June 2013 NC DWR Chlorophyll *a* Round Robin

Currently, 21 miles and 21,700 acres of surface waters in North Carolina are impaired due to chlorophyll *a*, a parameter used to assess algal productivity resulting from nutrient over enrichment (2012 Final 303(d) List). Reducing nutrient loading can result in significant costs savings to both the state and the stakeholders in the affected watersheds. It is important that the North Carolina Division of Water Resources (NC DWR) understands the quality of the data used to make management decisions.

There are currently no performance evaluation samples to test the entire chlorophyll *a* analysis method; therefore, 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 laboratories 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 seventh chlorophyll *a* round robin, which was performed July 16, 2013. Sixteen laboratories participated, each analyzing eight samples. All eight samples were collected from Raleigh area waterbodies. This compilation is being provided to the Laboratory Certification Program for their interpretation and use.

Methodology

Sample Collection

On July 16, 2013, NC DWR 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 DWR's Environmental Sciences Section (ESS).

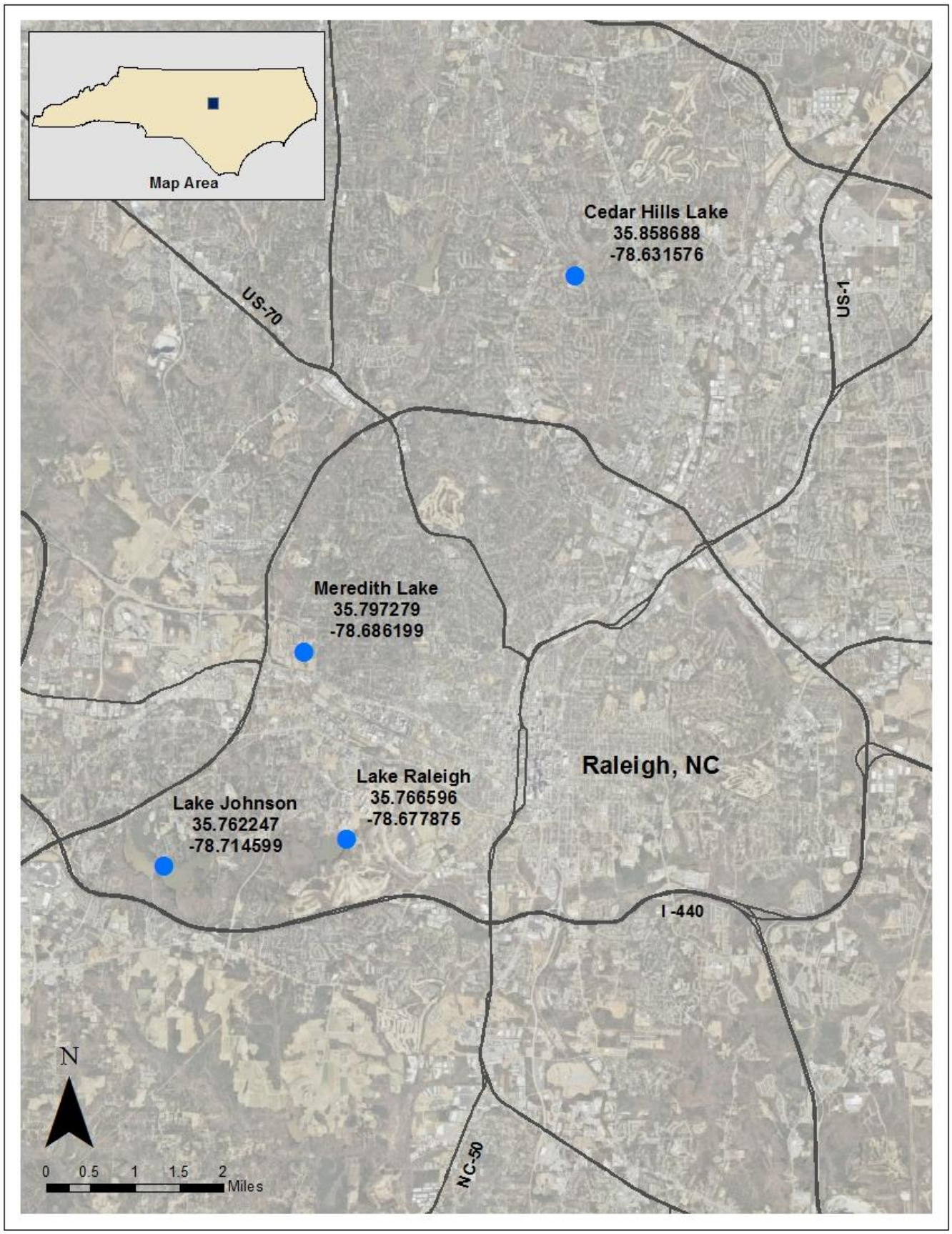
At ESS, each of the eight samples were split into sixteen 500 mL subsamples using a churn splitter. Every sample was churned for two minutes prior to splitting and was continually churned during the split. The splitter faucet was purged prior to sample collection. The order in which the subsamples were 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 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 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 -16.

Samples CRR-449 and CRR-949 had chlorophyll *a* concentrations that were well above the rest of the samples in the study and well above the class C surface water quality standard of 40 µg/L. The standard deviation of lab results for these two samples were also well above those for the rest of the samples. It appeared that the apparent outliers in the graph on page 12 may have been a reflection of the higher concentrations of those two samples. Graphical representations of the data analysis of just these two high concentration samples for all labs show the wide variance they contribute to the overall interpretation of the data (see pages 13 -14). When data from all samples excluding the higher concentration samples (CRR-449 and CRR-949) are graphed, the grouping of labs is quite good, with all labs performing with less than 5 µg/L standard deviation (see pages 15-16).

Sample Site Locations



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

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	Chlorophyll A SM18 10200H	7/17/13	N/A	2 °C	5 days
B	EPA 445.0 Fluorometer (Maceration)	7/16/13	0.6 °C	4.4 °C	6 days
C	Standard Methods 18th ed. Spectophotometric determination	7/16/13	2.6 °C	3.1 °C	40 hours
D	SM10200H Spectrophotometer	7/17/13	Room Temperature	2.7 ˚C	5 days 16 hrs 55 min
E	fluorometric (non-acidification) Welshmeyer 1994	7/16/13	4 °C	4.1 °C	7 days
F	Spectrophotometric Standard Method 10200H 1.(extraction); 10200H 2.(analysis)	7/16/13	filtered immediately after receiving	on ice	28 days
G	Standard Methods 10200 H.	7/16/13	3 °C	4.6 °C	17 hours
H	EPA 445.0	7/16/13	0.1 - 4.4 °C	Temp Blank 1.7 °C	7 Days
J	EPA 445.0	7/16/13	On ice	On ice	9 days
K	EPA Method 445 Uncorrected	7/16/13	20 - 22 °C	14.7 °C	approx. 2 days
N	EPA Method 445.0 modified	7/16/13	4 °C	not measured (samples on ice, not frozen; assume ca. 4 °C)	30 days
O	EPA 445.0, Rev. 1.2 (Fluorometric) (Aqueous)	7/16/13	3.8 °C	1.4 °C	16 days
P	EPA 445.0 Rev. 1.2	7/16/13	5.0 °C	3.6 °C	21.5 hrs
R	SM10200H	7/17/13	23 °C	1.8 °C	20 hrs
S	EPA 445.0	7/16/13	<5 °C	<5 °C	20 days
T	EPA Method 445.0	7/17/13	4 °C	0.3 °C	12 days

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	Samples are shaken well right before filtering.	7/17/13	N/A	500	6 - 14 min
B	Gently shook the bottle before dispensing the water into graduated cylinder	7/16/13	< 3 in Hg	100 mL	1 minute
C	Shaken	7/16/13	4-6 inches of mercury	100-250 mls	<5 min.
D	Sample bottle is vigorously shaken by hand before filtration.	7/17/13	Not measured	125 - 500	55 sec - 5 min 55 sec
E	Gently swirl before filtration	7/17/13	3 inches of mercury	25 mL (949 and 449); 50 mL for all other samples	3 to 7 minutes
F	Sample bottle inverted 3x	7/16/13	~7.0 in Hg	0.065-0.25 L	Typically less than 1 minute/sample; some up to 3 minutes.
G	Sample bottles are inverted 20 times to mix before measuring first aliquot. Sample bottle is inverted 10 times before measuring duplicate aliquot.	7/16/13	6 in. Hg	200 mL filtered for samples and duplicates except CCR 449 G & CCR 949 G: 50 mL filtered incl. dups.	Samples are filtered between 2 and 3 minutes.
H	Sample bottle shaken by hand for 5-10 seconds	7/17/13	< 6 in Hg	100 mL	2 minutes or less
J	Inverting bottles for 10 seconds	7/18/13	≤ 6 mm Hg	25-50 mL	10-30 seconds
K	Gently agitated by inverting sample several times	7/16/13	Vacuum filtration < 6 in. Hg (20kPa) (< 5 PSI).	150	25 min.
N	Briskly inverted bottle ~10 times	7/16/13	<= 5 in Hg	20-50 mL	10-20 min
O	Samples gently inverted 10 times	7/16/13	<5 in Hg	38 - 150 mL	Estimate 5-10 seconds/sample
P	Gently inverting sample bottle several times	7/16/13	5 in. of Hg	80 - 150ml	1 - 5 minutes
R	Shake for 30 seconds	7/17/13	Not Measured	125-500mL	1-4 min
S	Samples inverted 4 times	7/17/13	<20kPa	50-200mL	Up to 8 minutes
T	Shaking bottles vigorously prior to each aliquot measured	7/17/13	≤6 mm Hg	50-200 mL	About 30-90 seconds per sample

Laboratory ID	Type of Filters Used	Brand of Filters	Describe Filtering Technique (how were sample volumes measured, were sides rinsed)
A	Glass Microfiber 934-AH 47mm	Whatman	A volume of the sample is measured in a Class A volumetric cylinder and poured slowly into the filter funnel. The cylinder and the sides of the funnel are rinsed well.
B	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.
C	A/E Glass fiber 47mm	Millipore	Measured with 250 ml graduated cylinder, vacuum filtered, cylinder and funnel rinsed between uses
D	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.
E	GF/F (glass fiber) 25mm circles	Whatman	Volumes measured with graduated cylinder, sides of filter funnels were rinsed with deionized water
F	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.
G	0.7 um Glass Fiber Filters	Whatman	Each sample aliquot is measured in a graduated cylinder then poured into filtration apparatus. Graduated cylinder is rinsed twice with deionized water and added to filtration apparatus. Sides of filtration apparatus are rinsed twice with filtering of each sample.
H	47 mm Glass fiber GF/F	Whatman	Measured in a TD graduated cylinder; sides not rinsed
J	Glass Fiber, Pore size 0.7 mm 25 mm diameter	Millipore	Volume measured in a graduated cylinder, filter funnel sides not rinsed down
K	GF/F glass fiber, 47 mm, nominal pore size of 0.7 µm.	Whatman	Using 250 mL class B clean graduated cylinders
N	25 mm GF/F glass fiber	Whatman	Duplicate aliquots of 20-50 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.
O	GF/C 42.5mm	Whatman	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/F Glass Microfiber filters 47mm	Whatman	Measured with graduated cylinder. Graduated cylinder and funnels are rinsed.
R	Glass Fiber	VWR Glass Microfiber Filter, 696, 4.7mm	Measured with 500 mL measuring cylinder. Cylinder and filter flask rinsed three times with DI water.
S	Glass Fiber	Whatman GF/F	50 mL aliquots filtered in graduated cylinder. When filtration slows, final volume recorded and sides of cylinder rinsed 3x with DI water
T	glass fiber	Whatman (GF/F)	Measured in 100 mL glass graduated cylinder; poured into plastic filter tower; cylinder and filter towers sides both rinsed with DI

Laboratory ID	Light Conditions During Filtering	Extraction Solvent and Volume Used	Steeping Time	Was Grinding Used?
A	Under the hood with the hood lights off.	90% Acetone	3 hours	Yes
B	Overhead fluorescent lights	90/10 Acetone Water solution Acetone Honeywell B&J GC Water OmniSolv 25 mL	Approx 22 hours	Yes
C	Darkroom with green light	90% acetone with 10% deionized water. Purity = 99.7% @10mls used	23 hours	Yes
D	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 9 mL.	2 hrs 30 min	Yes
E	Sunlight through the windows, lab lights were turned off	90% Acetone : 10% water 7.5mL for each sample	24 hours	Yes
F	Ambient outside light; blinds closed with white posterboard covering windows as well, lights off	90% Acetone, 12 mL	2 hours in cold room (14.5°C) followed by 20 mins centrifuge (4°C at 2800 rpm); 2 hours 20 mins total	Yes
G	Overhead lights are turned off. Laboratory door is shut. Shades on window are turned down. The only light filters through the door window from the adjacent laboratory.	10ml per sample aliquot of Fisher Brand, 90% ACS reagent grade Acetone/10% ACS reagent grade water.	23 hours	Yes
H	Dark room with subdued LED green light	90% Acetone Optima 25 ml	18 hours	Yes
J	Very low ambient light from windows	Acetone, 90%, 10 mL	3 hrs	Yes
K	Dark Room with three 60-Watt green light bulbs was on.	90% Acetone, 25 mL	16:00	Yes
N	Lights turned off, blinds partially closed	90% HPLC grade acetone, ca. 10 mL extract volume (exact volume noted)	17 h	Yes
O	All overhead lights off, two small lamps with 25 watt green bulbs	90% acetone, Fisher Scientific Certified ACS, 14mL	19 hours	Yes
P	Dark room with green lights	Acetone ACS electronic grade; 90% v/v in DI water	17.5 hrs	Yes
R	Fluorescent Light	90/10 Acetone/MgCO3. Acetone is chromatography grade, MgCO3 is reagent grade. 10mL of Acetone/ MgCO3 solvent used to extract sample.	63 hrs	Yes
S	Dark Room with Red Lights	90% Acetone, Type 1 Water	21 Hours	Yes
T	Dimmed fluorescent (25% of full lab lighting)	90% acetone, 25 mL	20 hours	Yes

Laboratory ID	Description of Grinding Setup
A	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 25ml screw top centrifuge tube and an additional 7ml of 90% Acetone solution is added. Analysis occurs at room temperature.
B	Teflon® pestle with grooves in the tip attached to drill, 50-mL polypropylene conical tubes
C	Arrow 850 motor 1/10hp Kontes tissue grinder pestle 5224 and matching tube. No temperature control.
D	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 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.
E	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 (30 seconds). Temperature was not controlled.
F	Teflon (PTFE) tissue grinder with radial serrations on tip, powered by electric drill. Temperature not controlled - samples were removed from -20°C freezer, ground for approximately 30 seconds, and placed in dark box with ice packs.
G	Grinder: Lab Gen 125 by Cole Parmer with stainless steel rod and blade. Vessel: 50ml disposable polypropylene centrifuge tubes are used for both grinding and steeping.
H	Ground in a glass mortar. Pestle has round, serrated Teflon pestle. Unit powered by electric drive motor. Temperature monitored by feel. Sample not allowed to heat.
J	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 significantly as felt when holding the tube.
K	Tissue grinder, Teflon® pestle (50 mm X 20 mm) with grooves in the tip with ¼" stainless steel rod and yes temperature was controlled.
N	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
O	Stainless steel tip homogenizer, temperature was not controlled
P	Teflon pestle with radial serrations on tip; temperature controlled by touch
R	Drill press with a Teflon grinding tip. Not temperature controlled.
S	Tissue Grinder. Sample in plastic centrifuge tube. Temperature controlled to prevent evaporation.
T	Teflon grinding pestle attached to rotor; glass test tube; temperature controlled by feel

Laboratory ID	Samples Acidified? If so, Type, Concentration, and Volume	Type of Calibration Standard and Source
A	0.1 mL of 0.1 N HCl	NA
B	0.1 N HCl solution, 135 µL	Chl *a* Neat from Sigma
C	No	
D	Samples are acidified with 100 µL 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 this batch the standard read at 104% recovery.
E	No	Chl *a* pigment standard (Sigma Aldrich)
F	Yes, two drops of 6N HCl to 10 mL sample	90% acetone to zero the spectrophotometer
G	Yes. Acidified with 30 µL of 0.1 N HCl per 1 ml of sample.	NA
H	No	Turner Designs Fluorometric Chlorophyll Standard
J	No	Liquid Standard made with purified Chl *a* from Anacystis, Sigma Aldrich C6144-1mg
K	N/A	FIVE POINT CAL USED AS FOLLOW: 5.0, 10.0, 50, 100 AND 250 µg/L, source Chlorophyll *a* from Anacystis nidulans algae.
N	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.
O	No	Chl *a* from Anacystis nidulans, Sigma C6144
P	No	QCS intermediate Standard which will vary in concentration depending on the concentration of the stock received from the manufacturer.
R	0.1 mL of 0.1N HCl	N/A
S	No	Turner Designs Chlorophyll *a* and *b* Standard
T	No	Chlorophyll *a* from Anacystis (Sigma C6144)

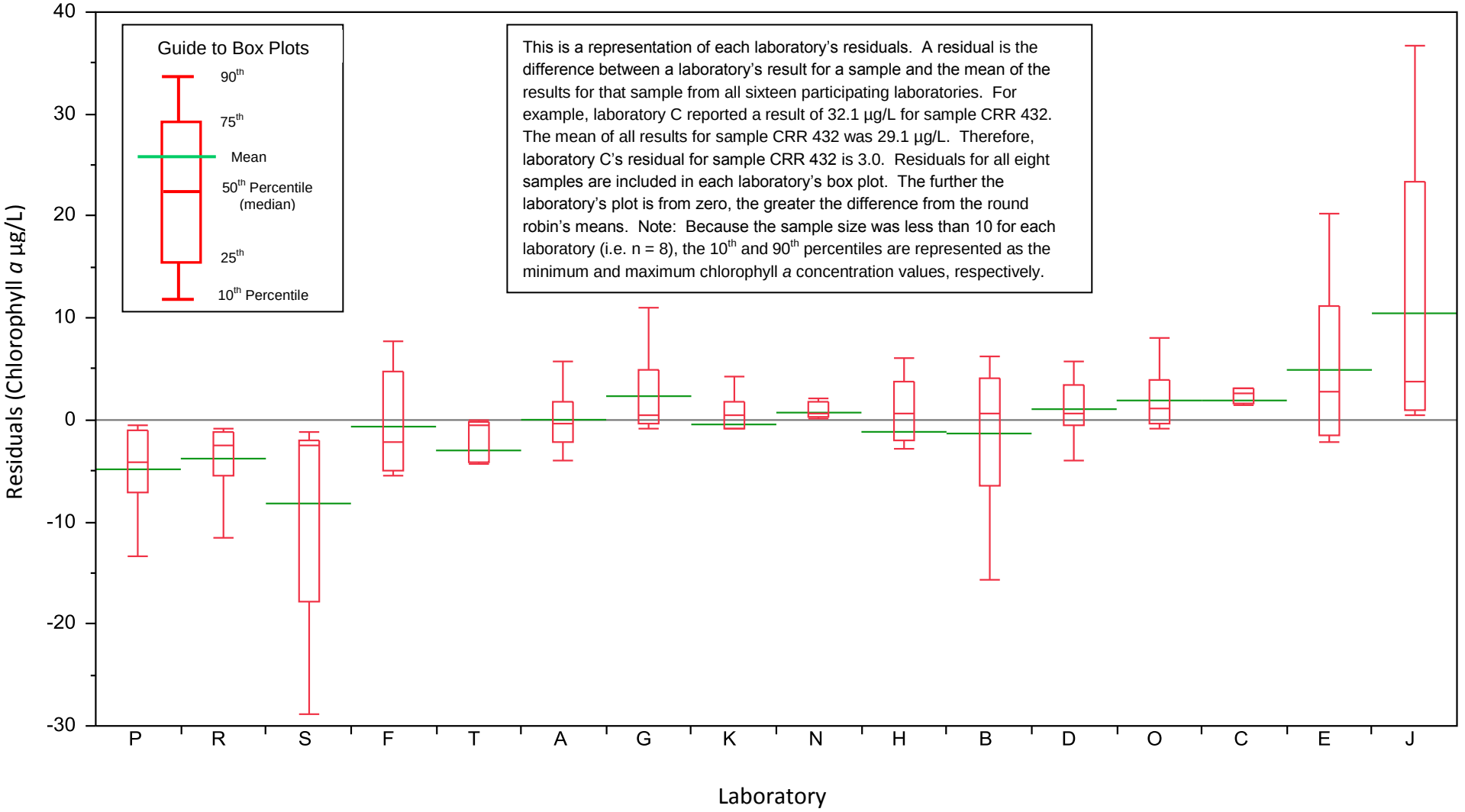
Additional information obtained from participating laboratories: time samples were filtered, filtering techniques, time samples were stored after filtering, make and model of instrument, instrument bandwidth(s), wavelength(s), time between acidification and analysis by instrument, notable differences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

July 2013 Chlorophyll *a* Round Robin Results

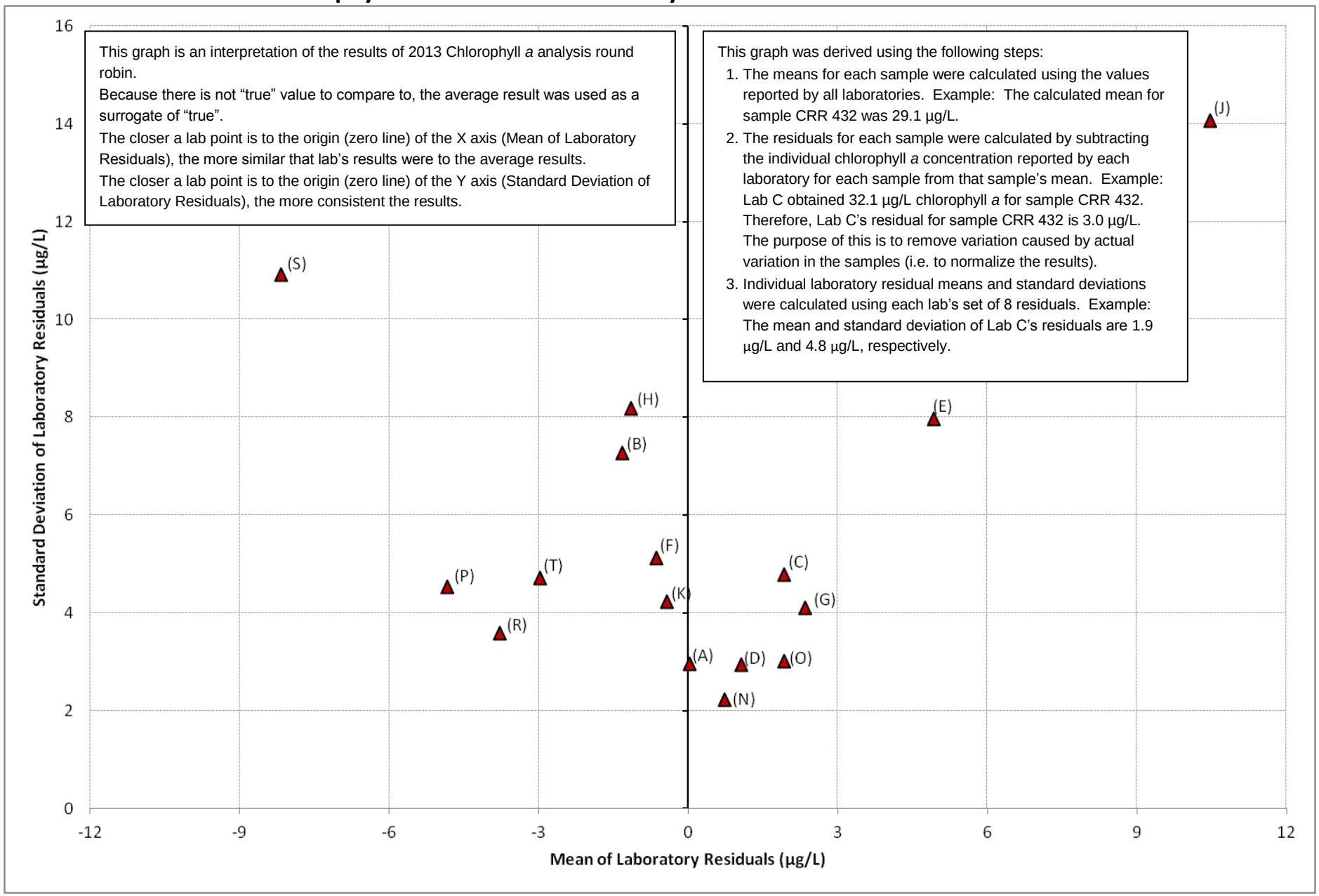
	Lake Johnson		Meredith Lake		Cedar Hills Lake		Lake Raleigh	
Laboratory ID	**CRR-422** *(µg/L)*	**CRR-642** *(µg/L)*	**CRR-220** *(µg/L)*	**CRR-432** *(µg/L)*	**CRR-449** *(µg/L)*	**CRR-949** *(µg/L)*	**CRR-590** *(µg/L)*	**CRR-735** *(µg/L)*
A	10.0	7.5	29.0	29.0	130	170	12.0	16.0
B	11.5	9.9	35.0	34.3	118	156	13.9	14.1
C	12.0	12.7	30.5	32.1	143	156	16.2	16.1
D	9.8	10.0	31.0	33.0	130	170	14.0	14.0
E	13.1	12.9	26.6	26.9	154	178	16.4	14.5
F	8.9	9.8	23.7	24.7	142	171	7.7	10.9
G	10.9	9.9	31.1	29.7	140	175	12.8	12.7
H	12.0	10.6	34.8	33.7	131	144	13.8	14.1
J	11.0	11.0	35.0	38.0	162	201	14.0	15.0
K	9.7	9.1	33.0	19.0	136	165	14.0	14.0
N	10.9	10.8	33.4	31.2	134	161	14.0	13.8
O	11.5	11.1	29.2	28.5	142	169	14.5	12.8
P	9.9	9.1	21.7	21.9	127	151	11.7	12.3
R	8.9	9.0	17.2	26.4	128	160	10.7	12.8
S	8.4	7.7	24.7	27.9	105	142	11.1	11.1
T	9.9	9.5	25.0	29.0	120	160	13.0	13.0
Median	**10.4**	**9.9**	**29.9**	**29.0**	**132.7**	**162.9**	**13.9**	**13.9**
Mean	**10.5**	**10.0**	**28.8**	**29.1**	**133.9**	**164.3**	**13.1**	**13.6**
Std Dev	**1.30**	**1.50**	**5.24**	**4.78**	**13.77**	**14.29**	**2.15**	**1.49**

Note: Data values are reported with laboratory's significant figures as sent.

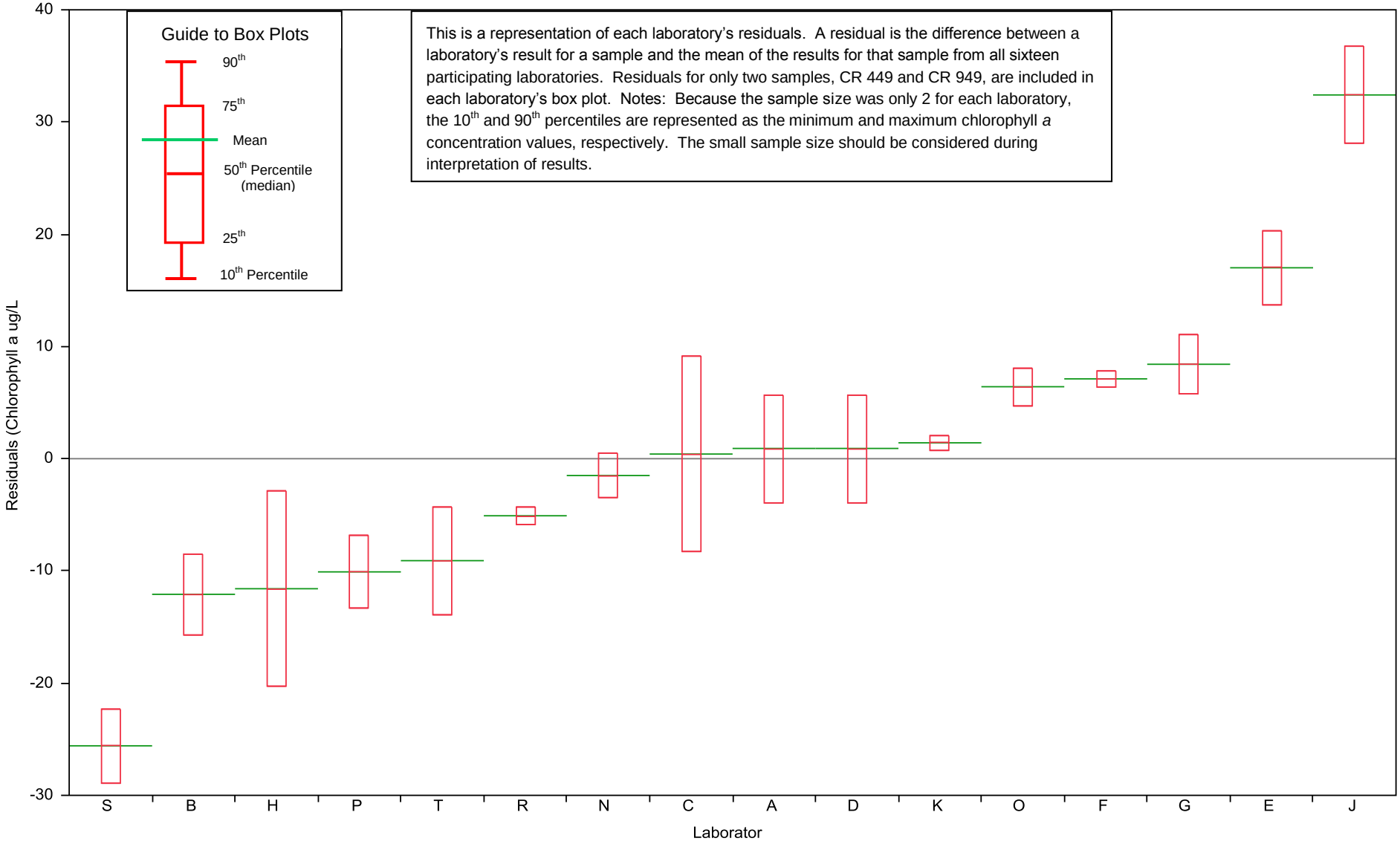
2013 Chlorophyll *a* Round Robin Box Plots of Laboratory Residuals



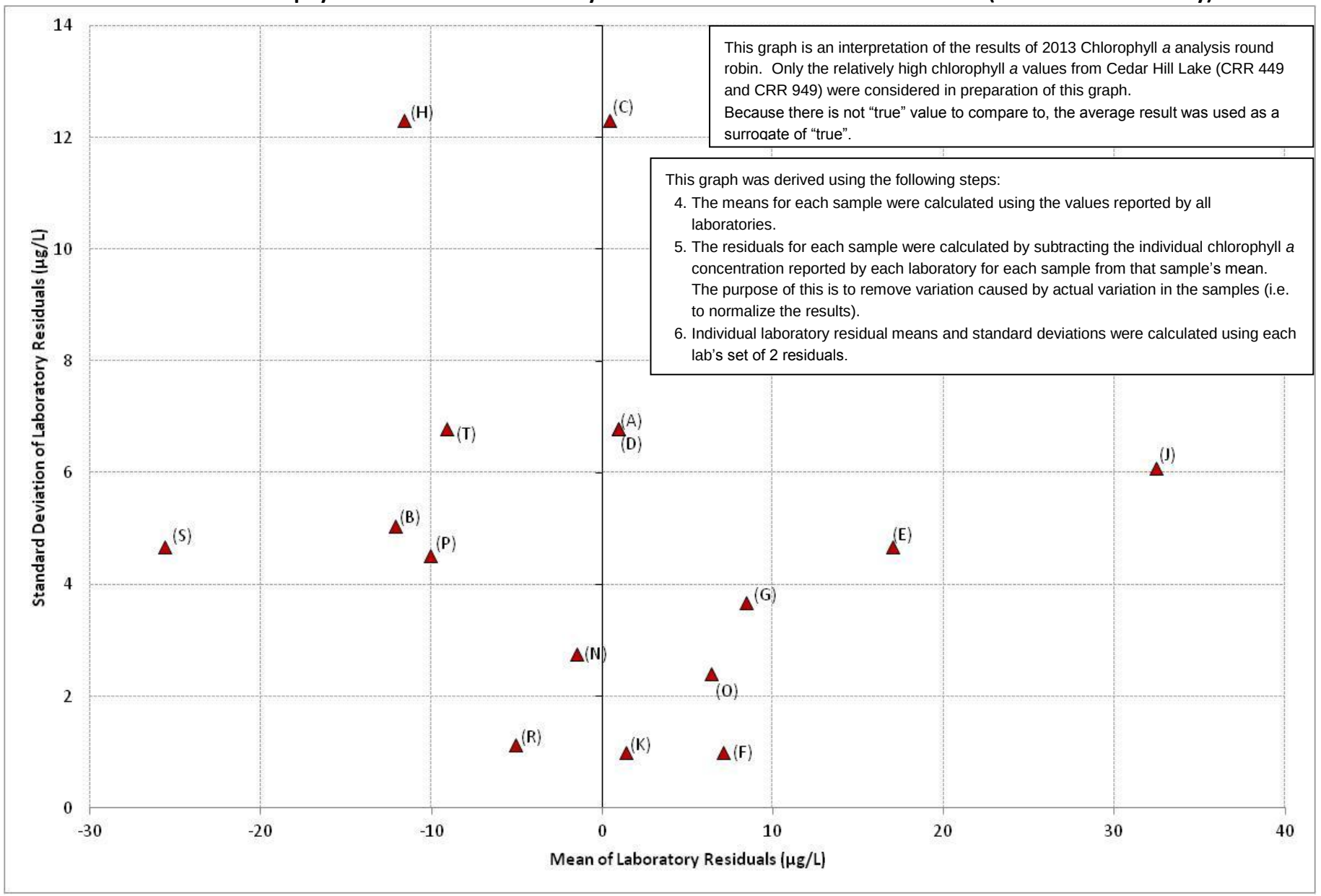
2013 Chlorophyll *a* Round Robin Laboratory's Residual Mean vs. Standard Deviation



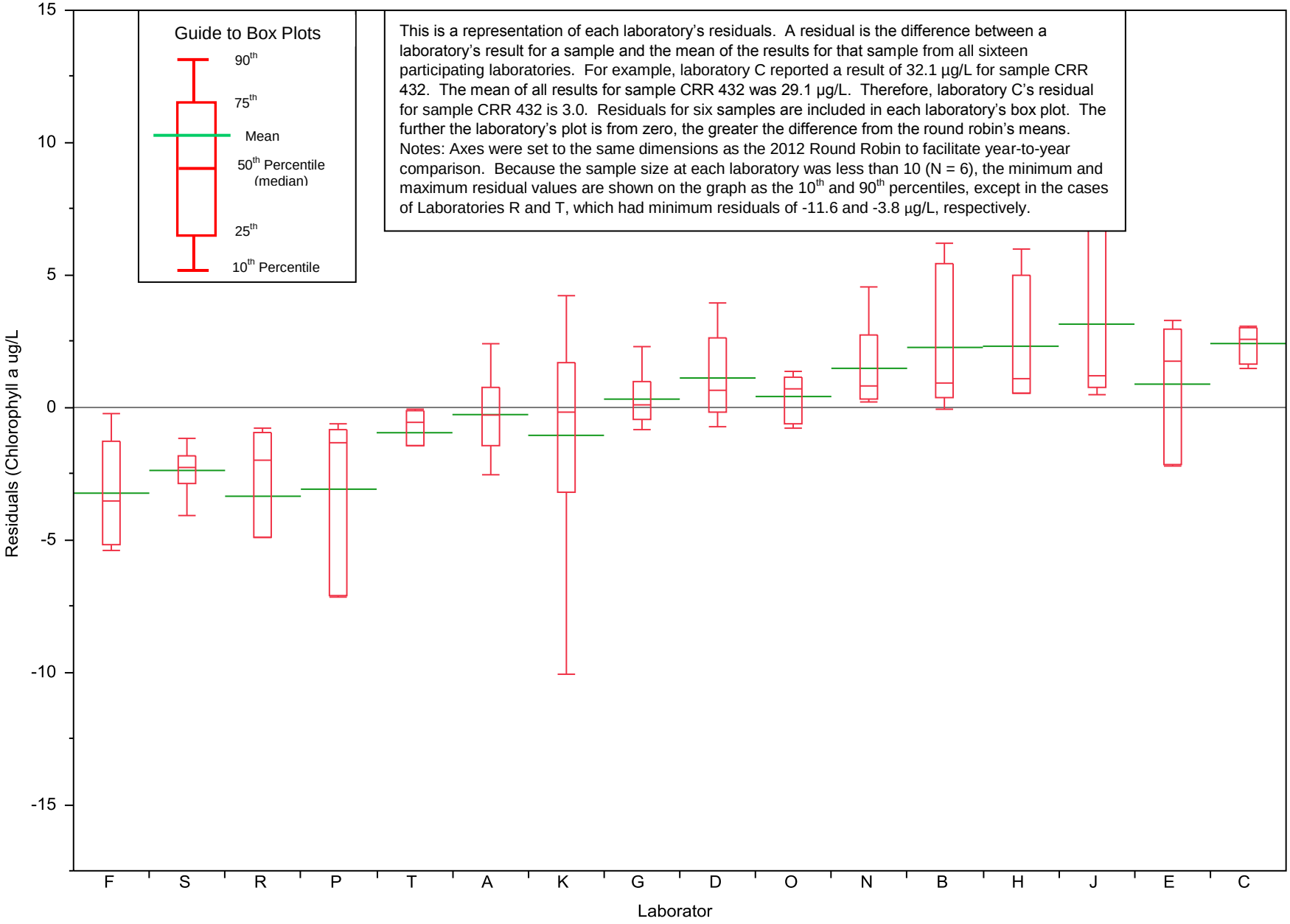
2013 Chlorophyll *a* Round Robin Box Plots of Laboratory Residuals (CR-449 & CR-949 Only)



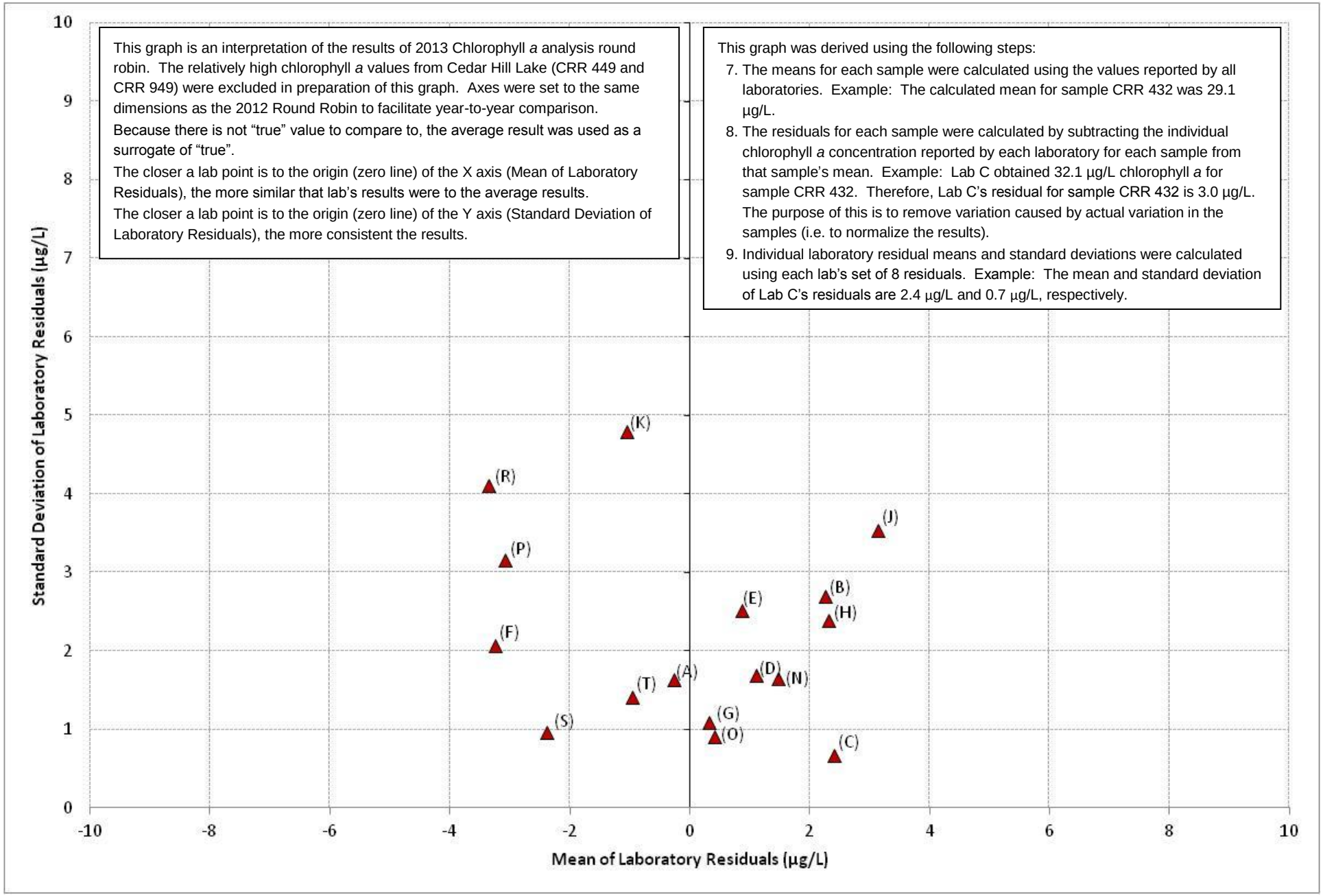
2013 Chlorophyll *a* Round Robin Laboratory's Residual Mean vs. Standard Deviation (CR-449 & CR-949 Only)



2013 Chlorophyll *a* Round Robin Box Plots of Laboratory Residuals (CR-449 & CR-949 Excluded)



2013 Chlorophyll *a* Round Robin Laboratory's Residual Mean vs. Standard Deviation (CR-449 & CR-949 Excluded)



In addition to these interpretations of the data, acceptance ranges were calculated for each sample using NELAC Proficiency Testing (PT) method, EPA/600/R-04/003. While some labs consistently generated higher (Lab J) or lower (Labs P, R, S, and T) results than the mean of results from all labs in this study, no lab's results were outside of the calculated "proficiency testing" range of natural log-transformed data ± 3 standard deviations (see table below).

2013 Lab ID	CRR 220	CRR 422	CRR432	CRR 449	CRR 590	CRR 642	CRR 735	CRR 949
A	29.0	10.0	29.0	130	12.0	7.5	16.0	170
B	35.0	11.5	34.3	118	13.9	9.9	14.1	156
C	30.5	12.0	32.1	143	16.2	12.7	16.1	156
D	31.0	9.8	33.0	130	14.0	10.0	14.0	170
E	26.6	13.1	26.9	154	16.4	12.9	14.5	178
F	23.7	8.9	24.7	142	7.7	9.8	10.9	171
G	31.1	10.9	29.7	140	12.8	9.9	12.7	175
H	34.8	12.0	33.7	131	13.8	10.6	14.1	144
J	35.0	11.0	38.0	162	14.0	11.0	15.0	201
K	33.0	9.7	19.0	136	14.0	9.1	14.0	165
N	33.4	10.9	31.2	134	14.0	10.8	13.8	161
O	29.2	11.5	28.5	142	14.5	11.1	12.8	169
P	21.7	9.9	21.9	127	11.7	9.1	12.3	151
R	17.2	8.9	26.4	128	10.7	9.0	12.8	160
S	24.7	8.4	27.9	105	11.1	7.7	11.1	142
T	25.0	9.9	29.0	120	13.0	9.5	13.0	160
Median	29.9	10.4	29.0	132.7	13.9	9.9	13.9	162.9
Mean	28.8	10.5	29.1	133.9	13.1	10.0	13.6	164.3
Std Dev	5.2	1.3	4.8	13.8	2.1	1.5	1.5	14.3
PT Min	15.6	7.2	17.0	97.5	7.5	6.3	9.7	126.8
PT Max	51.5	15.1	48.3	182.0	22.3	15.6	18.9	211.4