June 2012 NC DWQ Chlorophyll *a* Round Robin

Currently, 21 miles and 21,700 acres of surface waters in North Carolina are impaired due to chlorophyll *a*, a chemical parameter to assess algal productivity (2012 Final 303(d) List). These are impairments that require Total Maximum Daily Load (TMDL) development and increased regulation, often at significant costs to both the state and the stakeholders in the affected 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 to test the entire chlorophyll *a* analysis method, NC DWQ began a chlorophyll *a* round robin in August 2007 involving the state's certified laboratories as well as other academic and government laboratories. Seventeen participating laboratories in 2007 analyzed eight freshwater samples for chlorophyll *a* concentrations. The first Round Robin results indicated significant inconsistencies with the quality of the data. The division used the results of that round robin to work with laboratories and improve analyses.

The data presented within this report represents the sixth chlorophyll *a* round robin on June 20, 2012. Eighteen laboratories participated, each analyzing eight samples. All eight samples were collected from Raleigh Area waterbodies.

Methodology

Sample Collection

On June 20, 2012, NC DWQ staff collected a batch of eight surface water grab samples from four area waterbodies. The sample site locations are presented on page 2. Samples were placed in light protected carboys and transported on ice to NC DWQ's Environmental Sciences Section (ESS).

At ESS, each of the eight samples were split into eighteen 500 mL subsamples using a churn splitter. Every sample was churned for two minutes prior to splitting and was continually churned during the split. The splitter faucet was purged prior to sample collection. The order in which the subsamples were split from the main sample was randomized in an effort to control bias. Subsamples were put in amber HDPE bottles, then placed on ice and were either delivered to laboratories by NC DWQ staff (in-state laboratories) or shipped overnight (out-of-state laboratories) to meet holding times.

Analysis

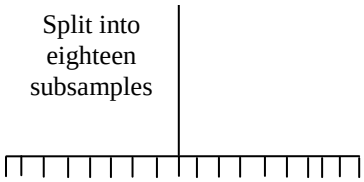
Participating laboratories were asked to analyze the eight samples according to their Standard Operating Procedures for chlorophyll *a* analysis and to complete a questionnaire concerning the analysis. The answers to most of the questionnaire's questions and the data from the study are found on pages 4 through 10. The answers to the questionnaire are entered as the laboratory presented them unmodified, except for spelling. Analyses of the data are presented graphically on pages 11 and 12.

Sample Site Locations

Lake Wheeler
35.69366, -78.70128

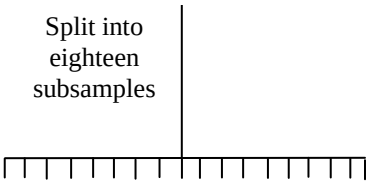
Sample CRR 974

Split into eighteen subsamples



Sample CRR 117

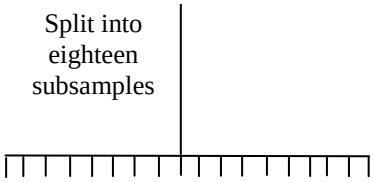
Split into eighteen subsamples



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

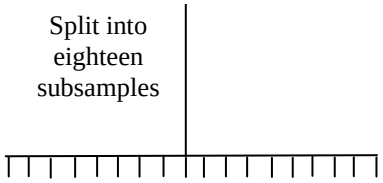
Sample CRR 233

Split into eighteen subsamples



Sample CRR 579

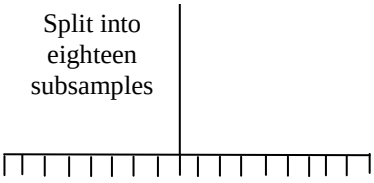
Split into eighteen subsamples



Harris Lake @ Crosspoint Landing
35.57309, -78.97594

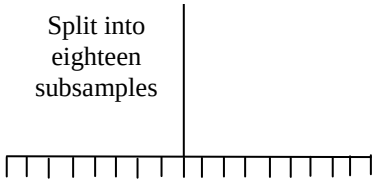
Sample CRR 258

Split into eighteen subsamples



Sample CRR 443

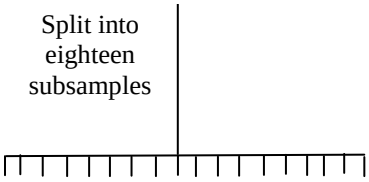
Split into eighteen subsamples



Jordan Lake @ Seaforth
35.79730, -78.68622

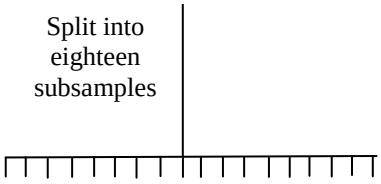
Sample CRR 163

Split into eighteen subsamples



Sample CRR 888

Split into eighteen subsamples



Participating Laboratories

Participating laboratories were referred to by random letter identification throughout the round robin. The order of letters are alphabetical and do not represent the following list.

Charlotte-Mecklenburg Utilities Division – Environmental Laboratory
Columbia Analytical Services
East Carolina University Department of Biology
Environment 1
Environmental Conservation Laboratories - Orlando
EPA Region IV, Science & Ecosystems Support Division
Florida Department of Environmental Protection
Meritech, Inc.
NC Division of Water Quality Laboratory
NCSU Center for Applied Aquatic Ecology
NOAA Center for Coastal Fisheries and Habitat Research
Pace Analytical (formerly Tritest, Inc.)
REI Consultants, Inc.
Raleigh, E. M. Johnson Water Plant
Research and Analytical Laboratories
UNC Institute for Marine Sciences
UNCW Center for Marine Sciences- Aquatic Ecology
USGS National Water Quality Laboratory

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	Temperature Samples Stored Prior to Filtering	Temperature Samples Received	Length of Time Samples were Stored after Filtering
A	SM10200H	06/21/12	23°C	0.3°C	144hrs
B	EPA 446.0	06/21/12	filtered immediately	1.5°C	19 days
C	SM10200H	06/20/12	filtered immediately after receiving	on ice	24 days
D	EPA 445.0	06/20/12	Samples were filtered immediately upon arrival and Sample ID number assigned	<5°C	15 days
E	EPA 446.0	6/20/12	3.0°C	4°C	7 days
F	EPA 445.0	06/20/12	Samples filtered upon receipt	5.4°C	2 days
G	EPA Method 445 Uncorrected	06/20/12	At Room temperature between 20 to 25 C°.	3.2°C	approx. 5 days and 18 hours
J	Chlorophyll A SM18 10200H	06/21/12	n/a	2°C	23h
K	SM 10200H	06/20/12	0.10 - 4.4 °C	1.9 - 4.7 °C	7 Days
L	EPA 445.0	06/20/12	on ice	on ice	two weeks
N	EPA 445.0 (Welschmeyer, non-acidification)	06/20/12	3.1°C	3.8°C	20 days
P	EPA 445.0 Fluorometer	06/20/12	3.2° C	11.5°C	6 days
R	EPA Method 445.0 modified	06/20/12	4°C	3.5°C	18 h
S	EPA 446	06/21/12	4°C	1°C	4 days
T	Standard Methods 18th ed. Spectrophotomteric Determination	06/20/12	1 °C 45 mins	3.4°C	28.5 Hours
U	EPA Method 446.0, 20th Edition: 10200 H	06/20/12	4.0°C	<4.0°C	Samples were extracted immediately after Filtering.
W	fluorometric (non-acidification)	06/20/12	n/a	8°C	5 days
Y	SM10200H Spectrophotometer	06/21/12	Room Temperature	2.9°C	4 days 17 hrs 30 min

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?
A	Shake for 30 seconds	06/21/12	Not Measured	355-500 mL	2-4.5min
B	shake	06/22/12	6 in Hg	466-531 mL	2-10 min
C	sample bottle inverted 3x	06/20/12	~7.0 in Hg	0.23-0.25 L	typically less than 1 minute/sample; some up to 3 minutes.
D	Samples inverted 4 times	06/20/12	< 6 in Hg	50-200mL	Up to 8 minutes
E	Samples were inverted several times and shaken in a circular fashion to homogenize.	6/20/12	< 6 in Hg	250mL	<1 min for each sample
F	Sample bottle inverted gently three times.	06/20/12	5 in Hg	150 mL	1-2 minutes
G	Gently agitated by inverting sample several times	06/20/12	< 6 in Hg	150 mL	25 min.
J	samples are shaken right before filtering	06/21/12	not measured	237 - 325 mL	10 - 27 minutes
K	Sample bottle well shaken by hand for 5-10 seconds	06/21/12	< 6 in Hg	100 mL	20 -25 seconds
L	invert sample gently for 10 seconds	06/20/12	< 6 in Hg	50 mL	10-30 seconds
N	Samples gently inverted 10 times before filtering	06/20/12	< 5 in Hg	82 - 156mL	time filtered was not recorded (estimate 5-10 seconds/sample)
P	Gently shook the bottle before dispensing the water into graduated cylinder	06/20/12	< 3 in Hg	100 mL	1 minute
R	briskly inverted bottle ~10 times	06/20/12	6 in Hg	50 mL	1-2 minutes
S	bottle shaken 4-5X	06/21/12	$\leq$ 6 in Hg	25 mL	1-2 minutes
T	shaken	06/20/12	4-6 in Hg	250 mL	<5 mins.
U	Gentle 180°inversion then back again 15 times.	06/21/12	6 in Hg	200 to 250ml	Approximately 1 to 2 minutes/per sample
W	gently mixed each sample before measuring sample volume to be filtered	06/20/12	5 in Hg	50-100 mL (attached on data sheet next tab)	1-2 minutes
Y	Sample bottle is vigorously shaken by hand before filtration.	06/21/12	Not measured	180 - 500 mL	30 seconds - 11 minutes

Laboratory ID	Type of Filters Used	Brand of Filters Used	Describe Filtering Technique (how were sample volumes measured, were sides rinsed)
A	1.2um	Whatman Glass- 696 Microfiber	Measured with 500mL measuring cylinder. Cylinder and filter flask rinsed three times with DO water.
B	934-AH (glass micro filter)	Whatman	weight difference; bottle rinsed
C	Whatman 934-AH glass fiber filters	Whatman	sample volume was measured in a graduated cylinder. Sides of cylinders and filter apparatus were rinsed with deionized water. Samples were filtered to dryness.
D	Glass Fiber	Whatman	50mL aliquots filtered in graduated cylinder. When filtration slows final vol. recorded and sides of cylinder rinsed 3X with DI water.
E	MG550HA	Munktell	Samples were measured using a class A graduated cylinder. Filtration performed in a dark room with all lights cut off. Only light entering room is through window to the rest of the lab. Sides were not rinsed in order to not put excess pressure on the filters. Manifold for filtration is glass.
F	GF/F glass fiber	Whatman	Sample volumes measured in a graduated cylinder. Sample poured into filtration apparatus containing Whatman filter under vacuum at 5 Torr. Side walls of cylinder and filter apparatus rinsed between samples.
G	GF/F filters glass fiber, 47 mm, with nominal pore size of 0.7 µm.	Colonial Scientific	By using Graduated cylinder, 250 mL and sides were rinsed
J	Glass Microfiber 934-AH 47mm	Whatman	The sample is poured slowly into the filter funnel until it no more sample is going thru the filter. The volume is measured after filtering. The sides are not rinsed, so it does not affect the volume.
K	47 mm glass fiber 0.7 micron	Whatman	Measured in a TD graduated cylinder; sides not rinsed
L	Glass Fiber, Pore size 0.7 mm 25 mm dia	Millipore	Volume measured in a graduated cylinder, filter funnel sides not rinsed down
N	Glass fiber	Whatman GF/C	After mixing, sample was poured into a graduated cylinder and volume was recorded. After pouring sample into filter funnel, the sides of graduated cylinder were rinsed twice and poured in funnel. The inside of the funnel was rinsed as the last step.
P	GF-75, 47 mm	ADVANTEC	Measured volume in a graduated cylinder. Filtered with a hand pump. Rinsed sides of filtration unit and graduated cylinder with DI water. Filtered the DI water through the filter.
R	25 mm Whatman GF/F	Whatman	duplicate aliquots of 50 ml were measured using a 50 ml graduated cylinder, sides of filter towers were not rinsed (we typically measure estuarine samples and do not rinse due to possible osmotic shock and cell lysis)
S	GF/F	Whatman	graduated cylinder; cylinder and filter tower both rinsed with DI
T	A/E glass fiber 47mm	Millipore	measured with 250ml graduated cylinder, vacuum filtered, cylinder and funnel rinsed between uses
U	47 mm Glass Fiber	HACH, Catalog #: 2530-00	Approximately 250ml of sample was measured in a graduated cylinder. Sample was poured into filtering apparatus. Vacuum was turned on. Graduated cylinder was washed with deionized water twice an added to filter. Sides of filtering apparatus were washed twice.
W	GF/F (glass fiber) 25mm circles	Whatman	samples were measured with a graduated cylinder and vacuum filtered, sides were not rinsed
Y	GF/C	Whatman	After being mixed, sample is poured into a 500 mL Class A graduated cylinder to be measured before filtration. Sample is vacuum filtered as quickly as possible. When filtration is nearing the end, 1-2 mL saturated MgCO3 solution is added. Funnel is rinsed thoroughly with DI Water. Filters are folded and wrapped in aluminum foil. Cylinder is thoroughly rinsed after each sample with DI water.

Laboratory ID	Light conditions during filtering	Extraction solvent and volume used	Steeping time	Was grinding used?
A	Florescent Light	90/10 Acetone/$MgCO_3$. The Acetone is chromatography grade and the $MgCO_3$ is reagent grade. 10mL of the Acetone/$MgCO_3$ solvent was used to extract the sample.	22hrs	yes
B	subdued	Aqueous acetone 90%; 10 mL	24 hours	Yes
C	ambient outside light; blinds closed, lights off	90% Acetone, 12 mL	2 hours in cold room (14.5oC) followed by 20 minutes in centrifuge (4oC at 2800 rpm) = 2 hours 20 minutes total	Yes
D	Dark room with red lights	90% Acetone : Water. DI water	20hr 40 min	Yes
E	Very little light in room. All lights in filtration room were cut off.	90% ACS grade acetone/10% deionized water. Each sample is extracted with 10mL extraction solution.	Overnight	Yes
F	Room converted to "Dark Room with only "Green" lights used throughout	Acetone: ACS Electronic Grade, diluted 1:10 with DI Water, 25 mls used per sample	24 hrs	Yes
G	Dark Room with three 60-Watt green light bulbs was on.	90% Acetone, 25 mL	6 hours 35 min.	yes
J	Florescent light	90% Acetone	2 - 9 hours	yes
K	Dark room with subdued green light	90% Acetone Optima, 25ml	17 hr, 36 min.	Yes
L	filtered light from windows	90% acetone, 10 mL	4 hours	yes
N	All overhead lights off, two small lamps with 25 watt green bulbs	90% acetone, Fisher Scientific Certified ACS, 14mL	23 hours	yes
P	Overhead fluorescent lights	90 % Acetone/water solution, Maceration- 25 mL	< 23 hrs	yes
R	lights turned off, blinds closed	90% reagent grade acetone	24h	yes
S	dimmed fluorescent light	10 mL 90% HPLC-grade acetone/10% deionized water	20 hours	yes
T	Darkroom with green light	90% acetone w/10% deionized water, Purity=99.7% @ 10mls used	23 Hours	yes
U	All lights were turned off, shutters at windows closed, and door was closed. Window in door remained uncovered, letting in filter light.	90% Acetone HPLC grade. 10 ml are used for each sample extraction.	23 hours	Yes
W	room lights off, ambient sunlight through the window	7.5mL of 90% methanol and 10% deionized water	24 hours	yes
Y	Filtration is done with regular overhead lighting. (Intensity Range 20-30 ft-candles)	90% Acetone with 10% MgCO3 solution. Extract has a final total volume of 8 mL.	22 hrs 35 min	yes

Laboratory ID	Description of grinding setup
A	Drill press with a teflon grinding tip. Not temperature controlled.
B	Glass tube grinder (30 mL) with teflon pestel; drill powered; cooled with ice bath.
C	Teflon (PTFE) tissue grinder with radial serrations on tip, powered by electric drill. Temperature not controlled - samples were removed from -20oC freezer, ground for approximately 30 seconds, and placed in dark box with ice packs.
D	Tissue Grinder. Sample in plastic centrifuge tube. Temperature controlled to prevent solvent evaporation.
E	Samples are ground with glass pestel in a glass tube until acceptably . Samples were then shaken for a few minutes to evenly distribute Chlorophyll into acetone solution. Temp is not monitored because no mechanical grainding is used.
F	Teflon pestle with radial serrations on lower part of pestle. Plastic tube, powered by electric drill. Temperature controlled by touch.
G	Tissue grinder, Teflon® pestle (50 mm X 20 mm) with grooves in the tip with ¼" stainless steel rod and yes temperature was controlled.
J	Samples are ground using a stainless steel tip in a glass test tube for 1 minute with 3 ml of 90% Acetone solution. Samples are then transferred into a 25ml screw top centrifuge tube and an additional 7ml of 90% Acetone solution is added. Analysis occurs at Room Temperature.
K	Ground in a glass mortar. Pestle has round, serrated Teflon tip. Unit powered by electric motor. Temperature monitored by feel. Sample not allowed to heat.
L	A teflon tip tissue grinder is attached to a motor and the filter is ground in a 50 mL centrifuge tube till completely macerated. Temperature does not rise signifcantly as felt when holding the tube.
N	stainless steel tip homogenizer, temperature was not controlled
P	hand drill, Pestle Tissue Grinder , 50 ml Conical tubes
R	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
S	glass grinding tube with teflon tissue grinder attached to variable speed mixer; temperature control by feel (stopped grinding before samples became warm)
T	Arrow 850 motor 1/10hp Kontes tissue grind pestle SZ 24 and matching tube. No temperature control.
U	Grinder: Cole Parmer Lab Gen 125 with stainless steel blade. 50ml disposable polyproplene centrifuge tubes are used for the grinding and steeping.
W	samples were poured into 100mL graduated cylinder and then into filter manifold. The cylinder and manifold was rinsed with deionized water between each sample. Teflon tissue grinder was used with a drill to grind the filter and 7.5mL of acetone completely (30seconds) Temperature was not controlled
Y	Filter is rolled up and placed in a 30 mL glass 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.

Laboratory ID	Samples Acidified? If so, type, concentration, and volume used	Type of calibration standard used and source
A	0.1ml of 0.1N HCL	N/A
B	yes; 0.1N HCl, 0.165 mL; Sample volume of 5.5 mL	NA
C	Yes, two drops of 6N HCL to 10 mL sample	90% acetone to zero the spectrophotometer
D	No	Turner Designs Chlorophyll A Std. High and low
E	Yes, with 0.1N HCl. 6.0mL of each sample was acidified with 180 µL HCl.	No calibration std used. But a Turner Designs std is used to show proper technique through acidification. Std conc is 18.9 µg/L.
F	No	QCS Intermediate Standard, which will vary in concentration depending on the concentration of the stcok as received from the manufacturer
G	N/A	High Chlorophyll a Standard, Dilution B (free of chlorophyll b)– (purchased from Turner Designs).
J	0.1ml of 0.1 N HCl	n/a
K	No	Turner Designs Fluorometric Chlorophyll Standard
L	no	Chla from Anacystis Purchased from Sigma Chemical
N	no	Chl a from Anacystis nidulans from Sigma (C6144)
P	0.1 N HCl solution, 135 uL	Chlorophyll A Neat (algea, free of Chlorophyll b) from Sigma
R	no	Primary standard was pure chl-a extracted from Anacystis nidulans (Sigma) dissolved in HPLC grade 90% acetone. This standard was used to determine chl-a equivalent of Turner Designs solid secondary standard which is used to account for instrument drift (should be minimal with LED excitation source). Solid standard is run immediately before measurement of environmental chl-a extracts.
S	no	Chlorophyll a from Anacystis, Sigma C6144.
T	No	N/A
U	90 uL of 0.1 N HCL were added to the Spectrophotmeter Cuvette. Then 3 mls of sample was added. Cuvette was gentle turned 180° - 3 times to mix.	Sigma-Aldrich Chlorophyll a #C57853 -1mg from Spinach.
W	no	Chlorophyll a from Sigma Aldrich
Y	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 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 101% recovery.

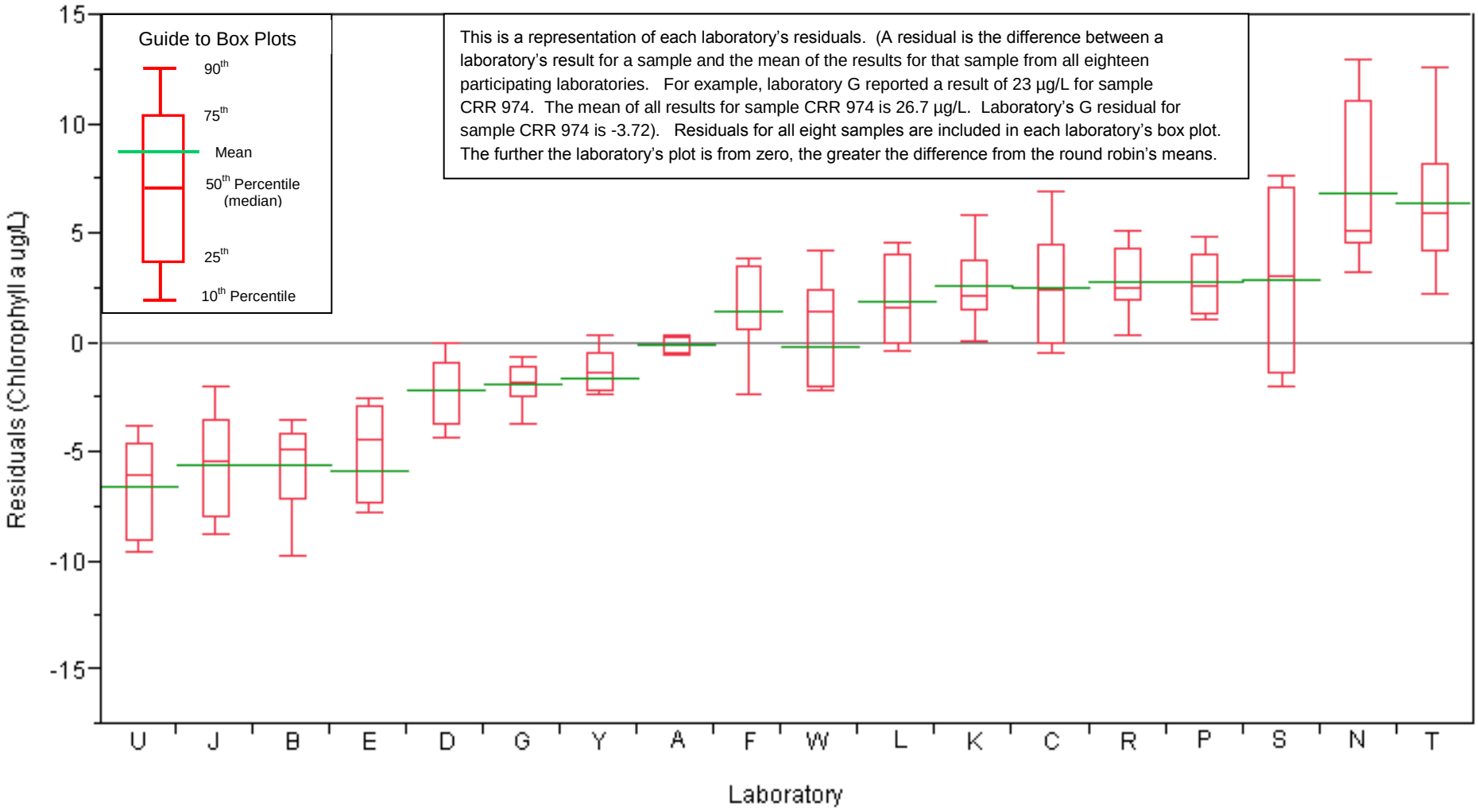
Additional information obtained from participating laboratories – time samples were filtered, type of filters used, filtering techniques, time samples were stored after filtering, make and model of instrument, instrument bandwidth(s), wavelength(s), time between acidification and analysis by instrument,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

June 2012 Chlorophyll *a* Round Robin Results

	Lake Wheeler		Jordan Lake - Robeson Creek		Harris Lake		Jordan Lake-Seaforth	
Laboratory ID	**CRR-974** *(µg/L)*	**CRR-117** *(µg/L)*	**CRR-233** *(µg/L)*	**CRR-579** *(µg/L)*	**CRR-258** *(µg/L)*	**CRR-443** *(µg/L)*	**CRR-163** *(µg/L)*	**CRR-888** *(µg/L)*
A	26.2	25.9	36.8	34.5	13.7	14	16.2	16
B	21.35	21.52	26.64	26.8	10.06	10.68	10.94	11.63
C	31.4	25.6	40.4	41.4	16.7	16.6	15.5	16.4
D	24.2	24.9	32.3	30.2	12.4	12.7	16	14
E	21.4	22	20.7	26.7	11.3	11.3	13.4	10.7
F	28.7	23.29	40.24	38	14.93	n/a	17.4	17.6
G	23	25	34	32	13	12	15	15
J	24.7	16.9	33.4	26.3	9.18	9.27	8.84	10.7
K	28.7	29.6	39.6	40.3	14.4	16.3	18.1	17.7
L	27	30	41	36	16	14	19	16
N	32.1	32.3	49.4	47	17.5	18.8	20.8	21
P	30.2	29.9	41.3	36.8	15.4	15.4	18.9	18.2
R	30.0	30.3	41.5	37.3	14.6	16.1	18.2	18.4
S	30	24	44	34	20	17	14	24
T	28.9	32.5	49	42.4	19.2	18.4	20.2	24.6
U	17.11	18.51	27.83	25.33	9.78	9.16	12.15	11.48
W	31.0	23.4	26.7	33.2	15.6	16.6	18.4	17.9
Y	25.0	26.0	31.0	33.0	14.0	13.0	15.0	14.0
Median	**27.9**	**25.3**	**38.2**	**34.3**	**14.5**	**14.0**	**16.1**	**16.2**
Mean	**26.7**	**25.7**	**36.4**	**34.5**	**14.3**	**14.2**	**16.0**	**16.4**

Note: Laboratory F had an error on CRR 443 and did not report a value. Data values are reported with laboratory's significant figures as sent.

2012 Chlorophyll *a* Round Robin Box Plots of Laboratory Residuals



Note: N=8 (except Lab F which had n=7)

2012 Chlorophyll *a* Round Robin Labortaory's Resdiual Mean vs. Standard Devation