July 2010 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 phytoplankton populations (2008 Draft NC Impaired Waters List). These impairments lead to the development of Total Maximum Daily Loads (TMDLs) 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 governmental laboratories. Seventeen participating laboratories analyzed eight surface water samples for chlorophyll *a* concentration. 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 to improve analyses.

The data presented within this report represent the fourth chlorophyll *a* round robin, which was held in July 2010. Sixteen laboratories participated, each analyzing eight samples. All eight samples were collected from Triangle area waterbodies.

Experimental

Sampling

On July 28, 2010, NC DWQ staff collected a batch of eight grab samples from four area waterbodies. The 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).

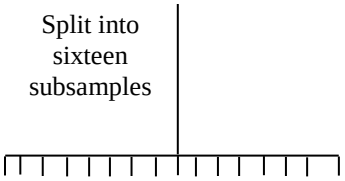
At ESS, each of the eight samples were split into sixteen 500 mL subsamples using a churn splitter. Every sample was churned for two minutes prior to splitting and was continually churned during the split. The splitter faucet was purged prior to sample dispensing. The order in which the subsamples were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

Analysis

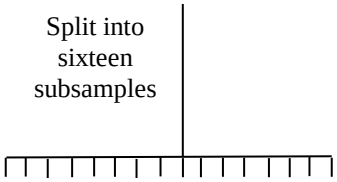
Participating laboratories were asked to analyze the eight samples according to their Standard Operating Procedures for chlorophyll *a* analysis. Each was also asked to complete a questionnaire concerning the analysis. The answers to the questionnaire and the data from the study are found on pages 4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

Lake Benson
35.672642, -78.7631847

Sample CRR 848

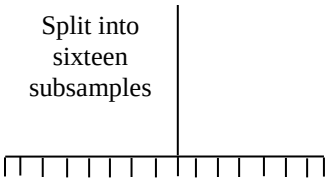


Sample CRR 096

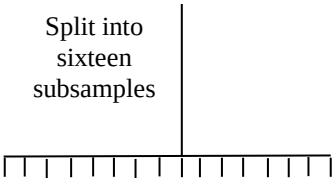


Raleigh Area Pond
35.79725, -78.68619

Sample CRR 425

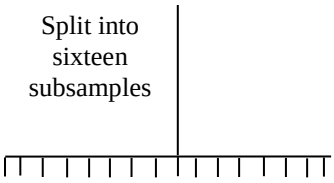


Sample CRR 266

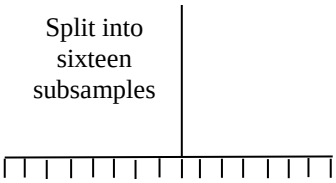


Lake Johnson
35.762208, -78.714553

Sample CRR 770

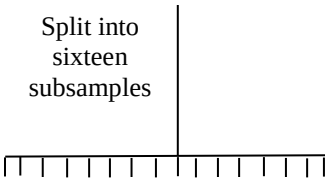


Sample CRR 615

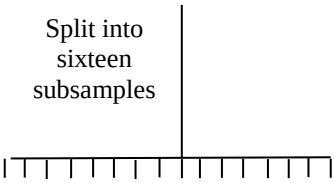


Harris Lake
35.57294, -78.97577

Sample CRR 293



Sample CRR 277



Participating Laboratories

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

CMU- Environmental Laboratory Services
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
Research and Analytical
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	Temperature Samples Received	Temperature Samples Stored Prior to Filtering	Length of Time Samples were Stored after Filtering
C	445.0 modified option Rev 1.2	7/28/2010	< 6 Celsius	Room temperature	13 days
E	EPA Method 445.0 modified	7/28/2010	4°C	4°C	8 days
F	EPA445.0 Fluorometric Method, Modified	7/29/2010	No temperature blank, ice remaining in cooler	approx. 4 deg C	4 days
G	EPA 445	7/28/2010	on ice	on ice	12 days
H	EPA 445.0	7/28/2010	3°C	1.5°C	1/2 day
J	SM 10200H Spectrophotometric Determination (18th ed)	07/28/10	2.1°C	1.3°C	1 day
L	SM 10200H	7/28/2010	8° C	3°C	21 Days
M	SM10200H Spectrophotometer	7/29/2010	2.3°C	~ 17° C	7 days
P	SM10200H	7/29/10	0.1 °C	4°C	15 hours
R	Spectrophotometric Standard Method 10200H 1.(extraction), and 10200H 2.(analysis)	07/28/10	0°C	N/A	overnight
S	SM10200H	7/28/2010	4 deg C +/-2	4 deg C on Hg thermometer, 4 deg C on IR thermometer	10 Days
U	Fluorometric (non-acidification) Welshmeyer 1994	7/28/2010	4 degrees C	N/A	5 days
V	EPA 445.0 Fluorometer	7/28/2010	not taken	<4°C	7 days (analyzed on 8.3.10)
W	SM10200H	7/28/2010	2.2 to 3.1 °C	0.1 to 4.4 °C	7 days
X	EPA 445, Fluorometer	7/28/2010	on ice	on ice	8 days
Y	EPA 445.0 (Welschmeyer, non-acidification)	7/28/2010	2.0 C	3.0 C	21 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?
C	Vigorous Shaking	7/28/2010	Did not exceed 6 in. Hg (i.e., 20kPa)	150 ml	2 - 5 minutes
E	inversion of container 4-5 times	7/28/2010	5 in Hg	50 mL	~ 1 minute
F	Invert 3-4 times	7/29/2010	≤ 6 in HG	100 to 250 mL	1 to 10 minutes
G	Invert 3-4 times	7/28/2010	15kpa	100-300 ml	3-4 min. not more than 10 min.
H	Invert sample bottle three times, then pour into graduate cylinder	7/29/2010	5 in. Hg	250 mls	Depends on the turbidity of the sample, but for the typical stream sample, filtering takes 1-2 minutes. Heavily turbid samples can take from 5-10 minutes. We generally do not filter longer than 10 minutes.
J	Shaken	7/29/2010	5 to 6 in.	250 mls	Approximately 1 minute. (45 secs. to 90 secs.)
L	Vigorous shaking	7/28/2010	Not Measured	150 mL	15 seconds to 1 minute
M	Sample bottle is vigorously shaken by hand before filtration.	7/29/2010	Not measured	CRR096M = 200 mL; CRR266M = 350 mL; CRR277M = 500 mL; CRR293M = 500 mL; CRR425M = 400 mL; CRR615M = 500 mL; CRR770M = 500 mL; CRR 848M = 200 mL	1 min 9 seconds to 4 minutes 1 second
P	Bottles were shaken for 5 seconds	7/29/2010	Not Measured	250-500mL	5-15mins
R	Bottle inverted 3 times	7/28/2010	~6.5 in Hg	0.24-0.25 L	Typically < 1 minute but some up to 1.5 minutes.
S	Shake well	7/28/2010	<6 Hg	250 mL for each sample	Approx 20-30 secs
U	Shaking of each sample before filtration for 5-10 seconds	7/28/2010	5 inches Hg	50-100mL (attached on data sheet next tab)	1-2 minutes
V	Gently inverted bottle 4-5 times before pouring sample.	7/28/2010	not measured; use low-vac hand pump	100	2 minutes
W	Shaken by hand. Three sample bottles were overfilled. Two of the worst were separated into another sample bottle, shaken and then recombined.	7/29/2010	<6 in Hg	100 mL	One minute or less.
X	Mildly shake sample for 5-10 seconds	7/28/2010	<6 mm Hg	25-50 mL	10 secs - 60 secs
Y	Samples were inverted several times	7/28/2010	<5 in Hg	34 - 160mL	time filtered was not recorded (estimate 10 seconds/sample)

Laboratory ID	Type of Filters Used	Brand of Filters Used	Describe Filtering Technique (how were sample volumes measured, were sides rinsed)
C	Glass fiber, 47 mm, with nominal pore size of 0.7 µm	Whatman™ GF/F filters	Graduated Cylinder
E	glass fiber filter	Millipore	Sample volumes measured with plastic graduated cylinder, no rinsing of sides
F	GF/F		Sample mixed, poured into graduated cylinder, then into filtration apparatus. Both rinsed with DI water after sample filtered
G	glass microfibre 47mm	Whatman	50ml at a time, grad cyl. Measured, sides rinsed, filter not sucked dry, blotted with tissue, filter folded in half with material inside
H	GF/F glass fiber filter	Whatman	We pour 250 mls of sample from the sample container into a graduate cylinder. Typically, the entire 250 mls is filtered. If the sample is turbid or otherwise will not allow the entire amount to go through the filter, we filter as much as possible, then read off of the graduate the amount filtered. The filter apparatus and graduate are rinsed before proceeding.
J	A/E Glass Fiber	Gelman	Samples were measured with graduated cylinder, poured into vacuum funnel, sides rinsed with DI water at end of filtration
L	Glass Fiber	Whatman	The samples are poured into a graduated funnel. The graduated marks have been verified by pouring volumes from a volumetric flask and marked for any discrepancies. With a filter in place a whole volume is placed in the funnel and a vacuum source is turned on to assist in filtering the sample. The vacuum is turned off when the sample volume reaches 50 mL and the remaining volume is simple filtered by gravity until the entire volume has passed through the filter. The walls of the funnel is rinsed with DI H2O.
M	Whatman GF/C	Whatman GF/C	After being mixed, sample is poured into a 500 mL Class A graduated cylinder to be meaured 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.
P	Glass Fiber	Whatman	Graduated cylinder was used to measure the sample filtered. If the full 500mL of sample was filtered, then the cylinder was rinsed. If only a portion was filtered then the cylinder was not rinsed.
R	Whatman 934-AH glass fiber filters	Whatman	Samples measured with a plastic graduated cylinder, no rinsing between samples.
S	934-AH	Whatman	In funnel; rinsed sides
U	GF/F (glass fiber) 25mm circles	Whatman	Samples were poured into 100mL graduated cylinder and then into filter manifold. The cylinder and manifold was rinsed with deionized water between each sample
V	GF75, 47mm	ADVANTEC (80905712)	Measured with a graduated cylinder. Yes, sides were rinsed.
W	Whatman GF/F	Whatman	Measured by graduated cylinder - sides not rinsed
X	A/E glass fiber	Gelman	Volume measured in a graduated cylinder, filter funnel is rinsed in sink between samples
Y	GF/C	Whatman	Samples were 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.

Laboratory ID	Light conditions during filtering	Extraction solvent/volume	Steeping time	Was grinding used?
C	Green light 25 W bulb	9:1 Acetone:DI water. 25 mL	5 hours	Yes
E	lights off, ambient light through closed blinds on windows	10-12 mL 90% acetone	24.25 hr	Yes
F	Dimmed fluorescent lighting	90:10 Actone:DI Water, 25 mL	Overnight, approx. 18 hours	Yes
G	red light	10 ml of 90% acetone/water	21 hr, 45 min	Yes
H	Black-out with green light	90/10 Acetone/DI water. 25 mls used per sample	23 hours	Yes
J	reduced laboratory light, light on behind me with door partially closed	90% acetone, 10% DI water @ 10 mL samples topped off at 10 mL	2:00 pm to 11:30 am overnight 21.5 hrs	Yes
L	subdued light (green light)	90% Acetone, 10 mL	Overnight	Yes
M	Filtration is done with regular overhead lighting.	90% Acetone with 10% MgCO3 solution. Extract has a final total volume of 8 mL.	4.5 hours	Yes
P	Flourescent lighting	Acetone/MgCO3, volume used 10mL	4hrs	Yes
R	Ambient outside light with lab blinds drawn.	90 % acetone, 12 mL	2.5 hours	Yes
S	Lights in lab were completely cut off	Acetone (MgCO3 is no longer used), 10 mL total	Overnight	Yes
U	light off in room minimal sunlight through window	90% methanol / 10% Deionized water	24 hours	Yes
V	Overhead fluorescent lighting	90% acetone, 10% water. Final volume is 20 mL extracted twice with 10 mL each.	0 (sonication method does not require steeping)	No
W	Subdued green light (LED)	90% Acetone. 25mL	Approx. 21 hours	Yes
X	filtered light from windows	90% acetone, 10 mL	6 hours	Yes
Y	All overhead lights off, two small lamps with 25 watt green bulbs	90% acetone, 14mL	22 hours	Yes

Laboratory ID	Description of grinding setup
C	® pestle (50 mm X 20 mm) with grooves in the tip with ¼" stainless steel rod long enough to chuck onto a suitable drive motor and 30 ml capacity glass grinding tub with no temperature control
E	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
F	Glass grinding tube, Teflon tipped pestle, variable speed mixer. Temp control by touch/feel
G	Temp. controlled tissue grinder, low speed drill
H	Teflon pestle with radial serrations on lower part of pestle. Pestle powered by electric drill in glass tube. Temperature controlled by touch.
J	glass/glass tissue grinder Arrow 850 motor 1/10 hp Kontes grinder pestle SA24 and matching tube -- no temperature control
L	Grinding was done with serrated pestle. Grinding lasted 30 seconds per sample and was determined by timer.
M	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.
P	Drill press with a Teflon grinding tip. Not temperature controlled.
R	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.
S	round bottom grinding tube with matching glass pestle; ~ 60 seconds
U	Teflon tissue grinder was used with a drill to grind the filter and 7.5mL of acetone completely (30seconds) Temperature was not controlled
V	N/A
W	Ground in a glass mortar using a rounded tip, serrated Teflon pestle using an electric drive motor. Temperature was monitored by feel - not allowed to get too warm.
X	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
Y	stainless steel tip homogenizer, temperature was not controlled

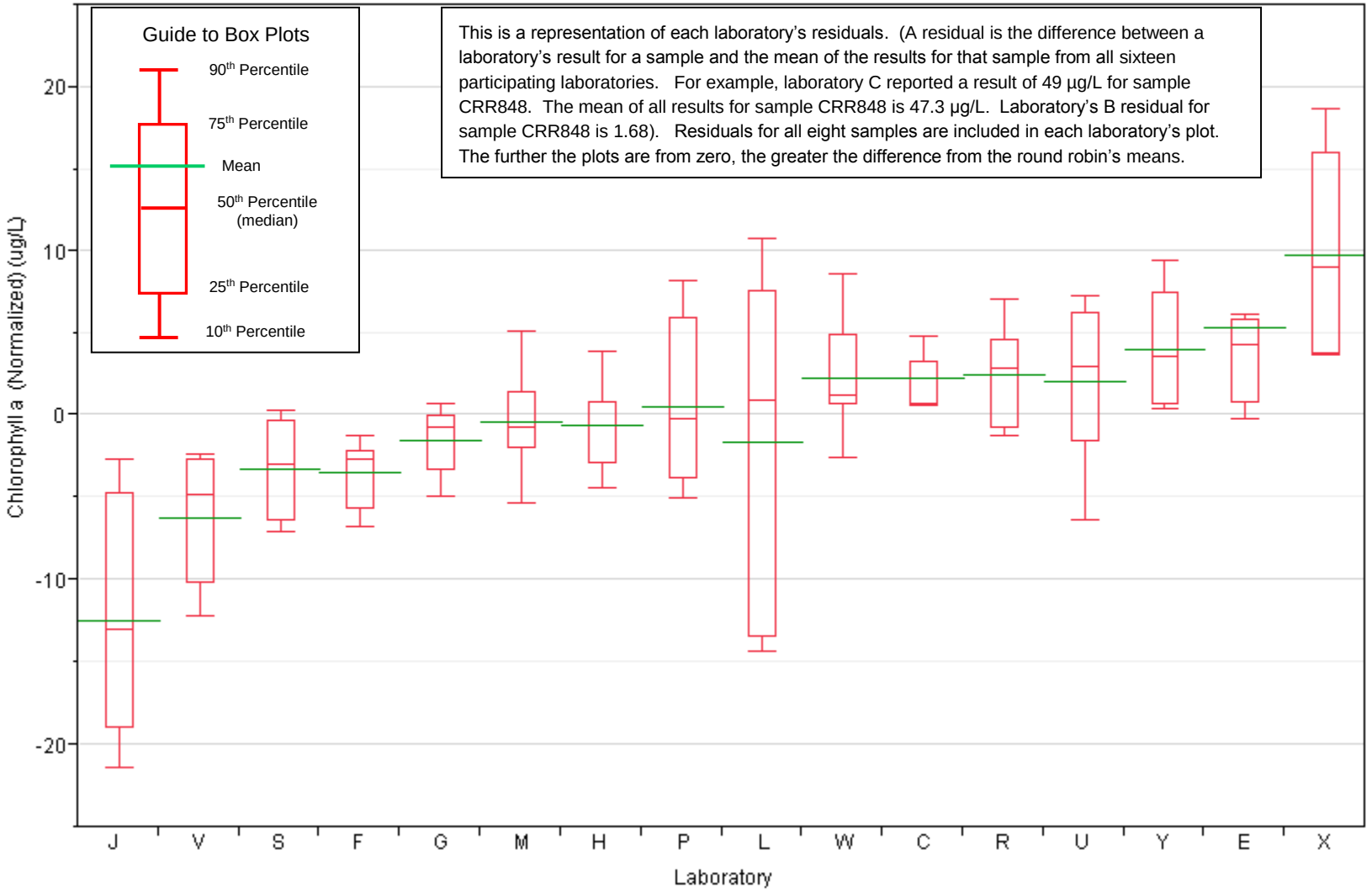
Laboratory ID	Samples Acidified? If so, type, concentration, and volume used	Type of calibration standard used and source
C	No	196 µg/L Turner instrument Corp
E	No	primary calibration using two concentrations of chl-a in 90% acetone prepared by Turner (last calibration on 7-26-10), solid fluorescence standard is used prior to each set of samples measured (and often during the middle of sample runs when the number of samples exceeds ~20) to correct for possible instrument drift
F	No	Chlorophyll a from Anacystis, Sigma C6144, a 200 ug/L calibration standard was made from stock solution on day of analyses
G	No	Turner Designs fluorometric chlorophyll std (high and low) verified with TD-700 solid standard
H	No	We calibrate using a blank, high and low standards. The standards are obtained from Turner Designs.
J	No	N/A
L	Yes 0.1 N HCl 100 uL per 5 mL	Sigma Aldrich Stock Standard
M	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.
P	Yes, 0.1N HCL. 0.3mLadded to 3mL of extract	None
R	Yes, two drops of 6N HCL to 10 mL sample	90% acetone to zero spec. Mixed in lab 8. No notable differences between samples.
S	Yes, 180 uL of 0.1N HCl was added to 6mL of extracted, centrifuged sample	27.7 ug/L Std used from Turner Designs
U	No	Sigma Aldrich Chlorophyll a standard
V	0.1 N HCl solution, 137 uL of acid to 4.5 ml of sample	Stock solution, Sigma C6144- 1 mg, Lot # 1449462 dissolved in 100 mL of 90% Acetone. The instrument is calibrated on the 200 ug/L standard because we use a 1 point curve. The other standards 800, 400, 100, 50, 10, and 5 ug/L are run to ensure the CCV is greater than or equal to .995.
W	No	Turner Designs Fluorometric Chlorophyll Standard
X	No	Purified Chla from Anacystis dissolved in 90% Acetone (Sigma-Aldrich Chemical)
Y	No	Primary - Chl a from Anacystis nidulans - Sigma (C6144) Secondary - Chl a from Spinach - Sigma (C5753)

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.

July 2010 Chlorophyll *a* Round Robin Results

	Lake Benson		Raleigh Area Pond		Lake Johnson		Harris Lake	
Laboratory ID	**CRR848** (µg/L)	**CRR096** (µg/L)	**CRR425** (µg/L)	**CRR266** (µg/L)	**CRR770** (µg/L)	**CRR615** (µg/L)	**CRR293** (µg/L)	**CRR277** (µg/L)
C	49.0	48.0	41.0	44.0	13.0	13.0	22.0	20.0
E	53.5	44.7	42.6	43.9	13.1	13.0	22.9	41.2
F	41.0	42.0	31.0	36.8	9.0	9.9	17.8	17.2
G	47.4	44.9	37.4	39.9	7.4	10.9	16.3	15.9
H	48.4	44.3	36.9	43.1	11.7	12.5	14.7	15.8
J	26.0	26.3	18.7	22.6	8.8	9.4	9.8	11.0
L	33.8	32.0	40.9	24.9	23.1	10.7	26.7	26.7
M	49.0	50.0	36.0	40.0	11.0	12.0	17.0	14.0
P	54.8	53.1	32.7	40.6	8.4	8.7	20.1	17.8
R	54.5	49.0	42.5	42.9	11.5	14.1	18.7	18.2
S	44.0	39.4	30.7	36.7	12.7	12.0	18.0	12.7
U	41.1	52.2	44.3	44.7	15.8	14.5	17.4	18.2
V	37.1	32.8	31.5	29.4	8.9	9.4	16.7	16.7
W	56.0	47.1	43.5	36.7	13.3	12.7	20.3	20.5
X	66.0	58.0	51.0	56.0	16.0	16.0	24.0	23.0
Y	55.6	54.3	42.0	44.5	13.0	13.1	21.9	19.7
Median	**48.7**	**46.0**	**39.2**	**40.3**	**12.2**	**12.2**	**18.3**	**18.0**
Mean	**47.3**	**44.9**	**37.7**	**39.2**	**12.3**	**12.0**	**19.0**	**19.3**

2010 Chlorophyll *a* Round Robin Box Plots of Laboratory Residuals



2010 Chlorophyll *a* Round Robin Labortaory's Resdiual Mean vs Standard Devation