2014 NC DWR Chlorophyll *a* Round Robin

Currently, 21 miles and 21,700 acres of surface waters in North Carolina are impaired due to elevated chlorophyll *a*, a chemical parameter used 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 Resources (NC DWR) 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 DWR 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 eighth chlorophyll *a* round robin on August 27, 2014. Seventeen laboratories participated, each analyzing eight samples. All eight samples were collected from Raleigh-area waterbodies (Figure 1).

Methodology

Sample Collection

On August 27, 2014, NC DWR staff collected a batch of eight surface water grab samples from four local waterbodies (Table 1). Samples were placed in light protected carboys and transported on ice to NC DWR's Water Sciences Section (WSS).

Table 1. Chlorophyll *a* Round Robin Sample Site Locations, 2014

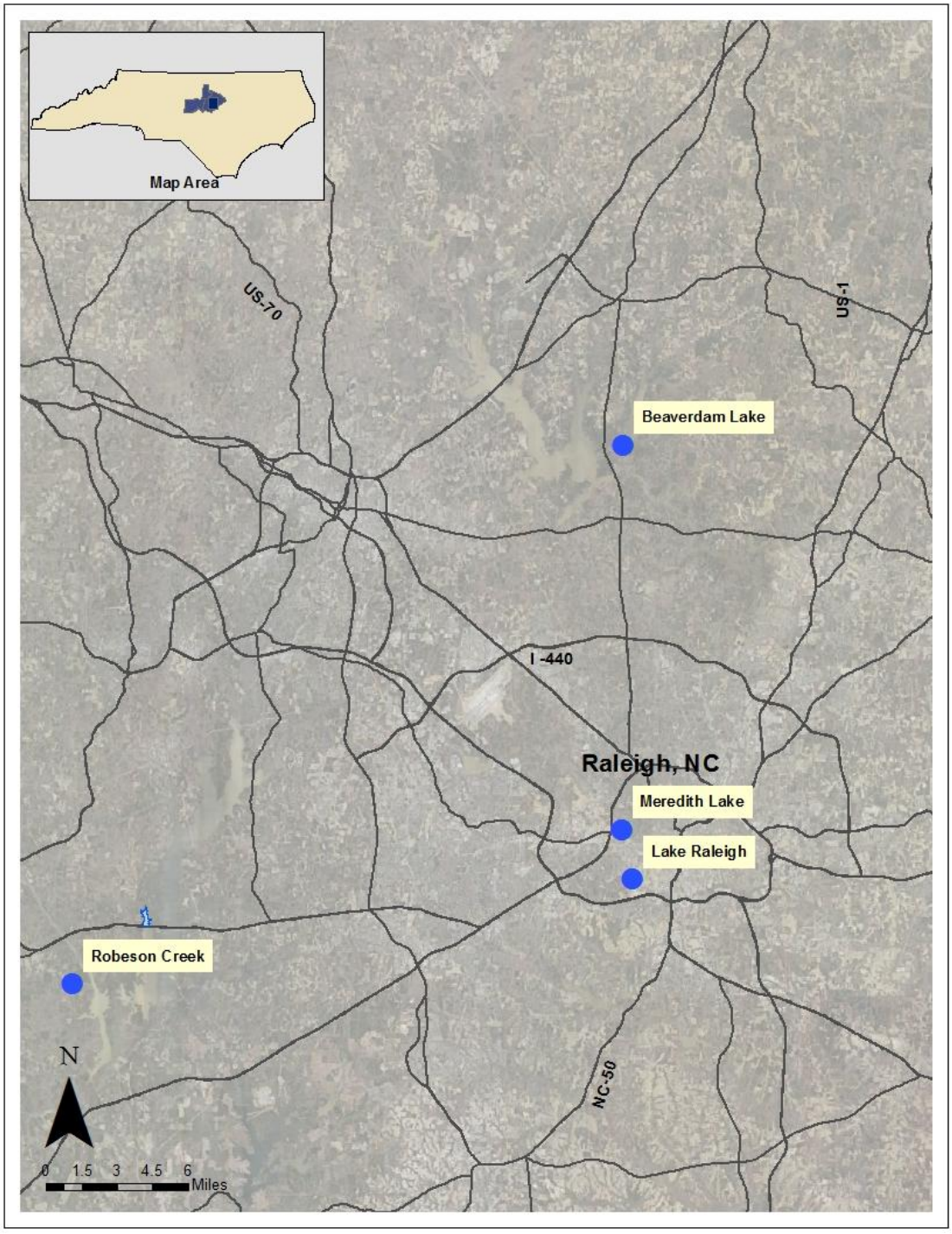
Waterbody	Samples	
Beaverdam Lake	CRR 204	CRR 280
Meredith Lake	CRR 275	CRR 341
Robeson Creek	CRR 382	CRR 434
Lake Raleigh	CRR 573	CRR 601

At WSS, each of the eight samples was split into seventeen 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 into amber HDPE bottles, then placed on ice and were either delivered to laboratories by NC DWR 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 11. Analyses of the data are presented graphically on pages 12 and 13. Final interpretation of the data is presented in Table 2 on page 14.

Figure 1.



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 order of the following list.

ALS Environmental – Jacksonville
ALS Environmental – Rochester
CMU – Environmental Laboratory Services
Environment 1, Inc.
Environmental Conservation Laboratories, Inc. – Orlando
Environmental Research Laboratory, Department of Biology, East Carolina University
EPA Region IV
Florida Department of Environmental Protection
Meritech, Inc.
NC Division of Water Resources Chemistry Laboratory
NCSU Center for Applied Aquatic Ecology
NOAA Center for Coastal Fisheries and Habitat Research
Raleigh, E. M. Johnson Water Plant
Research & Analytical Laboratories
UNC Institute of Marine Sciences
UNCW Center for Marine Sciences – Aquatic Ecology Lab
US Geological Survey National Water Quality Laboratory

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

Chlorophyll *a* Round Robin Analysis Details

Answers from Participants' Questionnaires

Lab ID	Method Used	Date Samples Received	Temperature Samples Received	Temperature Samples Stored Prior to Filtering	Date Samples Were Filtered
A	EPA Method 445.0 modified	08/27/14	2.4 C	4 deg C	08/27/14
D	EPA Method 445	27-Aug-14	buried in ice	on ice in 5.8°C refrigerator	28-Aug-14
E	Standard Methods 10200 H	8/27/2014	1.1°C	Not Stored - samples were filtered upon receipt	8/27/2014
H	EPA 445.0, Rev. 1.2	8/27/2014	7.4°C	2.8°C	8/27/2014
J	EPA 445.0, rev 2.1 modified option (in vitro)	8/27/2014	< 6.0 Celsius	23.0 Celsius	8/27/2014
K	EPA 445.0 Rev. 1.2	8/27/2014	0.4 deg. C	4.0 deg. C	8/27/2014
M	EPA Method 445.0	8/28/2014	0.1°C	4°C	8/28/2014
N	SM 10200 H - 2001	8/28/2014	2.0 C	Room Temperature	8/28/2014
P	SM10200H	8/28/2014	1.2 degrees C	0-6 degrees C	8/28/2014
Q	EPA 445.0	8/27/2014	Temp Blank 0.9°C	0.1-4.4°C	8/28/2014
R	SM 10200 H	8/27/2014	1.7C	<4C (45 mins)	8/27/2014
S	EPA 445.0	8/27/2014	3.9°C	4.0°C (8/28/14)	8/28/2014
T	SM10200H Spectrophotometer	8/28/2014	2.5 °C	Room Temperature	8/28/2014
U	SM 10200H	8/28/2014	3.4C	0-6 C	8/28/2014
V	fluorometric (non-acidification) Welshmeyer 1994	8/27/2014	4 degrees C	4°C	8/29/2014
X	EPA 445.0	8/27/2014	2.4 - 10.8 °C	3 °C	8/27/2014
Z	EPA 445.0	8/27/2014	on ice	on ice	8/27/2014

Lab ID	Type of Filters Used	Brand of Filters Used	Pressure at which Filtered	Volume of Sample Filtered	Homogenization Technique Prior to Filtering
A	25 mm GF/F glass fiber	Whatman	<= 5 in Hg	50 mL	briskly inverted bottle ~10 times
D	Whatman GF/F	Whatman	~7.0 in Hg	150ml-250ml	sample bottle inverted 3x
E	0.7 ul glass fiber filters	Whatman	6 in Hg	200 ml for all samples and duplicates	Samples bottles are inverted 30 times to mix before measuring each aliquot.
H	GF/C 42.5mm	Whatman	<5 in Hg	56 - 108 mL	Samples gently inverted 10 times
J	GF/F filters - glass fiber, 47 mm, nominal pore size of 0.7 µm.	Whatman	< -5.0 PSI	150 mL	lightly Shaked about 10 times
K	GF/F Glass Microfiber filters 47mm	Whatman	4.5 in. Hg	150 ml	shake vigorously several times
M	glass fiber	Whatman (GF/F)	≤6 mm Hg	50-100 mL	shaking bottles vigorously prior to each aliquot measured
N	Glass Microfiber 934-AH 47mm	Whatman	50 cmHg	500 ml	Samples are shaken well right before filtering
P	61631	Pall	N/A	235-470mL	Shaken
Q	47mm Glass Fiber GF/F	Whatman	< 6 in Hg	100 mL filtered for each sample	Sample bottle shaken by hand for 5-10 seconds
R	A/E Glass fiber 47mm	Millipore	4-6 inches of mercury	250 mls	Shaken
S	Glass Fiber	Whatman GF/F	-5kPa	50-100 mL	Samples inverted 4 times
T	GF/C	Whatman	Not measured	275 mL-475 mL	Sample bottle is vigorously shaken by hand before filtration.
U	47mm glass fiber	VWR	Not measured	100 mL	Shaking sample bottle
V	GF/F (glass fiber) 25mm circles	Whatman	5 inches Hg	50mL (attached on data sheet next tab)	Each sample was gently swirled for a few seconds before each replicate
X	GF-75, 47 mm	Advantec	< 3 in Hg	50 - 100 ml	Gently inverted the bottle several times
Z	Glass Fiber, Pore size 0.7 mm 25 mm diameter	Millipore	≤ 6 mm Hg	20 mL	Sample is mixed by gently shaking bottles for 10 seconds

Lab ID	Describe Filtering Technique (how were sample volumes measured, were sides rinsed, etc.)
A	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). Filters were folded and blotted dry with paper towel before storage.
D	sample volume was measured in a graduated cylinder. Sides of cylinders and filter apparatus were rinsed with deionized water. Samples were filtered to dryness.
E	Each aliquot is measured in a graduated cylinder then poured into the filtration apparatus. Graduated cylinder is rinsed twice with DI water and added to the filtration apparatus. Sides of filtration appratus are rinsed twice during the filtering of each sample.
H	After mixing, sample is poured into a graduated cylinder and volume is recorded. After pouring sample into filter funnel, the sides of graduated cylinder are rinsed twice and poured in funnel. The inside of the funnel is rinsed as the last step.
J	Samples measured using 250 Ml Plastic Cylinders
K	Volume measured with graduated cylinder. Grad. cylinder and filter funnel are rinsed.
M	measured in 100 mL glass graduated cylinder; poured into plastic filter tower; cylinder and filter towers sides both rinsed with DI
N	500 ml of the sample is measured in a class A volumetric cylinder and pored slowly into the filter funnel. The cylinder and the sides of the funnel are rinsed well.
P	Measured with 500mL measuring cylinder. Cylinder and filter flask rinsed three times with DI water.
Q	Measured in a TD graduated cylinder, sides not rinsed
R	measured with 250 ml graduated cylinder, vacuum filtered, cylinder and funnel rinsed between uses
S	50mL aliquots filtered in graduated cyliner. When filtration slows , final volume recorded and sides of cylinder rinsed 3x with DI water
T	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 $MgCO_3$ 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.
U	Sample volume measured in graduated cylinder. Poured onto filter. Grad cylinder rinsed and poured onto filter. After filtration, filter folded in half twice and wrapped in aluminum foil and placed in plastic bag with label prior to storage.
V	volumes measured with graduated cylinder, sides of filter funnels were rinsed with deionized water
X	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.
Z	Volume measured in a graduated cylinder, filter funnel sides not rinsed down

Lab ID	How long were samples filtered?	Lighting Conditions During Filtering	Extraction Solvent, Purity and Volume Used
A	1 min	lights turned off, blinds partially closed	90% HPLC grade acetone, ca. 10 mL extract volume (exact volume noted)
D	typically less than 1 minute/sample; some up to 3 minutes.	ambient outside light; blinds closed as well, lights off	90% acetone, 12ml
E	Each sample was flitered between 1 and 2 minutes	All lights are turned off. Laboratory door and blinds are closed. The only light entering the room filters through the door window and the sides of the blinds.	10 ml per sample aliquot of Ricca Brand 90% ACS Reagent grade acetone/ 10% ACS reagent grade water
H	5 - 10 sec	All overhead lights off, two small lamps with 25 watt green bulbs.	90% acetone, Fisher Scientific Certified ACS, 14mL
J	Between 1 to 9 min	In a dark room with green light on.	25 mL of 90 % acetone made from 99.4% pure Acetone .
K	1- 5 minutes	dark room with green lights	90% Acetone, Baker analyzed-ACS reagent grade, 25ml
M	about 30-60 seconds per sample	dimmed fluorescent (25% of full lab lighting)	90% HPLC-grade acetone, 25 mL
N	8 - 18 min	Room lighting	90% Acetone
P	2.75-3 minutes	Fluorescent lighting	90/10 Acetone/MgCO3. The acetone is chromatography grade and the MgCO3 is reagent grade and filtered through a 0.45um filter. 10mL of Acetone/MgCO3 solvent was used to extract the sample.
Q	3 minutes or less	Dark room with subdued green LED lighting	90% Acetone, Optima grade, 25 mL
R	<5min.	Darkroom with green light	90% acetone with 10% deionized water. Purity = 99.7% @10mls used
S	Up to 8 minutes	Dark Room with Red Lights	90% Acetone, Type 1 Water
T	All but one sample fell between 2 min 29 sec and 5 min 13 sec. Sample CRR 382 took 10 min 8 sec to filter.	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.
U	34-42 seconds	ambient lab lighting	acetone:DI (90:10)
V	3 to 7 minutes	sunlight through the windows, lab lights were turned off	90% Acetone : 10% water 7.5mL for each sample
X	1-2 minutes	Overhead fluorescent lights	90 % Acetone/ 10 % Water Solution,
Z	10-30 seconds	very low ambient light from windows	Acetone, 90%, 10 mL

Lab ID	Length of Time Samples were Stored after Filtering	Steeping Time	Was Grinding Used?
A	30 days	17 h	yes
D	18 days	22 hours	yes
E	Samples were immediately extracted after filtering.	21 hours	Yes
H	13 days	20 hrs	yes
J	about 24 hours	18 hours	Yes
K	7 days	18.5 hrs.	yes
M	approximately 2 hours	20 hours	yes
N	5 days	4hr 20min	yes
P	4.5 days	7hrs 10min	yes
Q	12 Days	19.75 Hours	Yes
R	Ground Immediately	Overnight 16:00 pm to 10:00 am	yes
S	14 Days	15.5 Hours	Yes
T	All but 1 sample were stored for 5 days 21 hrs 40 min. Sample CRR 204 was stored for 10 days 21 hrs 10 min.	18.5-9.0 hrs	yes
U	11 days	2+ hours	yes
V	7 days	24 hours	yes
X	11 days	Approx: 21 hrs	yes
Z	21 days	6 hrs	yes

Lab ID	Description of Grinding Setup
A	Kontes conical tip tissue grinder (PTFE pestle and glass mortar) coupled to Arrow Engineering electric stirrer, temperature was not controlled but grinding time was very short (ca. 15 s per sample)to prevent heating of the acetone/ filter slurry
D	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.
E	Grinder is a Lab Gen 125 by Cole Parmer with a stainless steel rod and blade. Vessels used are 50ml disposable polyproplene certrifuge tubes. These are used for both the griding and the steeping.
H	Pro Scientific stainless steel tip homogenizer, glass grinding vessel, temperature was not controlled
J	By using Tissue grinder, Teflon® pestle (50 mm X 20 mm) with grooves in the tip with ¼" stainless steel rod chuck onto a drive motor (counter based drill).
K	Teflon pestle with radial serrations on tip; temperature controlled by touch/feel
M	teflon grinding pestle attached to rotor; glass test tube; temperature controlled by feel
N	Samples are ground using a teflon tip in a glass test tube for 1 minute with 3 ml of 90% Acetone solution. Samples are then transferred into a 25 ml screw top centrifuge tube and an additional 7 ml of 90% Acetone solution is added. Analysis occurs at room temperature
P	Drill press with a teflon grinding tip. Not temperature controlled, ambient temp.
Q	Ground in a glass mortar. Pestle has round, serrated Teflon pestle. Unit powered by an electric drive motor. Temperature monitored by feel, sample was not allowed to heat.
R	Arrow 850 motor 1/10hp Kontes tissue grinder pestle SZ 24 and matching tube. No temperature control.
S	Tissue Grinder. Sample in plastic centrifguge tube. Temperature controlled to prevent evaporation
T	Filter is rolled up and placed in a 30 mL glass tube that is kept on ice between samples (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 grinding tube is rinsed with solvent until clean and added to the centrifuge tube. The grinder is rinsed with solvent a second time and added to the centrifuge tube. If slurry in centrifuge tube is less than 8 mL, the volume is brought up to 8 mL with solvent. If the slurry in the centrifuge tube is greater than 8 mL, solvent is used to bring the volume up to the nearest whole number. Samples are steeped in refrigerator.
U	Tissue Grinder with Teflon tip in glass vessel (wrapped in foil). Temperature not controlled. Slurry transferred to centrifuge tube.
V	samples were poured into 100mL graduated cylinder and then into filter manifold. The cylinder and manifold was rinsed with deionized water between each sample
X	drill with a Teflon® pestle with grooves, 50-mL polypropylene conical tubes
Z	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.

Lab ID	Samples Acidified? If so, Type, Concentration and Volume	Type of Calibration Standard and Source
A	No	Solid secondary standard (Turner Designs) used for daily calibrations. Solid standard concentration was determined (mean of 20 reads) after calibrating the fluorometer (last done 15 Aug 13) with dilutions of a primary standard made from chlorophyll a (Sigma; purified from Anacystis nidulans) dissolved in HPLC grade 90% acetone.
D	no	chlorophyll powder isolated from Anacystis nidulans dissolved in 90% acetone and spectrophotometrically analyzed using Jeffrey Method (1997) to determine concentration; purchased from Turner Designs
E	Yes, samples were acidified with 30ul of 0.1 N HCL per 1 ml of sample	N/A
H	No	Chl a from Anacystis nidulans, Sigma C6144
J	N/A	Five points direct LDR calibration standards curve with a blank. Two sources are used for the standards curve: sigma Aldridge and curve verifications: Turner design.
K	No	Fluorometric Chlorophyll standard, Turner Designs
M	no	chlorophyll *a* from Anacystis (Sigma C6144)
N	0.1ml of 0.1 N HCL	N/A
P	0.1mL of 0.1N HCL	N/A
Q	No	Turner Designs Fluorometric Chlorophyll Standard
R	No	
S	No	Turner Designs Chlorophyll A and B Standard
T	Samples are acidified with 100 uL of 0.1 N 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 the 1st batch, the standard read at 107% recovery. For the 2nd batch, the standard read at 101% recovery.
U	0.06 mL 0.1N HCl into a 2 mL extract. Measured with and without acidification.	Initial Calibration: Turner Designed foil wrapped sealed ampules at nominal concentrations of 20 and 200 ug/L, diluted as needed for a range of 4-200 ug/L. Daily cal check: Solid Secondary Standard Turner P/N 8000-952
V	no	chla pigment standard (sigma aldrich)
X	0.1 N HCl solution, 135 uL to 4.5 ml of sample	Chlorophyll *a* free of chlorophyll *b* Neat, Sigma
Z	no	Liquid Standard made with purified Chla from Anacystis, Sigma Aldrich C6144-1mg

Notes:

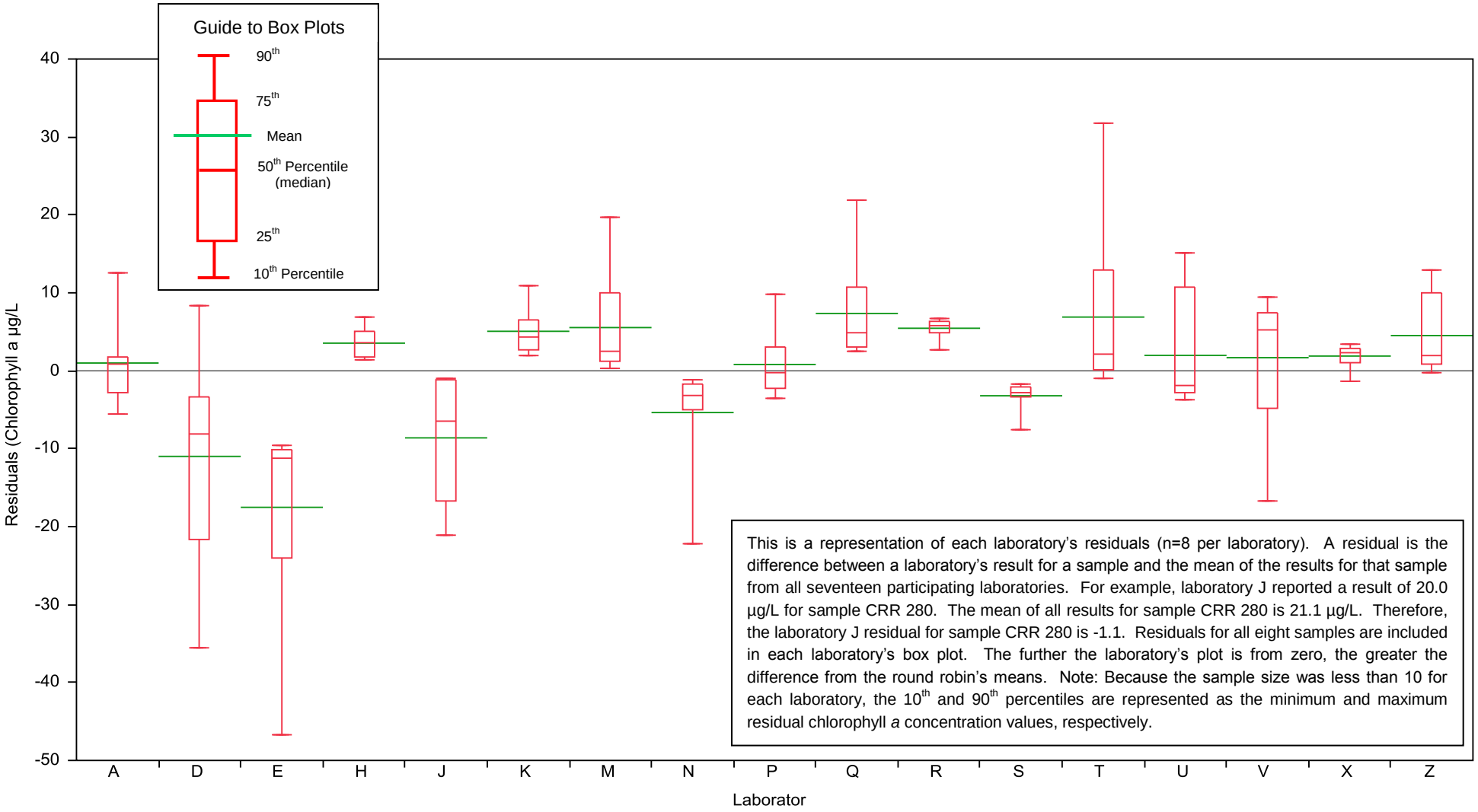
1. Answers to the questionnaire are entered as the laboratory presented them unmodified, except for spacing.
2. Additional information obtained from participating laboratories: time samples were filtered, make and model of instrument, instrument bandwidth(s) and wavelength(s), time between acidification and analysis by instrument, and notable differences between samples.

August 2014 Chlorophyll *a* Round Robin Results

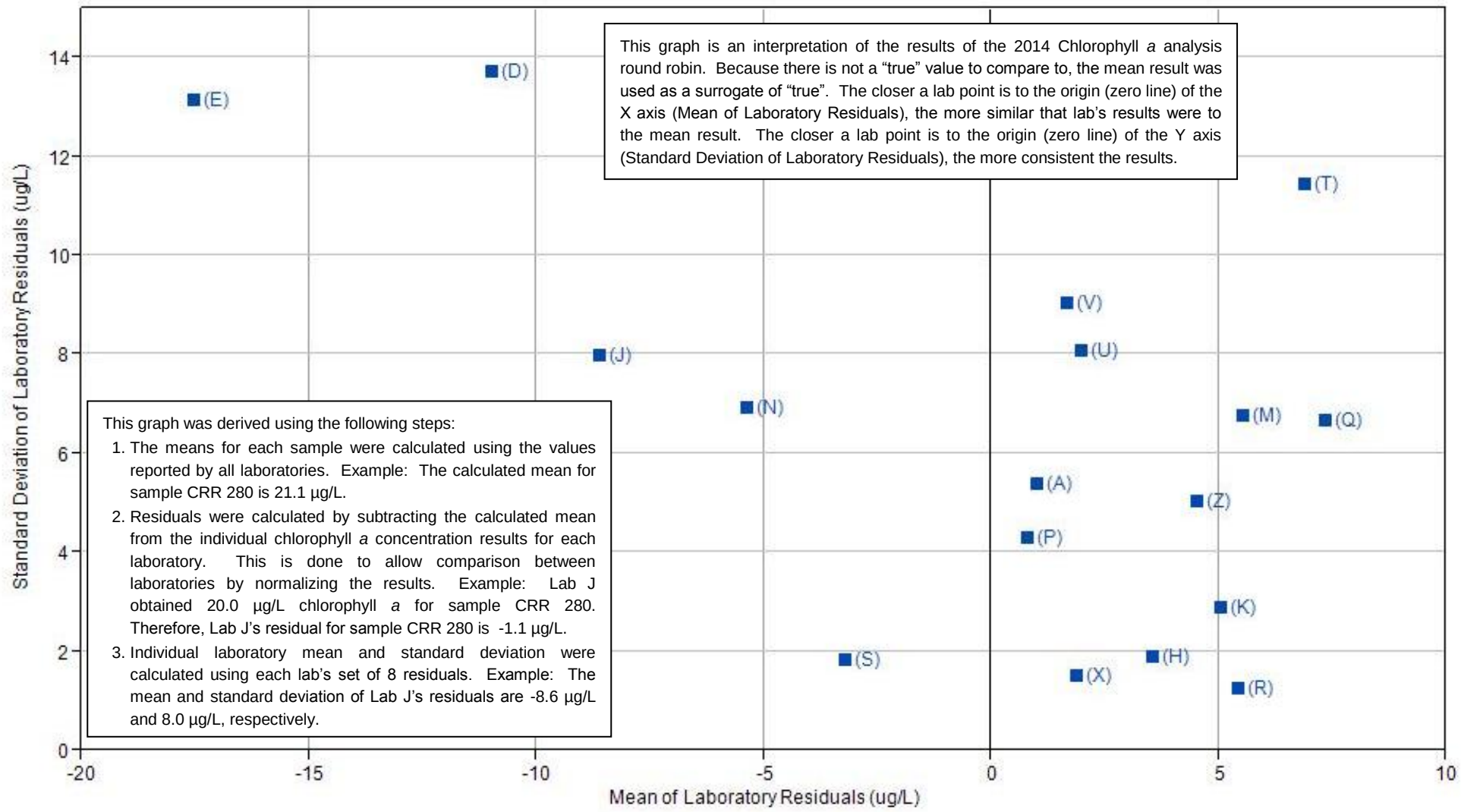
	Beaverdam		Meredith		Robeson Creek		Lake Raleigh	
Lab ID	CRR 204	CRR 280	CRR 275	CRR 341	CRR 382	CRR 434	CRR 573	CRR 601
A	22.24	21.42	96.49	97.77	30.59	35.44	18.69	18.19
D	17.88	16.68	59.38	62.63	16.79	21.12	30.61	19.88
E	11.47	10.95	56.53	51.50	17.78	20.03	12.20	12.23
H	22.7	22.6	89.2	105	32.4	38.1	24.0	27.2
J	20	20	66	77	15	33	16	17
K	23.35	25.63	87.96	109.01	35.32	40.62	24.20	27.28
M	21.3	22.4	95.4	117.8	31.7	39.6	23.5	25.5
N	19	20	80	76	26	29	18	22
P	18.1	20.5	87.9	108	25.2	34.0	22.0	23.6
Q	24.7	24.3	96.0	120	34.7	40.7	24.7	26.6
R	27.7	26.4	90.4	103	34.6	40.1	25.0	29.3
S	18.8	19.1	76.3	95.3	26.0	30.9	20.5	20.3
T	20	21	100	130	31	36	23	27
U	18.5	19.4	99.0	113	27.2	30.5	19.4	21.7
V	24.877	23.934	67.141	90.839	36.085	40.764	29.519	33.141
X	22.93	21.79	82.56	100.89	32.10	36.97	24.36	26.36
Z	23	23	95	111	35	34	23	25
Median	21.3	21.4	88.0	103.0	31.0	35.4	23.0	25.0
Mean	21.0	21.1	83.8	98.2	28.7	34.2	22.3	23.7

Note: Data values are shown with significant figures as reported by laboratories.

2014 Chlorophyll *a* Round Robin Box Plots of Laboratory Residuals



2014 Chlorophyll *a* Round Robin Laboratory Residual Mean vs. Standard Devation



DATA INTERPRETATION

Values reported by labs participating in the Round Robin, as well as the mean, median, and standard deviation (Std Dev) for each sample, are displayed in Table 2 below. Acceptance ranges (PT Min to PT Max) were calculated using NELAC Proficiency Testing (PT) methods[1] for microbiological parameters in non-potable water. One lab result (Lab E, Sampling Site CRR280) was outside of the expected "proficiency testing" range of natural log-transformed data ± 3 standard deviations.

Table 2. Chlorophyll *a* Round Robin 2014 Data Interpretation

Lab ID	CRR 204	CRR 280	CRR 275	CRR 341	CRR 382	CRR 434	CRR 573	CRR 601
A	22.24	21.42	96.49	97.77	30.59	35.44	18.69	18.19
D	17.88	16.68	59.38	62.63	16.79	21.12	30.61	19.88
E	11.47	10.95	56.53	51.50	17.78	20.03	12.20	12.23
H	22.7	22.6	89.2	105	32.4	38.1	24.0	27.2
J	20	20	66	77	15	33	16	17
K	23.35	25.63	87.96	109.01	35.32	40.62	24.20	27.28
M	21.3	22.4	95.4	117.8	31.7	39.6	23.5	25.5
N	19	20	80	76	26	29	18	22
P	18.1	20.5	87.9	108	25.2	34.0	22.0	23.6
Q	24.7	24.3	96.0	120	34.7	40.7	24.7	26.6
R	27.7	26.4	90.4	103	34.6	40.1	25.0	29.3
S	18.8	19.1	76.3	95.3	26.0	30.9	20.5	20.3
T	20	21	100	130	31	36	23	27
U	18.5	19.4	99.0	113	27.2	30.5	19.4	21.7
V	24.877	23.934	67.141	90.839	36.085	40.764	29.519	33.141
X	22.93	21.79	82.56	100.89	32.10	36.97	24.36	26.36
Z	23	23	95	111	35	34	23	25
Median	21.3	21.4	88.0	103.0	31.0	35.4	23.0	25.0
Mean	21.0	21.1	83.8	98.2	28.7	34.2	22.3	23.7
Std Dev	3.7	3.6	14.1	20.9	6.8	6.3	4.6	5.1
PT Min	11.4	11.4	47.8	46.0	12.2	17.7	11.2	11.2
PT Max	37.2	37.9	142.7	199.0	63.4	63.4	42.5	47.4

Note: Data values are shown with significant figures as reported by laboratories.

[1] EPA/600/R-04/003, table available at http://nelac-institute.org/fopt.php, full document available at http://nelac-institute.org/docs/2003nelacstandard.pdf