
M 1	Turner Designs TD-700	8/24/2007	436 nm	680 nm	no
M 2	Turner Designs 10AU	8/24/2007	340 - 500 nm	>665 nm	yes
N	Hach DR/2010	Factory calibration	N/A	N/A	yes, sample was acidified with 200 microliters of 0.1 N HCl
O	Milton Roy Spectronic 1201	no official calibration; use 90% acetone to check for zero	N/A	N/A	yes; 2 drops of 6N HCl
P	Turner Designs Flouromter	9/4/2007 14:00	436 nm	680 nm	no
U	Thermo Spectronic Helios Gamma	5/20/2003 -- instrument zeroed before analysis	N/A	Absorption wavelengths of 750, 664, 647, and 630 nm (spectrophotometer)	no
Y	Thermo Genesys 6	n/a	N/A	N/A	no

Other details collected – date samples were received, time samples were received, temperature samples were received, date samples were filtered, time samples were filtered, brand of filters used, volume of sample filtered, spectral bandwidth of instrument

Results of Chlorophyll a Analysis

Laboratory ID	Robeson Creek (Jordan Lake) Boat Access		Jordan Lake Bells Church Public Fishing Area		Raleigh Area Pond		Lake Benson at Buffalo Road		Yates Mill Pond
	sample 937 (µg/L)	sample 294 (µg/L)	sample 652 (µg/L)	sample 988 (µg/L)	sample 234 (µg/L)	sample 206 (µg/L)	sample 016 (µg/L)	sample 643 (µg/L)	sample 326 (µg/L)
A	91.4	87	43.7	41.9	74.3	57.4	55.1	56.1	51.1
B	73	59	41	35	50	53	32	41	35
C	85.0	78.7	46.2	43.3	66.9	64.4	60.3	54.4	42.7
D	42.9	63.7	30.7	29.1	21.2	26.5	30.7	23.4	23.5
F	76	79	37	35	57	53	46	45	36
G	20	21	10	9	14	13	12	11	10
I	77.06	76.92	35.78	33.2	58.37	59.18	44.91	43.48	36.42
K	83.1	82.8	40	37.4	62.6	57.2	49.4	48.7	43.6
L	91.6	91.4	40	41.1	88.6	85.2	54.3	58	37
M 1	71	69	33	30	59	51	43	41	36
M 2	70	67	32	30	56	51	43	40	37
N	95	92.9	51.3	44.8	68.4	55.5	63	53.4	38.4
O	68.09	74.49	34.04	37.25	48.06	62.08	47.72	38.21	36.04
P	50.5	49.8	24.8	26.9	42.8	37.8	31.7	28.6	26.9
U	112	113	52.5	50.4	89.4	70.4	72.9	66.9	43
Y	< 1.0	56	< 1.0	< 1.0	46.1	41.3	16.3	< 1.0	19.6

Residual Chlorophyll a (corrected for Sample variation)