June 2011 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 represents the fifth chlorophyll *a* round robin, which was held in June 2011. Seventeen laboratories participated, each analyzing eight samples. All eight samples were collected from Raleigh Area waterbodies.

Experimental

Sampling

On June 22, 2011, NC DWQ staff collected a batch of eight grab samples from four area lakes or ponds. 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).

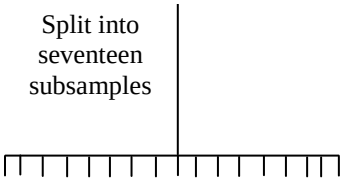
At ESS, each of the eight samples were split into seventeen 500 mL subsamples using a churn splitter. Every sample was churned for two minutes prior to splitting and was continually churned during the split. The splitter faucet was purged prior to sample dispensing. The order in which the subsamples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

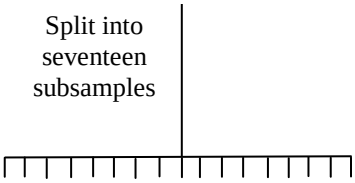
Participating laboratories were asked to analyze the eight samples according to their Standard Operating Procedures for chlorophyll *a* analysis. Each laboratory was also asked to complete a questionnaire concerning the analysis. The answers to the questionnaire and the data from the study are found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

Lake Wheeler
35.69366, -78.70128

Sample CRR 162

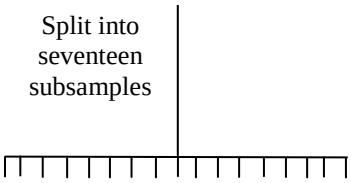


Sample CRR 047

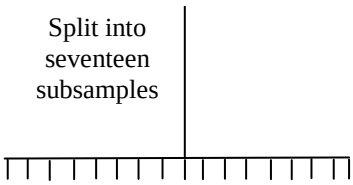


Harris Lake @ Crosspoint Landing
35.57309, -78.97594

Sample CRR 858

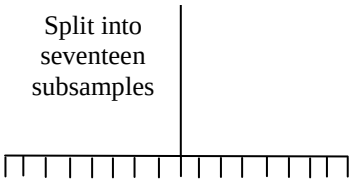


Sample CRR 553

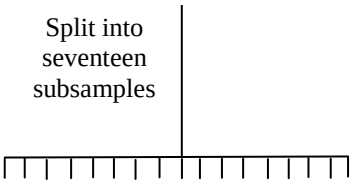


Lake Benson
35.67253, -78.63237

Sample CRR 171

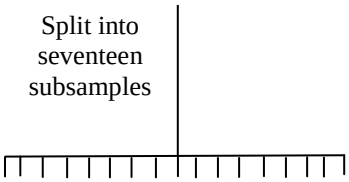


Sample CRR 191

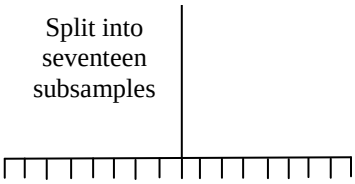


Raleigh Area Pond
35.79730, -78.68622

Sample CRR 772



Sample CRR 680



Participating Laboratories

The 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 – Hal Marshall Laboratory
Columbia Analytical
City of Durham Water and Wastewater Laboratory
NC Division of Water Quality Laboratory
East Carolina University Department of Biology
Environment 1
EPA Region IV, Science & Ecosystems Support Division
Florida Department of Environmental Protection
Meritech, Inc.
NCSU Center for Applied Aquatic Ecology
NOAA Center for Coastal Fisheries and Habitat Research
Raleigh, E. M. Johnson Water Plant
Research and Analytical Laboratories
Tritest, Inc.
UNC Institute for Marine Sciences
UNCW Center for Marine Sciences
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 Received	Temperature Samples Stored Prior to Filtering	Length of Time Samples were Stored after Filtering
B	Spectrophotometric Standard Method 10200H 1.(extraction), and 10200H 2.(analysis)	06/22/11	0°C	0°C	8 days
C	EPA 445.0	06/22/11	on ice	on ice	15 days
D	EPA 445.0	06/22/11	1.2°C	Filtered upon receipt	2 days
E	EPA 445.0 Fluorometer	06/22/11	4.85°C	4.44 °C	5 days
F	EPA 445.0	06/22/2011	< 5°C	-20°C	21 Days
G	EPA 445.0, modified	06/22/11	no temp blank	3°C	15 days
H	445.0 modified option Rev 1.2	06/22/11	< 6 Celsius	Room temperature	n/a
J	SM 10200H	06/23/11	0.2°C	4.5 °C	5 Days
K	SM10200H Spectrophotometer	06/23/11	4°C	room temperature (~21°C)	7 days, 18 hours, and 35 minutes
L	SM 10200 H 18th Ed. Spectrophotometric Determination	06/22/11	3.3°C	1.3°C	Overnight (3:00pm to 8:30am)
N	SM 10200H	06/22/11	≤ 4°C	0.10-4.4 °C	5 days
R	EPA 446	06/22/11	4°C	2-6 deg C	6 days. Samples were analyzed on 6-28-11.
S	EPA Method 446.0, 20th Edition: 10200 H	06/22/11	3.3°C to 5.2°C	0°C to 4°C	Samples were extracted immediately after Filtering.
U	SM 10200H	06/22/11	9^{o} C	3.5^{o}C	15 Days
W	EPA 445.0 Fluorometric Method, Modified	06/23/11	1.4^{o} C	4°C	4 days
X	EPA Method 445.0 modified	06/22/11	4.5°C	4°C	42 days, this was an abnormally long period. We were having fluorometer problems and halted all chl-a analyses until they were resolved
Z	Fluorometric (non-acidification) Welshmeyer 1994	06/22/11	6°C	N/A	12 hours

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	06/22/11	~7.0 in Hg	0.24-0.25 L	Typically less than 1 minute/sample; some up to 3 minutes.
C	Shake sample for 10 seconds	06/22/11	≤6 in Hg	50 ml	approx. 30 seconds
D	Sample bottle inverted gently three times.	06/22/11	5 Torr (0.2 in Hg)	250 ml	1-2 minutes
E	Gently shook the bottle before dispensing the water into graduated cylinder	06/22/11	not measured; use low-vac hand pump	100 ml	2 -4 minutes
F	Samples are inverted 4 times	06/22/11	<20 kPa	100-200 mL	Until samples slowed,no longer than 8 min.
G	Samples were inverted several times	06/22/11	<5 in Hg	53-118 mL	time filtered was not recorded (estimate 10 seconds/sample)
H	Agitated by inverting sample several times	06/22/11	Did not exceed 6 in. Hg (i.e., 20kPa)	150 ml	2 to <10 minutes each
J	Shake bottle	06/23/11	Not Measured	250 - 500 ml	> 1min < 5 min
K	Sample bottle is vigorously shaken by hand before filtration.	06/23/11	Not measured	200-500 mL	37 seconds (Field ID CRR858K) to 44 minutes 48 seconds (Field ID CRR162K)
L	Shaken	06/22/11	4-6 inches of mercury	250 ml (Dup 230ml)	< 10 minutes
N	Sample bottle well shaken by hand for 5-10 seconds	06/23/11	<6 in Hg	100 mL	20 -25 seconds
R	Samples were inverted several times, with multiple circular motions to further mix.	06/22/11	<6 Hg	250mL	approx 20 secs
S	Gentle 180°inversion then back again 4 times, then pour the entire sample into a 500ml Graduated cylinder.	06/23/11	6 in. Hg	165ml to 250ml	Approximately 2 minutes/per sample
U	Vigorous shaking	06/22/11	Not Measured	150 mL	15 seconds to 1 minute
W	Invert/Shake	06/23/11	≤ 6 in Hg	150 ml	1-8 min
X	Briskly inverted bottle ~10 times	06/22/11	6 in Hg	50 mL	1-2 minutes
Z	Teflon tissue grinder was used with a drill to grind the filter and 7.5mL of acetone completely (30seconds) Temperature was not controlled	06/22/11	5 inches Hg	50-100mL (attached on data sheet next tab)	1-3 minutes

Laboratory ID	Type of Filters Used	Brand of Filters Used	Describe Filtering Technique (how were sample volumes measured, were sides rinsed)
B	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.
C	Glass Fiber, pore size 0.7 µm	Millipore AP4002500, 25 mm dia.	Volume measured in a graduated cylinder, filer funnel sides not rinsed down
D	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.
E	GF75, 47mm	ADVANTEC (90828709)	Measured in a glass, 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.
F	Glass Fiber	Whatman	50mL aliquots were poured through the filtering apparatus until filtration slowed. The sides were rinsed with DI water.
G	GF/C 42.5	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.
H	Glass fiber, 47 mm, with nominal pore size of 0.7 µm	Whatman™ GF/F filters	By using Graduated cylinder, 250 mL and sides were rinsed
J	Glass Microfiber filters 1.2µm	Whatman, Cat # 1822-047	Samples were measured with a 500mL Class A measuring cylinder and then the cylinder was rinsed with DI.
K	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.
L	A/E Glass Fiber 47mm	Millipore	Samples were measured with 250 ml graduated cylinder, vaccum filtered, cylinder and funnel rinsed between uses.
N	47mm glass fiber, 0.7 micron	GE	Measured in a TD graduated cylinder; sides not rinsed
R	MG550-HA	Munktell	With lights cut off, samples were filtered using Tritest TSS filtration apparatus. Volumes were measured using graduations on funnel. Sides were not rinsed due to concern over degradation of Chlorophyll.
S	47 mm Glass Fiber	HACH, Catalog #: 2530-00	Entire sample was poured into a 500 ml graduated cylinder. Approximately 100 mls of sample were added to the filter. Vacuum turned on, and sample was slowly added to filter until a green/brown color was apparent, and approximately 1/2 sample was filtered. Cylinder walls were washed twice with deionized water, then vacuum is released. Volume filtered is read from graduated cylinder.
U	Glass Fiber	Whatman	Sample volumes were measured by the previously recorded markings on the filter apparatus
W	GF/F	Whatman	Graduated cylinder Sides Rinsed
X	Whatman 25 mm glass fiber filter	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)
Z	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

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	1 hour 40 minutes in cold room (14.5oC) followed by 20 minutes in centrifuge (4oC at 2800 rpm) = 2 hours total	Yes
C	filtered light from windows	90% acetone, 10 ml	5 hours	yes
D	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
E	Low ambient light in lab	90 % Acetone/water, 20ml	0 (sonication method does not require steeping)	No
F	Subduded light	90% Acetone:	22hr 55 min	yes
G	Overhead lights off, two desk lamps with green 25w bulbs	90% acetone (Fisher Certified ACS & Milli-Q Water), 14mL	22 hours	yes
H	Dark room, subdued light for analysis with 25 watt green light as background.	90% acetone, 25 mL	17 hours	Yes
J	Florescent Lighting	90:10 Acetone: MgCO3	25hrs	Yes
K	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.	2 hours 30 minutes	yes
L	Darkroom with green light	90% acetone w/ 10% deionized water, Purity = 99.7% @ 10 mls used	23.3 hours	Yes
N	Dark room with only subdued green light	90% Acetone; Optima; 25ml	21 hr. 10 min	Yes
R	Lights were off in the lab except for small lamp at far end to provide ability to see.	90% acetone/10% deionized water. Acetone is ACS, sub-micron filtered. Water is from Company system, which is kept at approx 18.2 megaohms.	Overnight	No
S	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
U	subdued light(green light)	90% Acetone, 10 mL	Overnight	Yes
W	Dimmed fluorescent lighting	Acetone, 90%, 25 ml	~18 hrs	Yes
X	lights turned off, blinds closed	90% reagent grade acetone	23 hr	yes
Z	lights off sunlight through windows	90% acetone 10% deionized water	24 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 removed from -20oC freezer, ground for approximately 30 seconds, and placed in dark box with ice packs.
C	A teflon tissue grinder is attached to a motor and the filter is ground in a 50 ml centrifuge tube. Temperature does not rise significantly to the touch.
D	Teflon pesstle with radial serrations on lower part of pestle. Glass tube, powered by electric drill. Temperature controled by touch.
E	Sonication
F	Tissue grinder attached to
G	homogenizer with stainless steel tip, glass vessel for grinding then transferred to 15mL centrifuge tube, not temperature controlled
H	® 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
J	Drill press with a teflon grinding tip. Not temperature controlled.
K	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.
L	Arrow 850 motor 1/10 hp. Kontes tissue grind pestle SZ 24 and matching tube. No temperature control.
N	Ground in a glass mortar using a rounded tip, serrated Teflon pestle, powered by an electric drive motor. Temperature monitored by feel. Sample not allowed to heat.
R	NA
S	Grinder: Cole Parmer Lab Gen 125 with stainless steel blade. 50ml disposable polyproplene centrifuge tubes are used for the grinding and steeping.
U	Grinding was done with serrated pestal. Grinding lasted 30 seconds per sample and was determined by timer.
W	Glass grinding tube, teflon tipped pestle, variable speed mixer. Temp control by feel
X	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
Z	Teflon tissue grinder was used with a drill to grind the filter and 7.5mL of acetone completely (30seconds) Temperature was not controlled

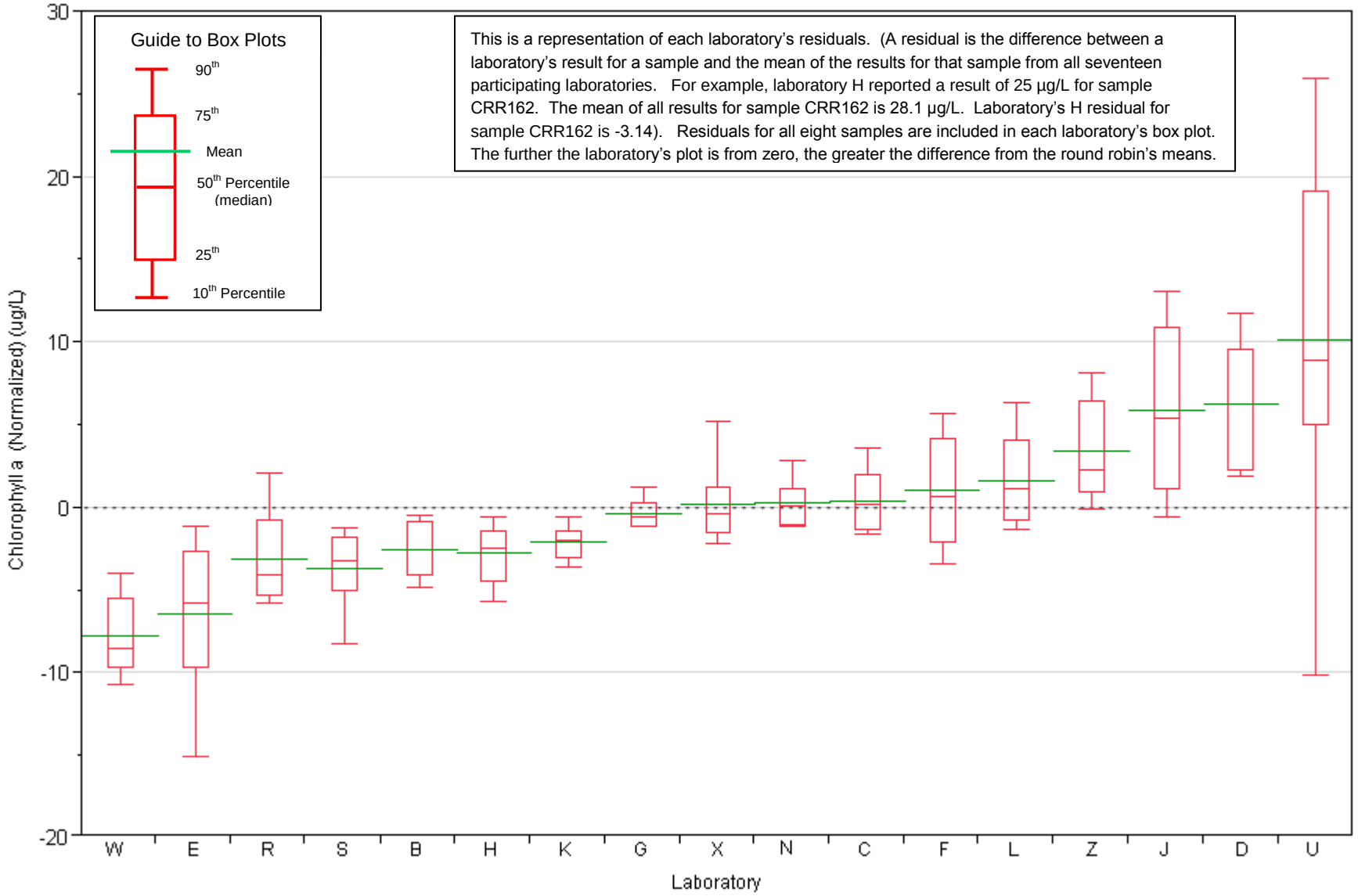
Laboratory ID	Samples Acidified? If so, type, concentration, and volume used	Type of calibration standard used and source
B	Yes, two drops of 6N HCL to 10 mL sample	90% acetone to zero the spectrophotometer
C	no	Purified Chla from Anacystis
D	No	QCS Intermediate Standard, which will vary in concentration depending on the concentration of the stock as received from the manufacturer
E	0.1 N HCl solution, 135 uL to 4.5 ml of sample	Stock solution, Sigma C6144- 1 mg, Lot # BCBD643OV 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 0.995.
F	no	Turner Designs
G	no	Primary - Chl a from Anacystis nidulans - Sigma (C6144) Secondary - Chl a from Spinach - Sigma (C5753)
H	No	161 µg/L Turner instrument Corp
J	0.3mL of 0.1N HCl into 3mL of sample extract	None
K	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 97% recovery.
L	No, pheophyton method not used.	N/A
N	No	Turner Designs Fluorometric Chlorophyll Standard
R	Yes. 180uL of 0.1N HCl added to each sample.	No calibration std used. But a Turner Designs std is used to show proper technique through acidification. Std conc is 32.2 ug/L.
S	90 uL of 0.1 N HCL were added to 3 ml of extracted sample in a labeled amber bottle, then shaken vigorously to mix.	Sigma-Aldrich Chlorophyll a #C57853 -1mg from Spinach.
U	Yes 0.1 N HCl 100 uL per 5 mL	Sigma Aldrich Stock Standard
W	No	Chlorophyll a from Anacystis, Sigma C6144. A 200ug/L calibration standard made from stock solution on day
X	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.
Z	no	Chlorophyll a (Sigma Aldrich)

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 2011 Chlorophyll *a* Round Robin Results

	Lake Wheeler		Harris Lake		Lake Benson		Raleigh Area Pond	
Laboratory ID	**CRR162** *(µg/L)*	**CRR047** *(µg/L)*	**CRR858** *(µg/L)*	**CRR553** *(µg/L)*	**CRR171** *(µg/L)*	**CRR191** *(µg/L)*	**CRR772** *(µg/L)*	**CRR680** *(µg/L)*
B	24.2	26	11.5	10.1	56.9	54.9	33.4	30.4
C	29	29	12	12	62	60	34	33
D	39.9	36.0	15.4	16.4	71.3	65.8	37.1	36.5
E	18.1	18.2	10.5	9.16	46.7	49	32.7	32
F	29.3	26.8	12.9	9.87	66.3	62.1	36.8	32
G	27.8	25.7	12.2	12.3	62.1	57.7	33.9	33.4
H	25	26	12	11	56	55	29	32
J	34.7	30.9	12.8	13.4	74.8	68.8	40.2	39
K	25	26	12	11	60	55	31	31
L	34.5	25.3	15.5	12.3	61.5	61.1	36.3	34.7
N	27.8	27.3	12.3	12.5	64.6	57.7	34.3	33.4
R	25.2	22.7	13.4	15.4	56.1	50.7	29.4	30.4
S	26.9	18.4	10.5	11.9	57.6	53.0	30.9	29.3
U	35.6	35.6	28.5	33.8	65.9	46.3	42.7	60.5
W	19	21	7.9	9.3	51	48	24	26
X	26.5	24.4	12.4	12.8	67.0	57.0	35.3	34.3
Z	30.1	33.2	15.9	13.2	69.9	57.9	34.6	40.3
Median	**27.8**	**26.0**	**12.3**	**12.3**	**62.0**	**57.0**	**34.0**	**33.0**
Mean	**28.1**	**26.6**	**13.4**	**13.3**	**61.7**	**56.5**	**33.9**	**34.6**

2011 Chlorophyll *a* Round Robin Box Plots of Laboratory Residuals



2011 Chlorophyll *a* Round Robin Labortaory's Resdiual Mean vs. Standard Devation