June 2008 NC DWQ Chlorophyll a Round Robin

Currently, 40 miles and 112,000 acres of surface waters in North Carolina are impaired due to chlorophyll a, a chemical parameter used to assess the phytoplankton population (2008 NC Impaired Waters List). These impairments lead to the development of TMDLs and increased regulation, often at significant costs to both the state and the stakeholders in the watershed. It is important that the North Carolina Division of Water Quality (NC DWQ) understands the quality of the data used to make these decisions.

Because of the lack of performance evaluation samples for the parameter to test the entire chlorophyll a analysis, NC DWQ conducted a chlorophyll a round robin in August 2007 involving the state's certified laboratories as well as other academic and governmental laboratories. Fifteen laboratories in all analyzed nine surface water samples for chlorophyll a concentration. Analysis of the results indicated significant inconsistencies with the quality of the data. The division used the results of that round robin to work with laboratories to improve analyses.

The data presented within this report represent the second chlorophyll a round robin which was held in June 2008. Eighteen laboratories participated, analyzing nine samples. Seven samples were collected from Triangle area waterbodies. Two of the nine were prepared *Selenastrum capricornutum* samples that are part of NC DWQ's attempts to develop chlorophyll a stock solutions to be used, along with the round robin studies, to assess laboratories' analyses.

Experimental

Sampling

On June 12, 2008, NC DWQ staff collected seven grab samples from three area waterbodies. The locations are presented on page 2. Staff measured field parameters (water temperature, dissolved oxygen, conductivity, and pH) at each location. Samples were placed in light protected containers and transported on ice to NC DWQ's Environmental Sciences Section (ESS).

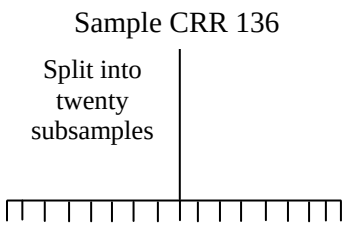
Two samples were prepared by NC DWQ's Aquatic Toxicity Unit (ATU) by mixing *Selenastrum capricornutum* concentrate (Aquatic Biosystems, Inc.) with soft synthetic fresh water prepared by ATU staff. Samples were well mixed prior to splitting.

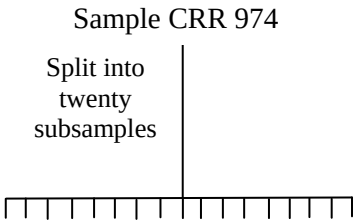
At ESS, all nine samples were split into twenty 500 mL subsamples using a churn splitter. (Eighteen subsamples were sent to laboratories for chlorophyll a analysis and two were analyzed by ESS staff for algal species.) Every sample was churned for two minutes prior to splitting and was continually churned during the split. The order in which the subsamples were split from the samples was randomized in an effort to control bias. Subsamples in brown HDPE bottles were placed on ice and were either delivered to laboratories by NC DWQ staff (in-state laboratories) or shipped overnight (out-of-state laboratories).

Analysis

Participating laboratories were asked to analyze the nine samples according to their Standard Operating Procedures for chlorophyll a analysis. Each was also asked to complete a questionnaire concerning the analysis. The answers to the questionnaire and the data from the study are found on pages 4 and 9, respectively. Analyses of the data are presented graphically on pages 10 and 11.

***Selenastrum capricornutum* Samples Prepared by NC DWQ**





Jordan Lake – New Hope Overlook Boat Launch
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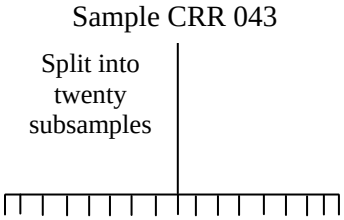
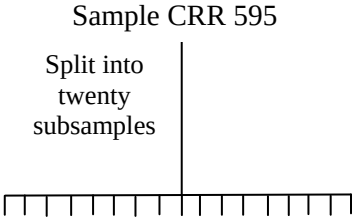
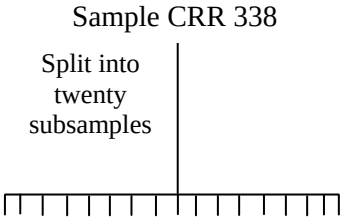
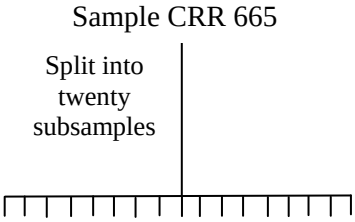


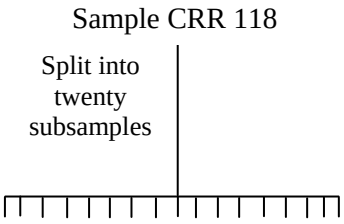


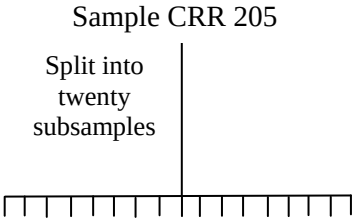
Harris Reservoir – Crosspoint Boat Launch
35.57304, -78.97601

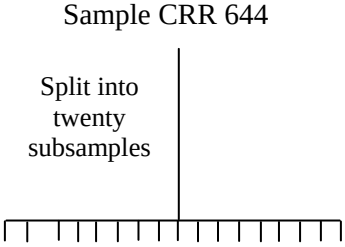




Lake Wheeler – Lake Wheeler Park Boat Dock
35.69326, -78.70078







Participating Laboratories

The laboratories were referred to by ID throughout the round robin.

Charlotte-Mecklenburg Utilities Division – Hal Marshall Laboratory
Columbia Analytical
City of Durham Water and Wastewater Laboratory
NC DWQ Laboratory
East Carolina University Department of Biology
Environment 1
EPA Science and Ecosystems Support Division
Florida Department of Environmental Protection
Meritech
NCSU Center for Applied Aquatic Ecology
NOAA Center for Coastal Fisheries and Habitat Research
REI Consultants
Research and Analytical
Tennessee Department of Health
Tritest
UNC Institute for Marine Sciences
UNCW Center for Marine Sciences
USGS

NC DWQ appreciates the time and cooperation of each participating laboratory.

Chlorophyll a Round Robin Analysis Details
Answers from Participants' Questionnaires

Laboratory ID	Method Used	Date Samples Received	Time Samples Received	Temperature Samples Received	Temperature Samples Stored Prior to Filtering
B	Standard Methods 10200H (spectrophotometric)	6/12/2008	15:40	2° C	filtered immediately
C	EPA 445.0 (fluorometric)	6/13/2008	10:10	1.1° C	samples were filtered shortly after arrival
D	EPA 445.0 (fluorometric)	6/12/2008	18:30	3.1° C	3.1° C
G	EPA 445.0 (fluorometric)	6/12/2008	13:50	7.2° C	4.0° C
I	EPA 445.0 (fluorometric)	6/12/2008	16:15	3.1° C	4.0° C
K	Standard Methods 10200H (spectrophotometric)	6/13/2008	9:30	0.3° C	20° C – room temperature to equilibrate prior to analysis
L	Welschmeyer 1994 (fluorometric)	6/12/2008	16:15	7.2° C	samples were filtered immediately
M	EPA 445.0 (fluorometric)	6/12/2008	15:00	0.8° C	1.4° C
O	Standard Methods 10200H (spectrophotometric)	6/12/2008	17:10	1.0° C	1.1° C
P	EPA 445.0 (fluorometric)	6/13/2008	10:00	1.8° C	1.8° C
Q	EPA 445.0 (fluorometric)	6/12/2008	16:00	4° C	2° C
R	Standard Methods 10200H (spectrophotometric)	6/13/2008	9:35	1° C	~ 20° C
T	EPA 445.0 (fluorometric)	6/12/2008	14:10	5.7° C	9.7° C
U	EPA 446 (spectrophotometric)	6/13/2008	13:11	1.5° C	filtered immediately
V	EPA 445.0 (fluorometric)	6/12/2008	14:45	3.8° C	2.3° C
W	Standard Methods 10200H (spectrophotometric)	6/12/2008	15:10	3° C	3° C
X	Standard Methods 10200H (fluorometric)	6/12/2008	16:35	0.4° C	0 – 4° C
Y	EPA 445.0 (fluorometric)	6/12/2008	16:20	2° C	2° C

Laboratory ID	Homogenization Technique for samples prior to filtering	Date Samples were Filtered	Pressure at which Samples were Filtered	Volume of Sample Filtered	How long were samples filtered?
B	sample bottle inverted 3x	6/12/2008	~5 in Hg	240 - 250 mL	typically less than 1 minute/sample; some up to 3 minutes.
C	bottles was inverted 4 times then gently shaken	6/13/2008	exact pressure unknown (not measured)	50 - 200 mL	2 - 10 minutes
D	inverted sample bottle about 5 times	6/12/2008	5 in Hg	25 - 50 mL	approximately 2 minutes/ sample
G	vigorous shaking	6/12/2008	<20 kpa (<5.9 in. Hg)	50 - 150 mL	2 - 5 minutes
I	inverted 3-4 times	6/13/2008	<20 kpa (<5.9 in. Hg)	50 - 300 mL	3 - 4 minutes, never more than 10 minutes.
K	Invert 3 - 4 times	06.13.08	not measured	122 - 500 mL	2 - 45 minutes
L	samples were moderately shaken by hand before filtering	6/12/2008	5 in. Hg	20 - 100mL	30 seconds to 2 minutes
M	slowly invert bottle several times	6/12/2008	5 in. Hg	86 - 250 mL	1 - 5 minutes
O	shaken	6/13/2008	not measured	250 mL	30 sec to 2 minutes
P	Invert 4-5 times	6/13/2008	5-6 in. Hg	10 - 50 mL	1 - 3 minutes
Q	manual agitation/shaking	6/13/2008	no measured	150 mL	3 - 5 minutes
R	Sample bottle is vigorously shaken by hand before filtration.	6/13/2008	Not measured	100 - 500 mL	~45 - 60 seconds
T	gently shook bottle before pouring sample	6/12/2008	not measured; use low-vac hand pump	100 mL	average of 40 seconds
U	shake	6/13/2008	6 in Hg	354 - 519 mL	10 minutes
V	Samples were inverted several times	6/13/2008	<5 in Hg	31 - 166 mL	time filtered was not recorded (estimate 10 - 15 seconds/sample)
W	shake well	6/13/2008	3 in Hg	250 mL	filter through quickly, 30 - 45 seconds
X	gentley shaken by hand.	6/13/2008	~5 in Hg	100 mL	< 30 seconds
Y	Shake bottle	6/12/2008	< 6 in Hg	50 mL	30 seconds to 5 minutes

Laboratory ID	Light conditions during filtering	Extraction solvent/volume	Steeping time	Was grinding used?
B	ambient outside light; blinds closed, lights off	90% acetone, 12 mL	2.5 hours	yes
C	a "yellow low light"condition existed while filtering	90% acetone 30 mL was the final volume for all samples and lab blank	20 hours	yes
D	dimly lit, blinds pulled over windows, no overhead lighting	90% acetone, 10 - 14 mL	25 hours	yes
G	green light 25 W bulb	9:1 acetone:DI water. 25 mL	18 hours, 10 min	yes
I	minimum light - red bulbs used	90% acetone, 10 mls	17 hours	yes
K	under florescent lighting	10mL of 90/10 acetone/$MgCO_3$ solution	12 hours	yes
L	sunlight through window was the only light in the room	7.5mL 90% acetone 10% deionized water	24 hours	yes
M	green light	90:10 acetone/DI water. 25 mls used for extraction	22 hours	yes
O	reduced laboratory light, light on behind me with door partially closed	90% acetone, 10% DI water @ 10 mL samples topped off at 10 mL	overnight (17 hours)	yes
P	dimmed fluorescent lighting	90:10 actone:DI Water, 10 mL	overnight (approx 18 hours)	yes
Q	subdued lighting using green light	90% acetone / 10 mLs	Overnight	yes
R	filtration is done with regular overhead lighting.	90% acetone with 10% $MgCO_3$ solution. extract has a final total volume of 8 mL.	4.5 hours	yes
T	fluorescent lighting in NC WSC lab	90 % acetone/water, 20mL	0 (sonication method does not require steeping)	no
U	subdued	aqueous acetone, 90 %; 10 mL	24 hours	no
V	all overhead lights off, small desk lamp on (60 watt soft white)	90% acetone, 14mL	22 hours	yes
W	turned off lab lights during filtration, subdued lighting from nearby room	aqueous acetone solution, 10 mL	overnight	yes
X	Dark, minimal light filtering through paper covering over door window, no lamps on in room.	90% Acetone, 25 mL	16+ hours	yes
Y	No overhead lights, some filtered light from window	90% acetone, 10 ml	approximately 3 hours	yes

Laboratory ID	Description of grinding setup
B	Teflon (PTFE) tissue grinder with radial serrations on tip, powered by electric drill. Temperature not controlled - samples were in dark box with ice packs, removed, ground for up to 30 seconds, then returned to the dark box.
C	The samples were taken out of their aluminum foil covers and cut with small sharp scissors into a plastic 50 mL centrifuge tube which already contained approximately 5 to 7 mL of 90% acetone. Next a glass grinding pestle was used to grind the pad up into fine pieces by hand. The pestle was rinsed as well as the other extraction tools back into the centrifuge tube. It was then capped with a screw-on cap and shaken vigorously for approximately 1 minute. The sides were rinsed down and volume brought to a final reading of 30 mL.
D	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
G	Teflon tip with drill press
I	Electric drill at slow speed is used for grinding - Wheaton tissue grinder with radial serrations. Temperature is held steady within 3 degrees C
K	Teflon tip which is cleaned with acetone between samples. Temperature is not controlled.
L	Teflon tissue grinder was used with a drill to grind the filter and 7.5mL of acetone completely (30seconds) Temperature was not controlled
M	Teflon pestle with radial serrations on lower part of pestle. Pestle powered by electric drill in glass tube. Temperature controled by touch.
O	glass/glass tissue grinder Arrow 850 motor 1/10 hp Kontes grinder pestle SA24 and matching tube -- no temperature control
P	Tissue grinder with teflon tip, no temp control except by touch
Q	Teflon Pestle with grooves in tip. Temperature not controlled but each sample was grind for 90 seconds.
R	Filter is rolled up and placed in a 30 mL glaas tube that is kept on ice (to minimize heat from friction). An Eberbach power unit with a Wheaton Tissue grinder is used to grind sample down with solvent. The slurry is added to a centrifuge tube. The 30 mL test tube is rinsed with solvent until clean and added to the centrifuge tube. The centrifuge tube is brought up to 8 mL with solvent, if needed. Samples are steeped in refrigerator.
T	Filters undergo sonication rather than grinding
U	Maceration with a spatula followed by vortex mixing.
V	stainless steel tip homogenizer, temperature was not controlled
W	round bottom grinding tube with matching glass pestle; ~ 60 seconds
X	Teflon Tissue Grinder in glass pestle , temperature not controlled but not allowed to get too warm
Y	a teflon tissue grinder is attached to a motor, temperature is not regulated except the we make sure not to grind hard enough to raise the temperature

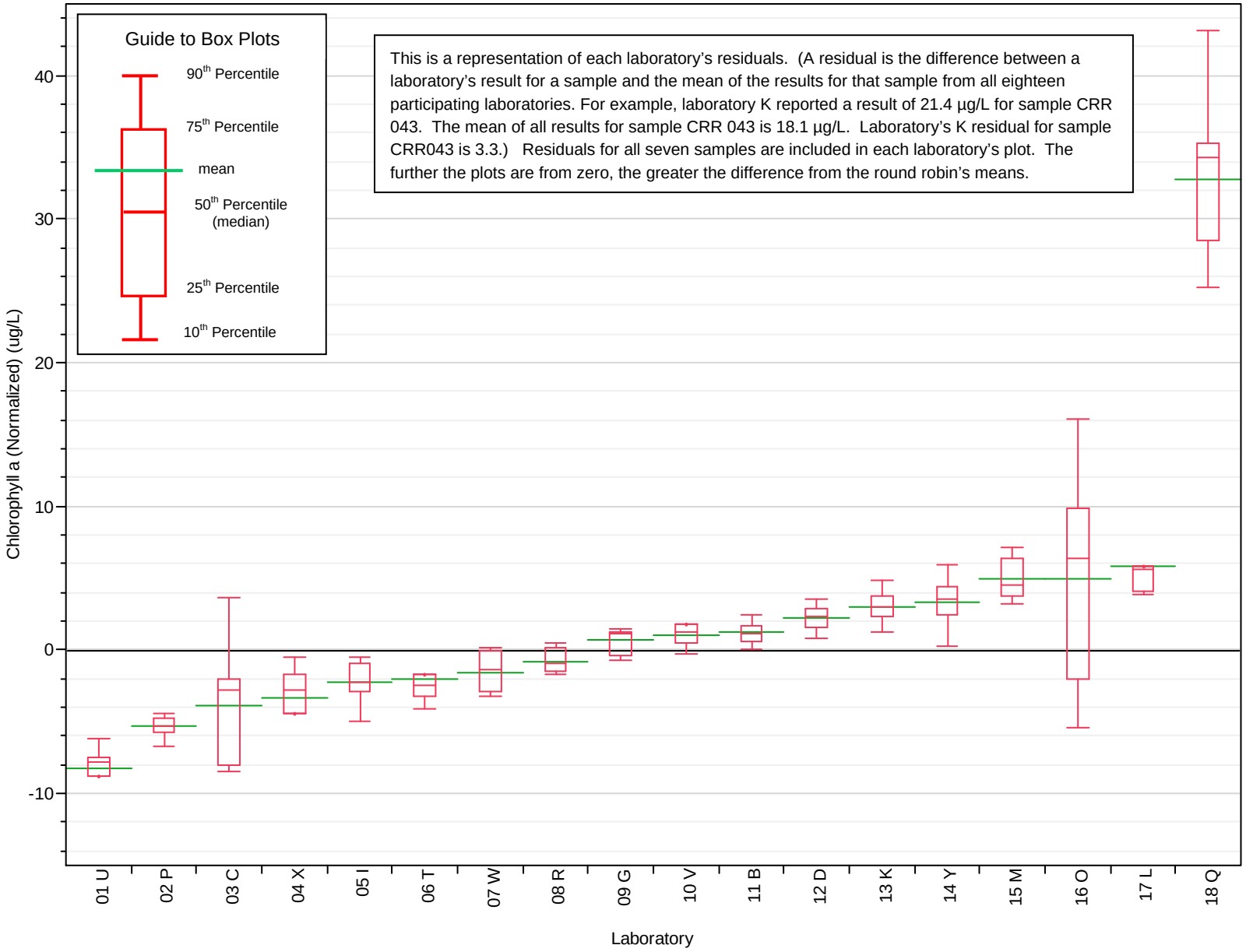
Laboratory ID	Samples Acidified? If so, type, concentration, and volume used	Type of calibration standard used and source
B	yes; 2 drops of 6N HCL/10mL extract	90% acetone to zero the spectrophotometer
C	no	Spinach, 1 mg chlorophyll a from which a 2000µg/L (ppb) was made as a stock standard solution. Product was purchased from Sigma Chemical Co.
D	no	2 concentrations of Turner Designs solid standard and a 90 % acetone zero
G	no	196 µg/L Turner instrument Corp
I	no	Turner Designs fluorometric Chlorophyll Standard - Dilution B High Conc., Dilution C Low Concentration
K	yes; 3.0mL of sample volume acidified with 0.3mL of 0.1N HCl	None
L	no	Chlorophyll a standard from Chromodex INC. was used
M	no	High Standard and Low standard (one tenth of High Standard value). Both standards obtained from Turner
O	yes; 0.1N HCl at a ratio of 0.03 mL acid per 1 mL sample	n/a
P	no	Chlorophyll a from Anacystis, Sigma C6144, a 200 µg/L calibration standard was made from stock on day of analyses
Q	n/a	Sigma-Aldrich
R	Samples are acidified with 100 uL of 0.1 HCl, mixed with a mini-mixer, and timed for 90 seconds.	A 0.20 mg/L concentration of chlorophyll-a standard is read at the beginning and end of each batch. The standard is made from Sigma Chlorophyll-a from spinach 5 mg powder (Cat# C5753-5MG). For this batch the standard read at 94 and 96% recovery.
T	0.1 N HCl solution, 137 uL to 4.5 ml of sample	Stock solution, Sigma C6144- 1 mg, Lot # 127K1032 dissolved in 100 mL of 90% Acetone. Calibration standards are 800, 400, 200, 100, 50, 10, and 5 ug/L
U	yes; 0.1 N HCl, 0.165 mL; Sample Volume of 5.5 mL	n/a
V	no	Primary - Chl a from Anacystis nidulans - Sigma (C6144) Secondary - Chl a from Spinach - Sigma (C5753)
W	yes; 200 µL of 0.1 N HCl	factory internal calibration curve; method blank to zero spectrophotometer
X	yes; 0.10N HCl 0.75 mL to 25 mL Sample	Turner Designs Calibration Standards for primary calibration, Solid stick for secondary daily reference.
Y	no	Purified Chlorophyll a from Anacystis dissolved in 90% Acetone (Sigma-Aldrich Chemical)

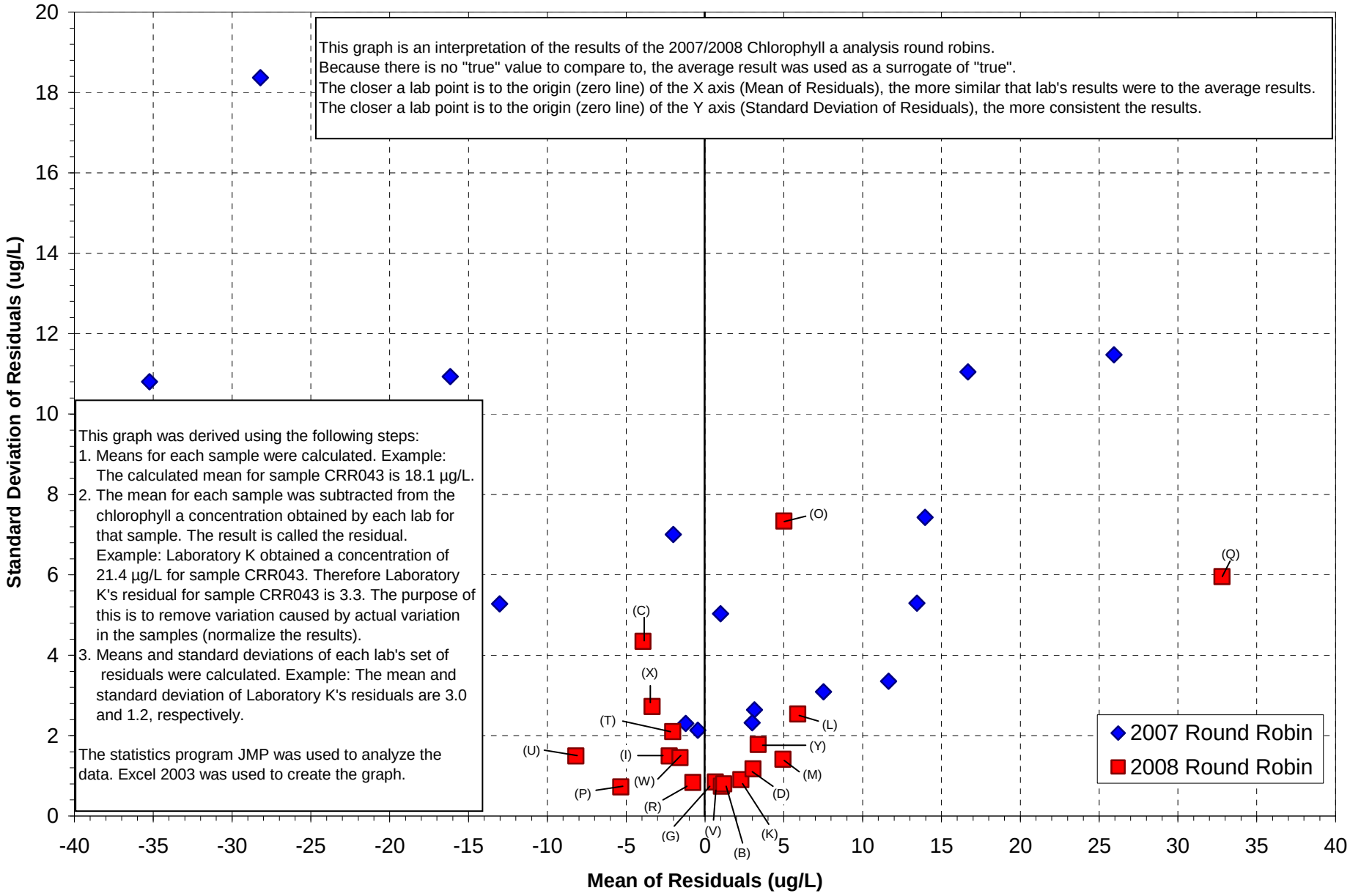
Additional information obtained from participating laboratories – time samples were filtered, type of filters used, filtering techniques, time samples were stored after filtering, make and model of instrument, instrument bandwidth(s), wavelength(s), time between acidification and analysis by instrument, and notable differences between samples.

June 2008 Chlorophyll a Round Robin Results

	Jordan Lake		Harris Lake		Lake Wheeler			*Selenastrum capricornutum* *	
Laboratory ID	CRR043 (µg/L)	CRR595 (µg/L)	CRR338 (µg/L)	CRR665 (µg/L)	CRR118 (µg/L)	CRR205 (µg/L)	CRR644 (µg/L)	CRR136 (µg/L)	CRR974 (µg/L)
B	18.9	17.7	16.8	18.6	20.5	22.4	20.9	158.9	152.8
C	8.445	8.31	14.01	15.45	11.565	18.15	24	183.6	195.6
D	20.03	19.51	18.92	19.77	21.74	21.42	21.93	175.11	152.21
G	18	18	18	18	20	20	20	44	43
I	15.6	16.3	15.1	14.7	16	15.7	18.1	179	187
K	21.4	20.2	19.8	18.7	22.7	23.2	22.7	223	193
L	22.1	28.1	20.9	23.3	24.6	24.5	25.1	166.4	168.2
M	23.6	23.1	20.6	20.7	24.3	24.8	24.9	175.3	195.3
O	11.1	14.7	24.7	23.8	21.2	36.8	30.2	313	302
P	11	11	12	13	14	14	15	120	150
Q	45	52	42	46	62	55	55	36	55
R	17	16	17	16	18	19	19	240	230
T	14.86	14.28	14.54	14.42	21.28	17.48	16.26	9.43	12.93
U	8.41	8.95	10.6	9.95	10.1	9.59	12.5	61.2	50
V	17.9	18.5	18	19.3	19.8	21.1	20.1	205	186.1
W	16.7	16.7	15.4	16.7	16	17.4	17.4	70.1	85.1
X	16	14	8	13	17	19	17	130	100
Y	19.4	19.2	21.2	21.6	22.4	26.6	20.6	197.6	146.2

* *Selenastrum capricornutum* samples were for research purposes only and were not used in any of the following graphical analyses.


Guide to Box Plots
90th Percentile
75th Percentile
mean
50th Percentile (median)
25th Percentile
10th Percentile
This is a representation of each laboratory's residuals. (A residual is the difference between a laboratory's result for a sample and the mean of the results for that sample from all eighteen participating laboratories. For example, laboratory K reported a result of 21.4 µg/L for sample CRR 043. The mean of all results for sample CRR 043 is 18.1 µg/L. Laboratory's K residual for sample CRR043 is 3.3.) Residuals for all seven samples are included in each laboratory's plot. The further the plots are from zero, the greater the difference from the round robin's means.
Chlorophyll a (Normalized) (ug/L)
40
30
20
10
0
-10
01 U
02 P
03 C
04 X
05 I
06 T
07 W
08 R
09 G
10 V
11 B
12 D
13 K
14 Y
15 M
16 O
17 L
18 Q
Laboratory


This graph is an interpretation of the results of the 2007/2008 Chlorophyll a analysis round robins.
Because there is no "true" value to compare to, the average result was used as a surrogate of "true".
The closer a lab point is to the origin (zero line) of the X axis (Mean of Residuals), the more similar that lab's results were to the average results.
The closer a lab point is to the origin (zero line) of the Y axis (Standard Deviation of Residuals), the more consistent the results.
This graph was derived using the following steps:
1. Means for each sample were calculated. Example: The calculated mean for sample CRR043 is 18.1 µg/L.
2. The mean for each sample was subtracted from the chlorophyll a concentration obtained by each lab for that sample. The result is called the residual. Example: Laboratory K obtained a concentration of 21.4 µg/L for sample CRR043. Therefore Laboratory K's residual for sample CRR043 is 3.3. The purpose of this is to remove variation caused by actual variation in the samples (normalize the results).
3. Means and standard deviations of each lab's set of residuals were calculated. Example: The mean and standard deviation of Laboratory K's residuals are 3.0 and 1.2, respectively.
The statistics program JMP was used to analyze the data. Excel 2003 was used to create the graph.
Standard Deviation of Residuals (ug/L)
Mean of Residuals (ug/L)
2007 Round Robin
2008 Round Robin
(O)
(Q)
(C)
(X)
(T)
(L)
(U)
(I)
(Y)
(W)
(M)
(P)
(D)
(R)
(G)
(V)
(K)
(B)