Blue-Green Algal Toxins Monitoring Protocol and Standard Operating Procedures

Algae Task Force
Spring 2004

Monitoring Plan

Background

The presence of *Cylindrospermopsis* has been identified in increasing concentrations in the Overton Arm and Boulder Basin of Lake Mead. This monitoring plan was developed under the direction of the Lake Mead Water Quality Forum Algae Subcommittee to track *Cylindrospermospsis* population trends and to address immediate, as well as long-term concerns about the presence of theses toxic blue-green algae in the Colorado River system. Sampling sites were selected for one or more of the following reasons: 1) *Cylindrospermopsis* was previously reported at the location, 2) the location is in a productive region of the lake, 3) the location is near a tributary to Lake Mead or 4) location is area of full body contact recreation. Microscopic identification of the algae will be done on each sample collected. Toxin analysis will be done, as warranted, based on algal counts and/or physical evidence of toxicity in the area.

Sampling Program

Beginning in July 2004, samples will be collected at 10 sites on Lake Mead from July through October during the following weeks: July 12 to July 16, 2004, July 26 to July 30, 2004, August 9 to August 13, 2004, August 30 to September 3, 2004, September 13 to September 17, 2004, and October 4 to October 8, 2004 (Figure 4). Samples will be collected for plant nutrients (ammonia, nitrate, nitrite, total phosphorus, and ortho phosphorus), *Cylindrospermopsis* counts, chlorophyll *a*, *b*, and *c*, and algal toxin analysis. Hydrolab profiles will be taken on the sites to provide general water quality data including turbidity. Field measurements of Secchi depth, wind speed, temperature, and if possible, light transmittance will be noted.

The samples will be distributed to the appropriate lab for nutrient analysis, *Cylindrospermopsis* counts and chlorophyll *a*, *b*, and *c* analyses. Turn around time for preliminary *Cylindrospermopsis* counts will be one day. If the preliminary counts are greater than 20,000 C*ylindrospermopsis* cells/mL at any location in Lake Mead raw and finished water samples from Alfred Merritt Smith Water Treatment Facility (AMSWTF) will be sent out for algal toxin analysis and the National Park Service (NPS) will be notified. The NPS will be asked to identify locations where full body contact recreation is occurring in the area with elevated *Cylindrospermopsis* counts. Samples will be collected from the designated site and sent out for algal toxin analysis. A sample will also be collected at Willow Beach to determine the concentration of *Cylindrospermopsis* in Lake Mohave.

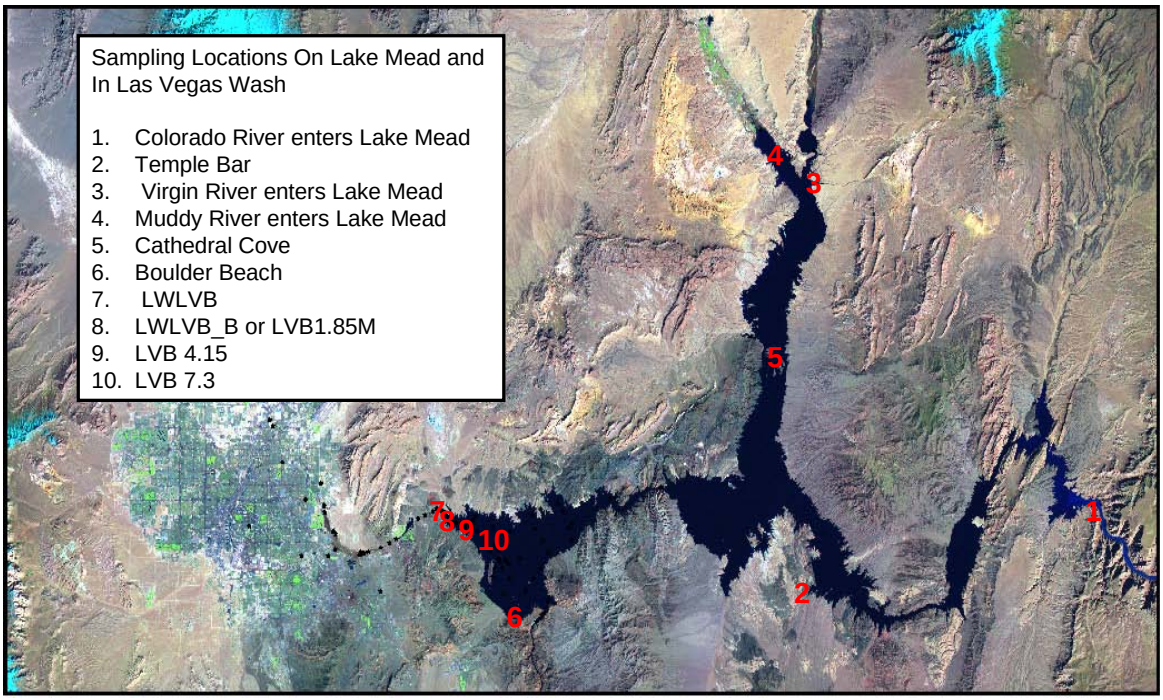

Figure 4: Monitoring Locations in Lake Mead

Additional samples will be taken in the Las Vegas Wash at two locations and in Lake Mohave at three locations with the August 30 to September 3, 2004 sampling event. In the Las Vegas Wash samples will be collected at LW 6.05 and LW 3.85. These samples will be analyzed for plant nutrients (ammonia, nitrate, nitrite, total phosphorus, and ortho phosphorus), chlorophyll *a*, *b*, and *c*, *Cylindrospermopsis* counts, and algal toxin analysis (Figure 5).

Hydrolab profiles will be taken on the sites to provide general water quality data including turbidity. Field measurements of Secchi depth, wind speed, temperature, and if possible, light transmittance will be noted.

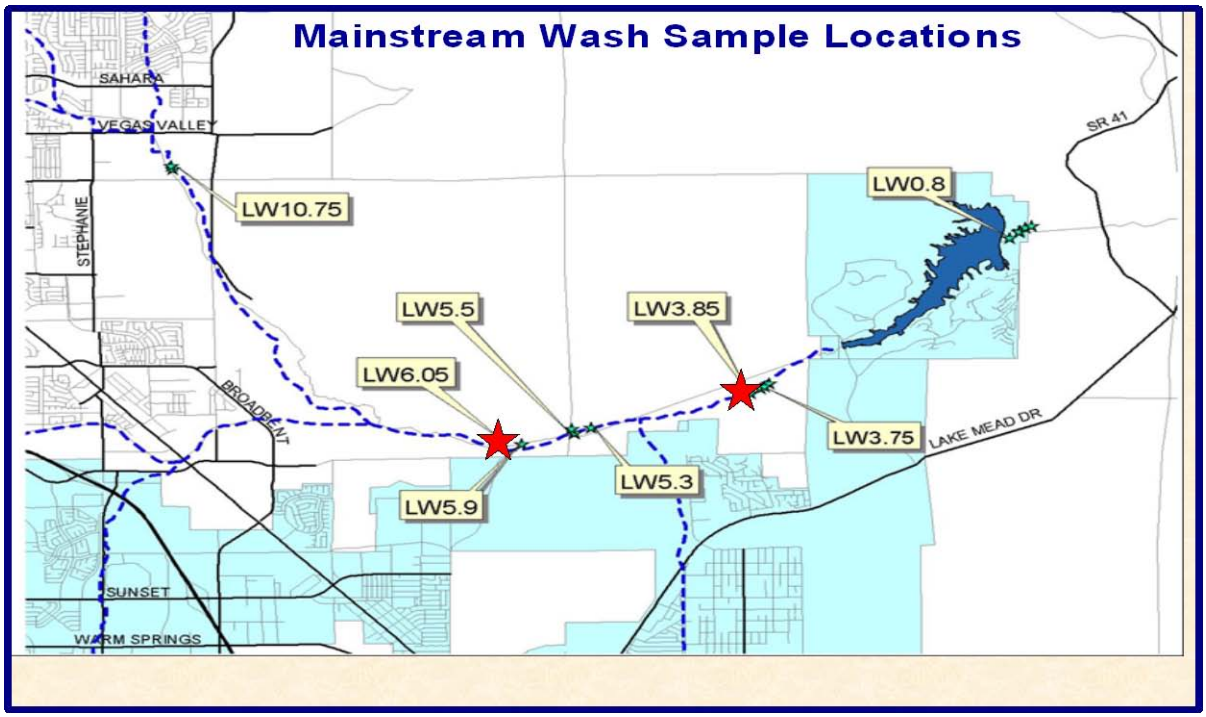

Figure 5: Las Vegas Wash Sampling Locations

Samples will be taken in Lake Mohave at three locations, Willow Beach, Cottonwood Cove and Davis Dam, with the August 30 to September 3, 2004 sampling event. These samples will be analyzed for plant nutrients (ammonia, nitrate, nitrite, total phosphorus, and ortho phosphorus), chlorophyll *a*, *b*, and *c*, *Cylindrospermopsis* counts, and algal toxin analysis (Figure 6).

Figure 6: Lake Mohave Sampling Locations

Entity Collecting Sample and Laboratory Performing Analyses

The entities responsible for sampling the different sites can be found in Table 1.

Table 1: Entities Responsible for Collecting Samples, Recording Field Data, and Performing Hydrolab Profiles

Location	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
1.Point where Colorado River enters Lake Mead	USBR	CLV	USBR	CLV	CLV	CLV
2. Temple Bar	USBR	CLV	USBR	CLV	CLV	CLV
3. Point where Virgin River enters Lake Mead	USBR	CLV	USBR	CLV	CLV	NDOW
4. Point where Muddy River enters Lake Mead	USBR	CLV	USBR	CLV	CLV	NDOW
5. Cathedral Cove	USBR	CLV	USBR	CLV	CLV	NDOW
6. Boulder Beach	NPS	NPS	NPS	NPS	NPS	NPS
7. LWLVB	USBR	USBR	USBR	CLV	USBR	CLV

Location	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
8. LWLVB_B or LVB1.85M	USBR	USBR	USBR	CLV	USBR	CLV
9. LVB 4.15	USBR	USBR	USBR	CLV	USBR	CLV
10. LVB 7.3	USBR	USBR	USBR	CLV	USBR	CLV
11. LW 6.05	X	X	X	SNWA	X	X
12. LW 3.85	X	X	X	SNWA	X	X
13. Willow Beach	X	X	X	NPS	X	X
14. Cottonwood Cove	X	X	X	NPS	X	X
15. Davis Dam at Lake	X	X	X	NPS	X	X

The entities responsible for sample analyses or sample send out for the different sites can be found in Table 2.

Table 2: Entities Responsible for Sample Analyses or Sample Send Out

Task	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
Plant nutrients (ammonia, nitrate, nitrite, total phosphorus, and ortho phosphorus)	USBR USBR to analyze NPS sample	USBR and CLV - CLV perform NPS sample	USBR USBR to analyze NPS sample	CLV CLV to analyze NPS sample and 2 samples from Wash, and 3 samples from Lake Mohave	USBR and CLV - CLV perform NPS sample	CLV CLV to analyze NPS and NDOW samples
Algae Counts (sample send out)	USBR USBR to send out NPS sample	USBR and CLV - CLV send out NPS sample	USBR USBR to send out NPS sample	CLV CLV to send out NPS sample and 2 samples from Wash, and 3 samples from Lake Mohave	USBR and CLV - CLV send out NPS sample	CLV CLV to send NPS and NDOW samples
Chlorophyll *a*, *b*, and *c*	USBR USBR to analyze NPS sample	USBR and SNWS - SNWS Laboratory to analyze CLV and NPS samples	USBR USBR to analyze NPS sample	SNWS SNWS – SNWS Laboratory to analyze CLV, NPS sample, and 2 samples from Wash, and 3 samples from Lake Mohave	USBR and SNWS - SNWS Laboratory to analyze CLV and NPS samples	SNWS - SNWS Laboratory to analyze CLV, NPS, and NDOW samples

Task	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
Collect Algal Toxin Samples	USBR and NPS	CLV, NPS, and USBR	USBR and NPS	CLV, NPS, and SNWA	CLV, NPS, and USBR	CLV, NDOW, and NPS
Collect SNWS raw and finished water algal toxin sample	SNWS Laboratory	SNWS Laboratory	SNWS Laboratory	SNWS Laboratory	SNWS Laboratory	SNWS Laboratory
Algal Toxin (sample send out or transport)	SNWA Watershed Division	SNWA Watershed Division	SNWA Watershed Division	SNWA Watershed Division	SNWA Watershed Division	SNWA Watershed Division
Algal Toxin Analysis	Either SNWA R&D or SUNY	Either SNWA R&D or SUNY	Either SNWA R&D or SUNY	Either SNWA R&D or SUNY	Either SNWA R&D or SUNY	Either SNWA R&D or SUNY
Hydrolab profiles	USBR	CLV and USBR	USBR	CLV	CLV and USBR	CLV and NDOW
Secchi depth	USBR	CLV and USBR	USBR	CLV	CLV and USBR	CLV and NDOW
Wind speed	USBR	CLV and USBR	USBR	CLV	CLV and USBR	CLV and NDOW
Temperature	USBR	CLV and USBR	USBR	CLV	CLV and USBR	CLV and NDOW
Light transmittance	USBR	CLV and USBR	USBR	CLV	CLV and USBR	CLV and NDOW

Appendix A lists the responsibility of each agency participating in the program

QA/QC Plan

In order to provide a defensible data set for this sampling activity, each sampling event will include one duplicate sample and one travel blank from each sample collector. Laboratories will be required to perform replicate analyses on 10 percent of the analyses performed in the laboratory.

Duplicate Samples

In each sampling event a duplicate sample will be taken at LVB 4.15. The entity collecting the sample at that location will be responsible for collecting the duplicate sample. Duplicate samples will be collected for all plant nutrients (ammonia, nitrate, nitrite, total phosphorus, and ortho phosphorus), chlorophyll *a*, *b*, and *c*, and algal toxins. Table 3 lists the entity responsible for sample duplicate collection.

Table 3: Entity responsible for duplicate sample collection

Duplicate Sample Collection	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
	USBR	USBR	USBR	CLV	USBR	CLV

Travel Blanks

Travel Blanks will be collected by each agency collecting samples. Travel blanks will be collected for all plant nutrients (ammonia, nitrate, nitrite, total phosphorus, and ortho phosphorus), chlorophyll *a*, *b*, and *c*, and algal toxins. The number of travel blanks for each sample collection can be found in Table 4.

Table 4: Number of travel blanks collected for each parameter

Travel Blanks	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
	2	3	2	3	3	3

Replicate Samples

Replicate samples will be performed on ten percent of the samples analyzed. Replicate samples will be performed on samples for all plant nutrients (ammonia, nitrate, nitrite, total phosphorus, and ortho phosphorus), chlorophyll *a*, *b*, and *c*, and algal toxins. The number of replicate samples required for each analysis and the agency responsible for performing the replicate sample is given in Table 5.

Table 5: Replicate Samples and Laboratory Responsible

Analysis	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
Ammonia	1 sample USBR	1 sample USBR and 1 sample CLV	1 sample USBR	2 samples CLV	1 sample USBR and 1 sample CLV	1 sample CLV
Nitrate	1 sample USBR	1 sample USBR and 1 sample CLV	1 sample USBR	2 samples CLV	1 sample USBR and 1 sample CLV	1 sample CLV
Nitrite	1 sample USBR	1 sample USBR and 1 sample CLV	1 sample USBR	2 samples CLV	1 sample USBR and 1 sample CLV	1 sample CLV
Total Phosphorus	1 sample USBR	1 sample USBR and 1 sample CLV	1 sample USBR	2 samples CLV	1 sample USBR and 1 sample CLV	1 sample CLV
Ortho Phosphorus	1 sample USBR	1 sample USBR and 1 sample CLV	1 sample USBR	2 samples CLV	1 sample USBR and 1 sample CLV	1 sample CLV

Analysis	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
Chlorophyll a, b, and c	1 sample USBR	1 sample USBR and 1 sample SNWS	1 sample USBR	2 samples SNWS	1 sample USBR and 1 sample SNWS	1 sample SNWS
Algal toxins	1 sample SNWA or SUNY	1 sample SNWA or SUNY	1 sample SNWA or SUNY	2 samples SNWA or SUNY	1 sample SNWA or SUNY	1 sample SNWA or SUNY

The total number of samples to be collected and analyzed, including all quality control samples, by agency can be found in Appendix B.

If *Cylindrospermopsis* is detected in any sample, the Phycologist will send out the slides for verification and the results of this verification will be sent with the sample results for documentation.

Two samples analyzed by the Southern Nevada Water Authority Research and Development Laboratory with detectable levels of algal toxins will also be sent out to State University of New York for verification. The samples will be sent to the State University of New York and analyzed for microcystin, anatoxin a, and cylindrospermopsin.

Sampling Procedure

The agency collecting the sample will arrive at the site and take the GPS location. The sampling crew will perform a Hydrolab profile from surface to bottom. They will also note the Secchi depth, wind speed, temperature, and when possible light transmittance.

A column of water will be collected using an integrated depth sampler from 0 to 5 meters. The water sample will be poured into a clean plastic container. From this container five bottles will be filled with various volumes of sample water. The first two bottles are for nutrients, one bottle will be preserved and one bottle will be left unpreserved and both bottles will be placed on ice. The preserved bottle will be preserved in the field with 1 mL of a 10% solution of sulfuric acid. The third bottle will be filled and prepared for phytoplankton analysis using a 25% gluteraldehyde solution. The fourth bottle will be filled and placed on ice for algal toxin analysis, and the fifth bottle will be filled and placed on ice for chlorophyll *a*, *b*, and *c* analyses. The trip blank will be opened and then closed at each sample site. The duplicate samples will be collected using the same method given above.

In the case of the beach samples, the sample collector will wade out into the water till the water reaches an approximate depth of 2 feet. The samples collector should, as much as is possible, make sure that any sediment that has been stirred up by their movement has either settled before the sample is collected or is moving away from the sample collection point. A Hydrolab will be used to take measurements of temperature, dissolved oxygen, conductivity, pH, and when possible turbidity. Secchi depth, wind speed, and air

temperature should also be noted. A clean bucket should be used to collect the water sample just below the surface. The samples should be treated in the same manner as given above. The trip blank will be opened at the beginning of the sample collection and closed at the end of the sample collection

When the samples are received in the laboratory the unpreserved nutrient sample should be filtered using a 0.45 um filter. The chlorophyll *a*, *b*, and *c* sample should be filtered using a Whatman GFC filter. Samples will be analyzed for plant nutrients, phytoplankton counts, algal toxins, and chlorophyll *a*, *b*, and *c* analyses using the laboratories standard procedures.

The Standard Operating Procedures for each entity collecting and analyzing samples can be found in Appendix C through H.

Field and laboratory analyses results should be sent to peggy.roefer@snwa.com for inclusion in the Lake Mead database.

Contact Information

In the event that individuals collecting samples require contact with others collecting samples or need any other information, contact information can be found in Table 6.

Table 6: Contact Information

Name	Cellular Number	Office Number
Randy Hadland	702-249-6078	702-229-2438
Bryan Moore	702-249-6181	702-293-8901
Dan Fischer	702-565-7753	702-229-2440
Chris Holdren	303-877-2110	303-445-2178
Peggy Roefer	702-249-3528	702-822-3359
Jason Kuchnicki		775-687-9450

Results

The results will be compiled and a summary report will be written.

Appendix A

Agency Responsibility

USBR Responsibility

Analyses	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
Nutrients Collect and Analyze	Collect 9 samples and analyze 10 samples (NPS sample) plus 2 travel blanks, 1 duplicate, and analyze 1 replicate	Collect 4 samples and analyze 4 samples plus 1 travel blank, 1 duplicate, and analyze 1 replicate	Collect 9 samples and analyze 10 samples (NPS sample) plus 2 travel blanks, 1 duplicate, and analyze 1 replicate	N/A	Collect 4 samples and analyze 4 samples plus 1 travel blank, 1 duplicate, and 1 replicate	N/A
Algae Counts Collect and Send Out	Collect 9 samples and send out 10 samples (NPS sample)	Collect 4 samples and send out 4 samples	Collect 9 samples and send out 10 samples (NPS sample)	N/A	Collect 4 samples and send out 4 samples	N/A
Chlorophyll a, b, and c Collect and Analyze	Collect 9 samples and analyze 10 samples (NPS sample) plus 2 travel blanks, 1 duplicate, and analyze1 replicate	Collect 4 samples and analyze 4 samples plus 1 travel blank, 1 duplicate, and analyze 1 replicate	Collect 9 samples and analyze 10 samples (NPS sample) plus 2 travel blanks, 1 duplicate, and analyze 1 replicate	N/A	Collect 4 samples and analyze 4 samples plus 1 travel blank and 1 duplicate	N/A
Collect Algal Toxin Samples	Collect 9 samples and leave in refrigerator at SNWS plus 1 travel blank, and 1 duplicate	Collect 4 samples and leave in refrigerator at SNWS plus 1 travel blank, and 1 duplicate	Collect 9 samples and leave in refrigerator at SNWS plus 1 travel blank, and 1 duplicate	N/A	Collect 4 samples and leave in refrigerator at SNWS plus 1 travel blank, and 1 duplicate	N/A
Collect SNWS raw and finished water algal toxin sample	N/A	N/A	N/A	N/A	N/A	N/A
Algal Toxin (sample send out or transport)	N/A	N/A	N/A	N/A	N/A	N/A
Analyze Algal Toxin Samples	N/A	N/A	N/A	N/A	N/A	N/A
Hydrolab profiles	Collect data at 9 locations	Collect data at 4 locations	Collect data at 4 locations	N/A	Collect data at 4 locations	N/A
Secchi depth	Collect data at 9 locations	Collect data at 4 locations	Collect data at 4 locations	N/A	Collect data at 4 locations	N/A
Wind speed	Collect data at 9 locations	Collect data at 4 locations	Collect data at 4 locations	N/A	Collect data at 4 locations	N/A
Temperature	Collect data at 9 locations	Collect data at 4 locations	Collect data at 4 locations	N/A	Collect data at 4 locations	N/A
Light transmittance	Collect data at 9 locations	Collect data at 4 locations	Collect data at 4 locations	N/A	Collect data at 4 locations	N/A

City of Las Vegas Responsibility

Analyses	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
Nutrients Collect and Analyze	N/A	Collect 5 samples and analyze 6 samples (NPS sample) plus 2 travel blanks and analyze one replicate	N/A	Collect 9 samples and analyze 15 samples (NPS sample and SNWA samples) plus 3 travel blanks, 1 duplicate, and 2 replicates	Collect 5 samples and analyze 6 samples (NPS sample) plus 2 travel blanks and analyze 1 replicate	Collect 6 samples and analyze 10 samples (NPS sample and NDOW) plus 3 travel blanks, 1 duplicate, and 1 replicates
Algae Counts Collect and Send Out	N/A	Collect 5 samples and send out 6 samples (NPS sample)	N/A	Collect 9 samples and send out 15 samples (NPS sample and SNWA samples	Collect 5 samples and send out 6 samples (NPS sample)	Collect 6 samples and send out 10 samples (NPS sample and NDOW)
Chlorophyll a, b, and c Collect	N/A	Collect 5 samples and drop off at SNWS for analysis plus 1 travel blank	N/A	Collect 9 samples and drop off at SNWS for analysis, plus 1 travel blank and 1 duplicate	Collect 5 samples and drop off at SNWS for analysis, plus 1 travel blank	Collect 6 samples and drop off at SNWS for analysis, plus 1 travel blank and 1 duplicate
Collect Algal Toxin Samples	N/A	Collect 5 samples and drop off at SNWS refrigerator plus 1 travel blank	N/A	Collect 9 samples and drop off at SNWS refrigerator plus one travel blank and one duplicate	Collect 5 samples and drop off at SNWS refrigerator plus one travel blank	Collect 6 samples and drop off at SNWS refrigerator and one duplicate
Collect SNWS raw and finished water algal toxin sample	N/A	N/A	N/A	N/A	N/A	N/A
Algal Toxin (sample send out or transport)	N/A	N/A	N/A	N/A	N/A	N/A
Analyze Algal Toxin Samples	N/A	N/A	N/A	N/A	N/A	N/A
Hydrolab profiles	N/A	Collect data at 5 locations	N/A	Collect data at 9 locations	Collect data at 5 locations	Collect data at 6 locations
Secchi depth	N/A	Collect data at 5 locations	N/A	Collect data at 9 locations	Collect data at 5 locations	Collect data at 6 locations
Wind speed	N/A	Collect data at 5 locations	N/A	Collect data at 9 locations	Collect data at 5 locations	Collect data at 6 locations
Temperature	N/A	Collect data at 5 locations	N/A	Collect data at 9 locations	Collect data at 5 locations	Collect data at 6 locations
Light transmittance	N/A	Collect data at 5 locations	N/A	Collect data at 9 locations	Collect data at 5 locations	Collect data at 6 locations

NDOW Responsibility

Analyses	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
Nutrients Collect and Analyze	N/A	N/A	N/A	N/A	N/A	Collect 3 samples and drop off in SNWS refrigerator plus one travel blank
Algae Counts Collect and Send Out	N/A	N/A	N/A	N/A	N/A	Collect 3 samples and drop off in SNWS refrigerator
Chlorophyll a, b, and c Collect	N/A	N/A	N/A	N/A	N/A	Collect 3 samples and drop off at SNWS for analysis plus one travel blank
Collect Algal Toxin Samples	N/A	N/A	N/A	N/A	N/A	Collect 3 samples and drop off at SNWS refrigerator plus one travel blank
Collect SNWS raw and finished water algal toxin sample	N/A	N/A	N/A	N/A	N/A	N/A
Algal Toxin (sample send out or transfer)	N/A	N/A	N/A	N/A	N/A	N/A
Analyze Algal Toxin Samples	N/A	N/A	N/A	N/A	N/A	N/A
Hydrolab profiles	N/A	N/A	N/A	N/A	N/A	Collect data at 3 locations
Secchi depth	N/A	N/A	N/A	N/A	N/A	Collect data at 3 locations
Wind speed	N/A	N/A	N/A	N/A	N/A	Collect data at 3 locations
Temperature	N/A	N/A	N/A	N/A	N/A	Collect data at 3 locations
Light transmittance	N/A	N/A	N/A	N/A	N/A	Collect data at 3 locations

Southern Nevada Water Authority - Southern Nevada Water System Laboratory

Analyses	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
Nutrients Collect and Analyze	N/A	N/A	N/A	N/A	N/A	N/A
Algae Counts Collect and Send Out	N/A	N/A	N/A	N/A	N/A	N/A
Chlorophyll a, b, and c Collect and Analyze	N/A	Analyze 6 samples (CLV and NPS) plus 2 travel blanks and one replicate	N/A	Analyze 15 samples (CLV, SNWA, and NPS) plus 3 travel blanks, 1 duplicate, and two replicates	Analyze 6 samples (CLV and NPS) plus 2 travel blanks and 1 replicate	Analyze 10 samples (CLV, NDOW, and NPS) plus 3 travel blanks, 1 duplicate, and 1 replicate
Collect Algal Toxin Samples	N/A	N/A	N/A	N/A	N/A	N/A
Collect SNWS raw and finished water algal toxin sample	Collect Raw and Finished water (2 samples) and put in SNWS refrigerator	Collect Raw and Finished water (2 samples) and put in SNWS refrigerator	Collect Raw and Finished water (2 samples) and put in SNWS refrigerator	Collect Raw and Finished water (2 samples) and put in SNWS refrigerator	Collect Raw and Finished water (2 samples) and put in SNWS refrigerator	Collect Raw and Finished water (2 samples) and put in SNWS refrigerator
Algal Toxin (sample send out or transfer)	N/A	N/A	N/A	N/A	N/A	N/A
Analyze Algal Toxin Samples	N/A	N/A	N/A	N/A	N/A	N/A
Hydrolab profiles	N/A	N/A	N/A	N/A	N/A	N/A
Secchi depth	N/A	N/A	N/A	N/A	N/A	N/A
Wind speed	N/A	N/A	N/A	N/A	N/A	N/A
Temperature	N/A	N/A	N/A	N/A	N/A	N/A
Light transmittance	N/A	N/A	N/A	N/A	N/A	N/A

Southern Nevada Water Authority – Watershed Division

Analyses	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
Nutrients Collect and Analyze	N/A	N/A	N/A	Collect 2 samples in Wash – Drop off in SNWS Refrigerator plus one travel blank	N/A	N/A
Algae Counts Collect and Send Out	N/A	N/A	N/A	Collect 2 samples in Wash – Drop off in SNWS Refrigerator plus one travel blank	N/A	N/A
Chlorophyll a, b, and c Collect and Analyze	N/A	N/A	N/A	Collect 2 samples in Wash – Drop off in SNWS Refrigerator plus one travel blank	N/A	N/A
Collect Algal Toxin Samples	N/A	N/A	N/A	Collect 2 samples in Wash – Drop off in SNWS Refrigerator plus one travel blank	N/A	N/A
Collect SNWS raw and finished water algal toxin sample	N/A	N/A	N/A	N/A	N/A	N/A
Algal Toxin (sample send out or transfer)	Send out 12 samples to SNWS R&D or SUNY plus 2 travel blanks and 1 duplicate	Send out 12 samples to SNWS R&D or SUNY plus 3 travel blanks and 1 duplicate	Send out 12 samples to SNWS R&D or SUNY plus 2 travel blanks and 1 duplicate	Send out 17 samples to SNWS R&D or SUNY plus 3 travel blanks and 1 duplicate	Send out 12 samples to SNWS R&D or SUNY plus 3 travel blanks and 1 duplicate	Send out 12 samples to SNWS R&D or SUNY plus 3 travel blanks and 1 duplicate
Hydrolab profiles	N/A	N/A	N/A	Collect 2 samples in Wash	N/A	N/A
Analyze Algal Toxin Samples	N/A	N/A	N/A	N/A	N/A	N/A
Secchi depth	N/A	N/A	N/A	Collect 2 samples in Wash	N/A	N/A
Wind speed	N/A	N/A	N/A	Collect 2 samples in Wash	N/A	N/A
Temperature	N/A	N/A	N/A	Collect 2 samples in Wash	N/A	N/A
Light transmittance	N/A	N/A	N/A	Collect 2 samples in Wash	N/A	N/A

NPS Responsibility

Analyses	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
Nutrients Collect and Analyze	Collect 1 sample Drop off in refrigerator at SNWS plus one travel blank	Collect 1 sample Drop off in refrigerator at SNWS plus one travel blank	Collect 1 sample Drop off in refrigerator at SNWS plus one travel blank	Collect 4 samples Drop off in refrigerator at SNWS plus one travel blank	Collect 1 sample Drop off in refrigerator at SNWS plus one travel blank	Collect 1 sample Drop off in refrigerator at SNWS plus one travel blank
Algae Counts Collect and Send Out	Collect 1 sample Drop off in refrigerator at SNWS	Collect 1 sample Drop off in refrigerator at SNWS	Collect 1 sample Drop off in refrigerator at SNWS	Collect 4 samples Drop off in refrigerator at SNWS	Collect 1 sample Drop off in refrigerator at SNWS	Collect 1 sample Drop off in refrigerator at SNWS
Chlorophyll a, b, and c Collect and Analyze	Collect 1 sample Drop off at SNWS for analysis plus one travel blank	Collect 1 sample Drop off at SNWS for analysis plus one travel blank	Collect 1 sample Drop off at SNWS for analysis plus one travel blank	Collect 4 samples Drop off at SNWS for analysis plus one travel blank	Collect 1 sample Drop off at SNWS for analysis plus one travel blank	Collect 1 sample Drop off at SNWS for analysis plus one travel blank
Collect Algal Toxin Samples	Collect 1 sample Drop off in SNWS refrigerator plus one travel blank	Collect 1 sample Drop off in SNWS refrigerator plus one travel blank	Collect 1 sample Drop off in SNWS refrigerator plus one travel blank	Collect 4 samples Drop off in SNWS refrigerator plus one travel blank	Collect 1 sample Drop off in SNWS refrigerator plus one travel blank	Collect 1 sample Drop off in SNWS refrigerator plus one travel blank
Collect SNWS raw and finished water algal toxin sample	N/A	N/A	N/A	N/A	N/A	N/A
Algal Toxin (sample send out or transfer)	N/A	N/A	N/A	N/A	N/A	N/A
Analyze Algal Toxin Samples	N/A	N/A	N/A	N/A	N/A	N/A
Hydrolab profiles	Collect 1 sample	Collect 1 sample	Collect 1 sample	Collect 4 samples	Collect 1 sample	Collect 1 sample
Secchi depth	Collect 1 sample	Collect 1 sample	Collect 1 sample	Collect 4 samples	Collect 1 sample	Collect 1 sample
Wind speed	Collect 1 sample	Collect 1 sample	Collect 1 sample	Collect 4 samples	Collect 1 sample	Collect 1 sample
Temperature	Collect 1 sample	Collect 1 sample	Collect 1 sample	Collect 4 samples	Collect 1 sample	Collect 1 sample
Light transmittance	Collect 1 sample	Collect 1 sample	Collect 1 sample	Collect 4 samples	Collect 1 sample	Collect 1 sample

Southern Nevada Water Authority – Research and Development Laboratory

Analyses	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
Nutrients Collect and Analyze	N/A	N/A	N/A	N/A	N/A	N/A
Algae Counts Collect and Send Out	N/A	N/A	N/A	N/A	N/A	N/A
Chlorophyll a, b, and c Collect and Analyze	N/A	N/A	N/A	N/A	N/A	N/A
Collect Algal Toxin Samples	N/A	N/A	N/A	N/A	N/A	N/A
Collect SNWS raw and finished water algal toxin sample	N/A	N/A	N/A	N/A	N/A	N/A
Algal Toxin (sample send out or transfer)	N/A	N/A	N/A	N/A	N/A	N/A
Analyze Algal Toxin Samples	Analyze up to 12 samples depending on cell count or have them sent out by SNWA Watershed Division plus 2 travel blanks, 1 duplicate, and 1 replicate	Analyze up to 12 samples depending on cell count or have them sent out by SNWA Watershed Division plus 3 travel blanks, 1 duplicate, and 1 replicate	Analyze up to 12 samples depending on cell count or have them sent out by SNWA Watershed Division plus 2 travel blanks, 1 duplicate, and 1 replicate	Analyze up to 17 samples depending on cell count or have them sent out by SNWA Watershed Division plus 3 travel blanks, 1 duplicate, and 1 replicate	Analyze up to 12 samples depending on cell count or have them sent out by SNWA Watershed Division plus 3 travel blanks, 1 duplicate, and 1 replicate	Analyze up to 12 samples depending on cell count or have them sent out by SNWA Watershed Division plus 3 travel blanks, 1 duplicate, and 1 replicate
Hydrolab profiles	N/A	N/A	N/A	N/A	N/A	N/A
Secchi depth	N/A	N/A	N/A	N/A	N/A	N/A
Wind speed	N/A	N/A	N/A	N/A	N/A	N/A
Temperature	N/A	N/A	N/A	N/A	N/A	N/A
Light transmittance	N/A	N/A	N/A	N/A	N/A	N/A

Appendix B
Total Number of Samples Collected and Analyzed

Sample Collection

Analysis	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
Ammonia	USBR – 12* NPS – 2**	USBR – 6* NPS – 2** CLV – 5**	USBR – 12* NPS – 2**	CLV – 12* NPS – 5** SNWA – 3**	USBR – 6* NPS – 2** CLV – 5**	CLV – 8* NPS – 2** NDOW – 4**
Nitrate	USBR – 12* NPS – 2**	USBR – 6* NPS – 2** CLV – 5**	USBR – 12* NPS – 2**	CLV – 12* NPS – 5** SNWA – 3**	USBR – 6* NPS – 2** CLV – 5**	CLV – 8* NPS – 2** NDOW – 4**
Nitrite	USBR – 12* NPS – 2**	USBR – 6* NPS – 2** CLV – 5**	USBR – 12* NPS – 2**	CLV – 12* NPS – 5** SNWA – 3**	USBR – 6* NPS – 2** CLV – 5**	CLV – 8* NPS – 2** NDOW – 4**
Total Phosphorus	USBR – 12* NPS – 2**	USBR – 6* NPS – 2** CLV – 5**	USBR – 12* NPS – 2**	CLV – 12* NPS – 5** SNWA – 3**	USBR – 6* NPS – 2** CLV – 5**	CLV – 8* NPS – 2** NDOW – 4**
Ortho Phosphorus	USBR – 12* NPS – 2**	USBR – 6* NPS – 2** CLV – 5**	USBR – 12* NPS – 2**	CLV – 12* NPS – 5** SNWA – 3**	USBR – 6* NPS – 2** CLV – 5**	CLV – 8* NPS – 2** NDOW – 4**
Chlorophyll a, b, and c	USBR – 12* NPS – 2**	USBR – 6* NPS – 2** CLV – 5**	USBR – 12* NPS – 2**	CLV – 12* NPS – 5** SNWA – 3**	USBR – 6* NPS – 2** CLV – 5**	CLV – 8* NPS – 2** NDOW – 4**
Algal toxins	USBR – 12* NPS – 2**	USBR – 6* NPS – 2** CLV – 5**	USBR – 12* NPS – 2**	CLV – 12* NPS – 5** SNWA – 3**	USBR – 6* NPS – 2** CLV – 5**	CLV – 8* NPS – 2** NDOW – 4**

*Duplicate and Travel Blank
**Travel Blank only

Sample Analysis

Analysis	7/12-7/16	7/26-7/30	8/9-8/13	8/30-9/3	9/13-9/17	10/4-10/8
Ammonia	USBR – 15 samples*	USBR – 7 samples* CLV – 9 samples**	USBR – 15 samples*	CLV – 21 samples*	USBR – 7 samples* CLV – 9 samples**	CLV – 15 samples*
Nitrate	USBR – 15 samples*	USBR – 7 samples* CLV – 9 samples**	USBR – 15 samples*	CLV – 21 samples*	USBR – 7 samples* CLV – 9 samples**	CLV – 15 samples*
Nitrite	USBR – 15 samples*	USBR – 7 samples* CLV – 9 samples**	USBR – 15 samples*	CLV – 21 samples*	USBR – 7 samples* CLV – 9 samples**	CLV – 15 samples*
Total Phosphorus	USBR – 15 samples*	USBR – 7 samples* CLV – 9 samples**	USBR – 15 samples*	CLV – 21 samples*	USBR – 7 samples* CLV – 9 samples**	CLV – 15 samples*
Ortho Phosphorus	USBR – 15 samples*	USBR – 7 samples* CLV – 9 samples**	USBR – 15 samples*	CLV – 21 samples*	USBR – 7 samples* CLV – 9 samples**	CLV – 15 samples*
Chlorophyll a, b, and c	USBR – 15 samples*	USBR – 7 samples* SNWS – 9 samples**	USBR – 15 samples*	SNWS – 21 samples*	USBR – 7 samples* SNWS – 9 samples**	SNWS – 15 samples*
Algal toxins	SNWA R&D or SUNY – up to 15 samples*	SNWA R&D or SUNY – up to 16 samples*	SNWA R&D or SUNY – up to 15 samples*	SNWA R&D or SUNY – up to 21 samples*	SNWA R&D or SUNY – up to 16 samples*	SNWA R&D or SUNY – up to 15 samples*

*Travel Blank, Duplicate, and Replicate

**Travel Blank and Replicate

Appendix C

QA/QC Information for Sample Collection and Sample Analysis

Interagency Method Survey

Interagency Survey of QA/ QC Procedures for City of Las Vegas and US Bureau of Reclamation - Denver

City of Las Vegas
USBR

General

1. What field QA/QC methods do you use? For example trip blanks, duplicate or replicates.

Trip Blanks- Verify cleanliness of sample bottles, filtering equipment, preservation, and handling techniques. Split Replicates- Verify handling techniques including filtering of samples and preservation.
Trip blanks and split samples and some duplicates are collected for nutrient samples.

2. For each method, what information does it provide? A trip blank for example would tell if there is contamination from sampling bottles, airborne contamination, contamination in your DI or MilliQ water, etc.

Blanks- look at value compared to detection limit. Replicates- relative percent difference.
Trip blanks are used to check for contamination during sample collection, handling, and storage. Split samples check for differences between laboratories and between analytical methods (the two USBR labs use different methods for nutrient samples). Duplicates check precision of the results obtained.

3. How do you analyze your QA/QC data? Relative percent differences, control values, etc.

Field QA/QC data is used primarily as a check for contamination. Lab QA/QC data includes RPD, spikes and external standards to verify data accuracy.

4. What do you do if you find a QC problem? Simply report the value with a qualifier, retest, etc.

If there is a QC problem we report the data and then qualifying it. We also then make sure to resolve the problem.
Samples are retested if possible. Blank corrections are applied if it appears there is a consistent source of contamination, i.e. sample bottles.

Multi-parameter probe

1. What procedure is used to calibrate before going to the filed, and after return from the field?

D.O.- air saturation/ mg/L, BP verified against wall-mounted barometer, temp. NIST checked, pH 2-point calibration 7 and 9, conductivity 2-point calibration air and 2767 lab made standard, turbidity 2 point calibration milli-Q and 50 NTU standard. Then checks are made with pH 7 buffer and 1000 us/cm purchased standard.
Manufacturer's directions are used to calibrate the Hydrloab. The unit is calibrated daily, with post-calibration if the unit appears to be drifting (very infrequent). Periodic depth calibration is performed as necessary.

2. What is the maintenance schedule? Is it documented?

Monthly cleaning using alcohol for pH and turbidity sensor, change out DO membrane and solution, Change pH reference solution and check flow through junction.
The Hydrolab is calibrated before each sampling event and maintenance is performed as needed. Maintenance is not routinely documented, although specific problems are noted in field books.

3. Are data checked before releasing to others or before input to database?

All Hydrolab data is downloaded and formatted and by the Aquatic Biologist and then double checked by the QA officer before putting in to the database
The data are checked before entry into the database.

4. Where are data stored, and in what format?

We keep all the original hard copies, store the data electronically as excel files, then place the data in our Access database and finally we send the data to the interagency website database.
The data are stored in spreadsheets in both QuattroPro and Excel formats. All downloaded data files from the Hydrolab are also stored in the original .txt format. Data is periodically stored in the SNWA database. All laboratory data is stored in the format received from the laboratories – spreadsheets and/or hard copy.

Chlorophyll

1. Are samples composite or grab samples?

Integrated 5 or 2.5 meter column
Samples are collected as surface grabs and 0-5 m composites.

2. What size of hose is used?

A pool hose (approx. 2” diameter) marked at 1-m intervals is used to collect the composites.

3. What is the depth of the sample?

Surface grabs are collected at all stations. The composite is usually 0-5 m, unless the station is too shallow.

4. How are filters treated and preserved?

GFC filters that are primed with $MgCO_3$
Samples are stored on ice and filtered immediately upon return to the laboratory. Filters are folded in half, blotted dry, placed in a paper coin envelope, and kept frozen prior to analysis.

5. How are samples preserved?

Wrapped in foil and kept at 4C (30 day holding time)
Frozen filters are kept in paper envelopes to minimize moisture and typically analyzed within 1 or 2 days after collection.

6. What is the holding time for the sample?

Typically 1-2 days, almost never more than 1 week.

7. What are the holding conditions for the sample?

See above.

<u>Secchi transparency readings</u>

1. What size disc is used? Is it black and white or all white? What size?

30 cm diameter, painted flat black and white
30 cm diameter, painted flat black and white.

2. Does the same individual take measurements?

.
No
No, although the same individual usually takes all readings on a given day, and all readings are taken by the same group of about four people.

3. Does anyone check the measurement, ever?

Sometimes
Readings are rarely taken by a second person. Differences between two people are usually about the same as two readings taken by the same person as a result of changing conditions.

4. Is a viewscope used? If not, what consideration is made for choppy waves and sun glint?

A viewscope is used when needed- i.e. large glare or rough conditions.
A viewscope is used except when Secchi depth is less than 0.5 m. Reading both with and without the viewscope are reported.

Phytoplankton (algae) sampling

1. How are samples collected? With a net or directly from a composite water sample?

Phytoplankton samples are collected from a composite water sample at most stations, at as surface grabs near the inflows.

2. If from a composite water sample, what depths does that sample include and how is it collected?

The same composite used for the chlorophyll *a* samples is used for phytoplankton.

3. What depths are collected, 0 to 5 meters or other?

0-5 meters

4. What size net is used, mesh size of net, and mesh size of cup? These are critical.

N/A

5. How fast is the net pulled upward through the water column? Are the speeds consistent?

N/A

6. How are samples preserved and how fast are they preserved?

Samples are placed on ice immediately after collection and are preserved with 25% glutaraldehyde to a final concentration of approximately 1% (about10 mls/L) upon return to the laboratory, usually within 6 hours of collection.

Other

1. Are weather conditions noted during each sampling event?

Yes- temp, wind speed and direction and cloud cover
Yes. Air temperature, wind speed and direction, and general weather conditions are noted.

2. Is the sampling carried out during the same time of day and in the same order of sites for each event?

Yes always from least to most concentrated sites
Samples are collected at about the same time of day and stations are usually collected in the same order, although wind can cause variations.

3. Other comments?

GPS coordinates are measured for each sampling station on each date.

Appendix D

QA/QC Information for Sample Collection and Sample Analysis

United States Bureau of Reclamation Procedures

UNITED STATES BUREAU OF RECLAMATION METHODS AND MATERIALS

Locations

Table 1 describes all sampling location used by the Bureau of Reclamation (Reclamation) for limnological studies on Boulder Basin. Names of all sampling stations in Lake Mead were changed in 1999 through the efforts of the Interagency Sampling Committee, a group open to all agencies with interests in Lake Mead. The old sampling site designations used in previous studies are also included for reference to older data sets.

Sites in bold were sampled in 1999. Limnological sampling was done at a series of sites (LVB0.8 - CR342.25) in a linear transect from the Las Vegas Wash inflow (LVB0.8) to the immediate forebay of Hoover Dam (CR342.25). All sites were located at the deepest point of the thalweg or historical stream channel determined using a depth finder. Sites were sampled a minimum of once monthly, October through March, and twice monthly, April through September.

At each site, the limnological methodology was replicated for each station and on each date. Surveys were carried out at nearly the same time of day on every occasion. Weather permitting; surveys began at LVB0.8 at about 0800 hours. Sampling continued Adownstream@ toward Hoover Dam (CR342.25) which was surveyed between as near 1300 hours as practicable. Occasionally the deeper open water stations were sampled first to avoid predicted winds.

Reservoir Elevation

Data used to calculate historical reservoir elevations was obtained from the Bureau of Reclamation, Boulder Canyon Operations Office. Those data were put into a spreadsheet and daily averages used to plot reservoir surface elevations.

Analytical Procedures

Standard analytical procedures are used for Bureau of Reclamation samples. Methods are summarized in Table 2.

Physical-Chemical Profiles

Measurements were done from surface to bottom of temperature, dissolved oxygen, pH, specific conductance, and turbidity. Data were collected at 1 m-depth intervals at least through the thermocline, then 2 to 5 m thereafter depending on uniformity of the water column. Profile data were collected using a Surveyor 4 Sonde unit connected to a Surveyor 4 data recorder. Both units are products of Hydrolab Corporation[7]. The Sonde unit was temperature compensated for conductivity and, at a given temperature, repeatability of measurements was within 0.2 percent.

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Table 1 – Bureau of Reclamation Sampling Locations for Boulder Basin of Lake Mead

New (Old) ID	Location*	Description
LVB 0.8 (LV00)	36E 07' 47.72" N 114E 52' 55.37" W	Within 30 meters of Las Vegas Wash Discharge to within the flow, moving station depending on the depth of the lake. Depth is <2m**.
LVB1.5 (LV01)	36E 07' 51.78" N 114E 52' 18.19" W	Within channel on top of Ashelf@ that becomes flow when lake elevation low. Depth is 3 to 10 meters when sampled.
LVB1.8 (LV02)	36E 07' 42.93" N 114E 51' 58.40" W	Between the rocky points north side of bay in deepest hole. Depth is 20 to 25 m, similar to City=s LM2 North.
LVB2.15 (LV03)	36E 07' 31.83" N 114E 51' 45.04" W	Just off old swim beach, center channel at opening to inner bay. Depth is 25 to 30 m.
LVB2.3 (LV04)	36E 07' 26.63" N 114E 51' 36.81" W	Las Vegas Bay Boat Harbor, center channel. Used mostly in 1991-93. Depth is 25 to 30 m.
LVB2.6 (LV05)	36E 07' 13.88" N 114E 51' 29.71" W	Off of the Las Vegas Bay Boat Launch Ramp, center channel straight off end of tire barrier (not reliable measure). Depth is 30 to 35 m.
LVB2.7 (LV06)	36E 07' 10.78" N 114E 51' 20.64" W	First marked buoy, was "I" before 1991, now marked "C." Used mostly in 1991-93. Depth is 30 to 35 m.
LVB3.1 (LV07)	36E 07' 06.16" N 114E 50' 55.48" W	Halfway between first two buoys. Used mostly in 1991-93. Depth is 35 to 40 m.
LVB3.5 (LV08)	36E 07' 03.41" N 114E 50' 30.83" W	Second marked buoy, was "H" before 1991, now "B." Depth is 45 to 50 m.
LVB3.85 (LV09)	36E 07' 01.04" N 114E 50' 07.34" W	Halfway between second and third buoys, "Twin Peaks." Used mostly in 1991-93. Depth is 55 to 60 m.
LVB4.15 (LV10)	36E 07' 00.08" N 114E 49' 46.77" W	Third marked buoy, was "G," now marked "A." Depth is 55 to 60 m.
LVB4.45 (LV11)	36E 06' 49.50" N 114E 49' 31.84" W	Old buoy "F" now gone, small rocky islands emerge. Used mostly in 1991-93. Depth is 65 to 70 m.
LVB4.95 (LV12)	36E 06' 35.58" N 114E 49' 11.19" W	Old buoy "E" now gone, flagged island that emerges when lake is lower. Recently flagged as a boat lane, Red and Green #5. Depth 75 to 80 m.
LVB5.7 (LV13)	36E 06' 11.58" N 114E 48' 30.69" W	Old buoy "D" now gone, between fish hatchery and volcanic islands. Used mostly 1991-93. Depth is 90 to 95 m.
LVB7.3 (LV14)	36E 05' 27.71" N 114E 47' 14.97" W	Old buoy "C" now gone, deepest portion of thalweg directly between Saddle & Black Islands about 2.3 km off of Saddle Island Intake area. Depth is 120 to 125 m.
CR346.4 (LV15)	36E 03' 39.47"N 114E 44' 19.50" W	Deepest area between Sentinel Island and Fortification Hill in old thalweg of the Colorado River. Depth is 145 to 150 m.
CR352 (LV16)		Deepest area center at Calville Bay. Depth is 145 to 150 m. Used infrequently.
CR342.25 (LV17)	36E 01' 03.25" N 114E 44' 09.50" W	Deepest area at Hoover Dam. Center of buoy line. Depth is 140 to 145 m.

*Coordinates determined by GPS (NAD 1983).

**Depths determined at a Lake Mead surface elevation of 1211'.

Table 2 – Bureau of Reclamation Analytical Methods

Parameter	**Method**	**Reference** [1]
Temperature	Thermistor, in situ	2550 B
Dissolved Oxygen Concentration	Membrane electrode, in situ	4500 – O G
Dissolved Oxygen Saturation	Membrane electrode/thermistor, in situ	4500 – O G
Conductivity	Conductivity cell, in situ	2510 B
pH	Electrometric, in situ	4500 – H^+B
Turbidity	Nephelometric, in situ	2130 B
Secchi depth	Visual observation, in situ	N/A
Orthophosphate total	Ascorbic acid colorimetric method	4500 – P E
Ammonia - N	Nesslerization	4500 – NH_3C
Nitrate - N	Uv spectrophotometric method	4500 – NO_3 B
Chlorophyll a	Spectrophotometric method	10200 H
Phytoplankton	Enumeration	10200 F
Zooplankton	Enumeration	10200 G

[1] Method reference's are from Standard Methods for the Examination of Water and Wastewater, 18th Edition, APHA, AWWA, WEF, 1992, unless otherwise stated

Water Transparency and Color

Water transparency at each sampling site was measured with the naked eye using a standard black and white Secchi disc in early years. In the later years (>1995) a solid white Secchi disc was used and two readings were always recorded. One with the naked eye and a second using a meter long viewscope that was black inside and included a tilted clear plastic base. Use of the viewscope greatly reduces the influences of wind (and resulting wave action), and variable light thus equalizing readings from location to location and time to time. In every case the readings with a viewscope are either equal to (< 4 m readings), or greater than (> 4 m readings) those recorded with the naked eye. A plastic measuring tape marked in centimeters and meters was used to lower the disc until it was no longer visible.

Color is a qualitative measure, although a numerical value is assigned. A color strip developed for use by volunteers who monitor lake conditions in Ohio was used during this study. The basic principal of a color strip is to make a qualitative notation of whether lower Secchi readings are due to sediment (brown in color) or algae (various shades of green). The intensity of each is distinguished by the scale of numbers assigned from 1 through 11. The value A1" denotes clear water. Colors 2-6 are increasing intensities of green associated with algal blooms, and colors 7- 11 are increasing intensities of brown colors associated with silt in the water column.

Nutrients

Nutrients (inorganic phosphate, total [PO_4-P], ammonia [NH_3-N], and nitrate [NO_3-N]) were collected from the surface and from depths of 1 m, 3 m and the plume depth. Surface samples were collected as grabs and a Van Dorn sampler was used to collect samples from the other depths. Sample analyses were performed by the Bureau of Reclamation=s Lower Colorado Regional Laboratory in Boulder City, Nevada.

Additional samples for analyses of organic and inorganic nitrogen and phosphorus were collected for analysis by the Clark County Sanitation District (CCSD) Water Quality Laboratory beginning in November 1999 and will continue on a quarterly basis. Methods used by CCSD allow for lower detection limits for the nutrients analyzed and this sampling will continue on a quarterly basis. The results from these surveys, which are continuing on a quarterly basis, will be reported beginning with the year 2000 monitoring report.

Selenium

Samples for analysis of selenium were collected from surface and plume of each sampling location beginning in April 1999. These samples are part of a one-year investigation of a basin-wide investigation of the fate and transport of selenium in the entire Colorado River basin. The Principal Investigator for the project is Mr. Jerry Miller, Water Quality Specialist, Upper Colorado Region, Bureau of Reclamation, and the project is supported by Congressionally appropriated funds to Reclamation. Results will be discussed in the 2000 monitoring report.

Perchlorate

Collection by Reclamation of water samples from Lake Mead for analysis of perchlorate (ClO4) began in early 1998. Samples are collected during each of the regular sampling events from surface and the plume. Additional samples were collected on May 11, 1999, when Station LVB2.6 was sampled at one-meter intervals from surface to bottom. Since the discovery that Las Vegas Wash, via shallow groundwater sources, contributes perchlorate (a by-product of rocket fuel production) to the watershed, monitoring of this inorganic compound has become important. Additionally, its presence serves as a good marker of how inflows from Las Vegas Wash behave limnologically within Boulder Basin. Data from both 1998 and 1999 are included in this report. The Southern Nevada Water Authority, Southern Nevada Water System Chemistry Laboratory located at Saddle Island on Boulder Basin provided sample bottles and performed all perchlorate analyses for this study using ion chromatography. Mr. John Fronk, Chemistry Laboratory Supervisor, provided results from those analyses.

Bacteria

Samples for bacterial analyses were collected during each sampling event and from each sampling location in 1998 and 1999. Bacteria samples were also collected from the surface and plume and were analyzed for the presence and enumeration of fecal coliform bacteria and specifically the E coli component. More intensive sampling was done on six dates in 1998 and 1999, once at Station LVB2.15 and five times at LVB2.6. Samples for these events were collected at one-meter intervals on either side of the plume, and from surface to bottom of the water column. The Southern Nevada Water Authority, Southern Nevada Water System Microbiology Laboratory at Saddle Island Properly provided sterile collection bottles and performed all bacterial analyses for the study. Results in this report are those provided by Ms. Peggy Roefer, Microbiology Laboratory Supervisor.

Chlorophyll *a*

Water samples for analyses of chlorophyll *a* were collected at each location. A grab sample was collected from surface and a Van Dorn sampler was used to collect water from depths of 1 and 3 meters. A composite sample from the surface to 5 meters was collected using a flexible hose manufactured for use in cleaning swimming pools. The pool hose was rinsed by filling the hose and discarding the first composite sample collected at each location. All samples were decanted into 1-L Nalgene bottles and placed into a cooler with ice. Immediately upon returning to the laboratory, 650 mL samples were filtered through Whatman GF/C fiberglass filters using a Millipore Corporation manifold and vacuum pump apparatus at 238.4 kilopascals. The filters were suction-dried and then folded in half (with sample surface inside) and pressed with paper towels to assure maximum drying. Filters were placed in individual manila coin envelopes and stored in the laboratory freezer until processing.

Spectrophotometric processing of chlorophyll *a* samples was always performed within two weeks after collection and usually within 7 days. Extraction methods used followed procedures described by Holm-Hansen and Reimann (1978). This method uses acetone as the extracting agent. A Shimadzu Model UV2101PC spectrophotometer was used to measure absorbance. Chlorophyll *a* concentrations were calculated from analytical results using the trichromatic equations reported by Jeffery and Humphrey (1975). This laboratory method is also described in APHA (1992) as Method 10200 H (2).

Phytoplankton

Since 1994, plankton samples have been collected from the same composite water used for the chlorophyll *a* samples. The sample collected from LVB0.8 was always a grab surface sample because of the shallow depth of this station. Samples were placed on ice immediately following collection and were preserved with 1 % glutaraldehyde solution upon return to the laboratory.

Identification, enumeration, and biovolume calculation of phytoplankton was performed by Dr. Ann St. Amand and her staff at PhycoTech, Inc., St. Joseph, Michigan. The HPMA method for producing algal sample slides provides an optically clear background while permanently infiltrating and preserving the sample for archival purposes (Bergquist

1985, Crumpton 1987, St. Amand 1990). Wet samples are always maintained in case clarification of identification is necessary. The microscope used to process all samples is an Olympus BHT, research-grade compound microscope equipped with Nomarski optics (100x, 200x, 400x, and 1000x), Phase Optics (400x), epifluorescence, and a trinocular head for photography. The magnification used depends on the size of the dominant taxa and the size and number of particulates. The goal is to count at multiple magnifications in order to correctly enumerate and identify taxa present that may vary by several orders of magnitude in size. If the sample is dominated by cells below 10-20 :m or the cells are fragile and difficult to identify, the majority of counting will be completed at 400x-1000x. The goal, regardless of magnification, is to enumerate and identify a minimum of 400 natural units per sample exclusive of miscellaneous microflagellates. QA/QC is performed on approximately every 10th sample, for random distribution and identification verification.

Measuring biovolume included measuring the greatest axial linear dimension (GALD), which is the length, width and depth of different aspects of the colony or cell. Cell and colony shapes are approximated to a geometric figure and or figures and the appropriate calculations made. These measurements provide a general indication of the edibility of an algal cell for zooplankton, with GALD measurement of <30 Φm usually considered edible and GALD >30 Φm usually considered inedible. Up to a total of 30 natural units (sometimes higher on exceptionally variable taxa) are measured for each taxa depending on variability and number encountered.

All data are recorded on physical count sheets and the raw data is transferred to a data analysis and management program. A proprietary program written for PhycoTech in C language records all measurements, does all calculations for concentration and biovolume and then generates reports. The program allows the retention of 100% of the input data for future reference and error checking.

For presentation purposes species of algae were grouped under the major groups: blue-green (Cyanophyta), green (Chlorophyta), diatoms (Bacillariophyceae) , yellow-brown (Chrysophyta), euglenoids (Euglenophyta), dinoflagellates (Pyrrhophyta), cryptomonads (Cryptophyta), and miscellaneous (includes microflagellates, unidentifiable cysts, and Gonyostomum semen which is in the division Chloromonadophyta).

Zooplankton

Zooplankton samples were collected with a Wisconsin-style plankton net with 80 Φm mesh towed from 5 m below the surface to the top of the water column. Zooplankton samples were collected at all sampling stations except for LVB0.8 and were placed on ice immediately after collection and then preserved with Lugol=s solution upon return to the laboratory. All analyses were performed by Dr. John Beaver, BSA Environmental Services, Beachwood, Ohio. One mL aliquots were placed in a Utermohl chamber and zooplankton were counted at 100X until at least 200 individuals were tallied. Identification followed Edmundson (1959), Ruttner-Kolisko (1974), and Pennak (1989). For presentation purposes, species and or genera were grouped to the major kinds of

zooplankton: copepods, cladocerans, ostracods, and rotifers. These summaries are presented in this report.

Appendix E

QA/QC Information for Sample Collection and Sample Analysis

City of Las Vegas Standard Operating Procedures

City of Las Vegas

Environmental Division Laboratory
Standard Operating Procedures
Lake Mead Sampling

PRINCIPLE

The Lake Mead Sampling program is a compilation of standard operating procedures that define the steps necessary for collecting NPDES permit required samples.

BACKGROUND

The City of Las Vegas (CLV), Clark County Sanitation District (CCSD) and the City of Henderson (COH) have entered into a cooperative agreement concerning the water quality monitoring on Lake Mead and Las Vegas Wash in compliance with their NPDES Discharge Permits. The Lake Mead sampling is conducted by the CLV, the Las Vegas Wash sampling and analysis is conducted by the COH and the laboratory analyses on all of the lake samples and TKN, TOC and Chl-*a* on the wash samples are conducted by the CCSD.

City of Las Vegas	Clark County Sanitation District	City of Henderson
Lake Mead Sampling Collection of Samples **Hydrolab Profiles at All Stations** **Temperature** **Dissolved Oxygen** **pH, &** **Conductivity** **Secchi Depth** **Air Temperature** **Light Transmittance** **Light Transparency** **Wind Direction and Speed** **Zooplankton Sample Collection** **Zooplankton Analysis**	**All Chemical Analyses on Lake Mead Samples** **Chl-*a*, Phae-*a*, TDS, ALK, Cl-, SO4, TP, DisOP, Nitrate/Nitrite-N, Nitrite-N, Nitrate-N, Ammonia-N, TC, FC, TSS, TN, HPC, E. Coli, Color & Turbidity** **Chemical Analyses on Las Vegas Wash Samples** **TKN**	**Las Vegas Wash Sampling Collection of Samples** **Profiles at All Stations** **Temperature** **Dissolved Oxygen** **pH** **Conductivity, &** **Turbidity** **Air Temperature** **Wind Direction and Speed** **Analyses on Las Vegas Wash Samples** **TP, DisOP, Nitrite-N, Nitrate-N, Nitrate/Nitrite-N, Ammonia-N, TSS, TDS, NTU, Cl-, SO4, T/C, F/C, & Toxic Parameters listed in Table 1 of Attachment A**

APPLICATION AND PERMIT REQUIREMENTS

The lake monitoring is performed weekly March through October and monthly November through February. Lake Mead sampling stations LVB1.8M and LVB1.85M are sampled weekly. LVB2.7, LVB3.5 and BB3 are sampled biweekly. CR346.4 and CR350.0SE0.55 are sampled monthly.

In the Water Quality Standards established by NDEP, limits were set on the levels of chlorophyll-a and unionized ammonia in the lake samples. The unionized ammonia standard of a 4 day average concentration is not to exceed 0.04 mg/L and a single value not to exceed 0.45 mg/L at stations LVB1.8M. The chlorophyll-a standard of a monthly mean of not more than 45 □g/L and a mean summer (July-September) value not to exceed 40 □g/L was set at stations LVB1.85M. In addition to monitoring these parameters for water quality standards, several other constituents are also analyzed, including: fecal coliform, nitrate -N, nitrite-N, ammonia-N, total phosphorus (TP), dissolved ortho-phosphorus, total dissolved solids (TDS), total suspended solids (TSS), TN, alkalinity, color, HPC, E. Coli, Fecal Coliforms, turbidity, sulfate and chloride.

In addition, to collecting the field samples the CLV is responsible for measuring temperature, dissolved oxygen, pH, conductivity, light transmittance, and light transparency of the water at each of the sampling stations. The wind direction, air temperature and wind speed are also recorded at each station. Zooplankton samples are collected biweekly at stations LVB1.8M, LVB1.85M, LVB2.7, LVB3.5, BB3 and monthly at CR396.4 & CR350.0SE0.55. These samples are analyzed by the CLV.

SAMPLING LOCATIONS

Stations LVB1.8M and LVB1.85M represent the Inner Las Vegas Bay in Lake Mead. Stations LVB2.7 and LVB3.5 represent the Middle Las Vegas Bay area. With Boulder Basin being represented by station BB3 and CR346.4. The background concentrations to Boulder Basin are measured at CR350.0SE0.55. An historical database has been maintained by the Limnology Research Center at UNLV since 1976 for stations LM 2, 5, and 8 (now LVB1.8M, 3.5 and CR346.4 respectively). Stations LM 3 and 4 (now LVB1.85M and LVB2.7 respectively) have been sampled since 1979. The location of stations LVB2.7, 3.5, and CR346.4 have remained the same since they were first established. Stations LVB1.8M and 1.85M have been moved periodically to maintain an approximate sampling depth of 10 m at LVB1.8M and 16-18 m at LVB1.85M.

SAMPLE BOAT OPERATION

The boat is transported on a trailer from the CLV Water Pollution Control Facility to the lake for each sample episode. Prior to leaving the facility boat operating condition is verified using the Lake Mead Sampling & Boat Routine Inspection Form (please see attached). All of the sampling equipment is loaded into the boat and secured before leaving for the lake. The bilge pump, blower, oil, drain plug, running lights, and anchors are checked and verified to be in operable condition periodically. The jet drive is lubed periodically.

Boat Launching Procedures:
The trailer is backed down just to the water. The boat safety chains and trailer lights are disconnected at this time.

The boat operator then gets into the boat and turns the engine blower on.

The trailer is then backed down the launch ramp into the water. It is important to keep the trailer straight and level as it enters the water. The trailer will stop backing up when the water level reaches the top of the trailer tire wells.

It is important that the blower be run for at least 1 minute prior to turning on the engine. The boat is then started in neutral and allowed to idle for a couple of minutes. The boat operator will then turn the bilge pump on and check the pump strainer to make sure it is not leaking.

The trailer is then backed further into the water until the boat is just afloat. The boat is then shifted into reverse and backed off of the trailer. The trailer is then parked in the parking area.

General Boat Operations:
Only persons who have successfully completed the United States Coast Guard Boat Safety and Seamanship Course or the NDOW Boating Safety Course may operate the boat.

When in a wakeless area (marinas and the Inner Las Vegas Bay) the boat is operated at 900-1100 rpm or approximately 5 mph. Under no circumstances is the boat to be shifted at an rpm greater than 900. When stopping the boat, it must be slowed down gradually so the boat wake does not back flood into the exhaust manifold. When at a station, it is critical that the boat be kept in position while sampling. If it is calm or if the boat can be held by the anchor, turn the boat off. If the wind is blowing and the boat is unable to be anchored, back into the wind and idle the boat in neutral and reverse to hold it in position. The boat is operated according to standard U.S. Coast Guard Navigational Rules, including coming to the assistance of boaters in distress. Under no circumstances is the boat be used for any purpose (water skiing, fishing, sun bathing, etc.) other than water quality sampling.

After the sampling is completed the boat is loaded on the trailer according to the following steps:

The trailer is backed down the launch ramp into an open spot. It is important to keep the trailer straight and level as it enters the water. The trailer will stop backing up when the water level reaches the top of the trailer tire wells. The boat operator then idles the boat on to the trailer, making sure to keep the boat straight, until the bow of the boat is firmly against the nose guard on the trailer. The operator of the boat then connects the winch and safety chains to the bow of the boat. If the boat operator is not able to connect the winch or safety chains, then the truck driver puts the truck into park, engages the parking brake, and assists in connecting the winch and chains. When the boat is secured, it is shut off, and the trailer is pulled out of the water slowly. The boat operator makes sure that the bilge pump, blower, and radio are turned off. The antennas are lowered. The trailer is then driven to the CCSD to

deliver the samples and then back to the CLV facility. When we arrive back at the facility all the sampling equipment is unloaded and the boat logbook is filled out. The boat and truck are filled with gas after each run.

SAMPLING SAFETY PROCEDURES

Both the designated boat operator and a stand-by operator are on the boat for each run. All boat operators are required to have successfully passed the United States Coast Guard Boat Safety and Seamanship Course or the NDOW Boating Safety Course.
The boat is equipped with a fire extinguisher, water resistant flare kit, marine band radio, cellular phone, first-aid kit, and life jackets for everyone on board.

Prior to each sampling run the National Weather Service Office at McCarran Airport will be called to get an up-to-date weather forecast for Lake Mead. If any of the following conditions are forecasted the run is postponed:

winds of 25 mph or greater
winds of less than 25 mph when gusts are predicted to exceed 25 mph
rain or thunderstorm activity.

Winds of less than 25 mph are a nuisance to work in, however they do not pose a safety hazard. If at any time during a run the weather conditions change and the safety of the crew is at risk, return to the marina and finish the sampling when conditions improve. The Aquatic Biologist and/or boat operator (in the absence of the Aquatic Biologist) makes this decision.

Prior to use of the boat all safety equipment will be checked and verified as available and in operable condition. This verification shall include, but is not limited to the following: verify fire extinguisher is full (gauge on green), count life jackets, check first aid kit for missing contents, and test marine band radio.

Whenever the boat is in motion everyone will remain seated.

Life jackets are to be worn whenever the boat is going faster than wakeless speed. The Aquatic Biologist may also decide to have the crew wear life jackets while sampling if conditions dictate.

All members of the sampling crew including alternates should be capable of swimming. CPR and basic first aid training should be given to all members of the sampling crew and renewed biannually.

In the event that one of the crew members has to enter the water, another crew member will monitor the activity with a life saving/flotation device in hand. The boat is equipped with twice the floatation necessary for its size, therefore in the event that the boat should sink the bow will remain above water giving the crew something to hold on to. In addition, the life jackets are a bright color, which is easily seen in the water. The flare kit is in a water resistant container stored just behind the drivers seat.

SAMPLE COLLECTION

Sampling Preparation

Prior to each sampling run the chain of custody sheets are prepared and the sample containers are tagged and put into individual bags for each station. The tags must contain the following information: station, chain of custody number and line number, number of grabs, if preserved, filtered or unfiltered, depth, collectors initials and date. At this time we are using the CCWRD's tags and custody sheets because they are analyzing the samples.

Sample Types:
Integrated Sample (0-5 m or 0-2.5 m)
Integrated water samples are taken from the surface to a depth of 5 or 2.5 m with a flexible hose 4.5 cm in diameter. The bottom end of the hose is weighted and has a line running to the surface. At each station the hose is flushed by lowering it into the water and raising it out. The hose is then slowly lowered into the water until the 5 or 2.5 m mark is just below the surface. Make sure that the hose is in a vertical position. Place the stopper in the top end of the hose, forming a seal, and raise the weighted end with the rope. Place the weighted end into a 5-gallon polyethylene carboy, which has been rinsed with surface water, and release the stopper thereby draining the water into the carboy. Repeat this procedure until an adequate volume of sample has been obtained. The integrated sample is then thoroughly mixed by shaking before each container is filled. All sample containers are rinsed with sample prior to being filled.

Discrete Samples:
Discrete water samples are collected from selected depths, which represent regions within the metalimnion (transition zone from warm to cold water), or hypolimnion (cold unmixed layer). The sample is collected using an opaque, non-metallic, polyvinlychloride (PVC) van Dorn water bottle, which is suspended from a line, marked in 1-meter increments. The van Dorn bottle is lowered to the selected depth and a weight (messenger) is sent down the line to trip the closing mechanism on the bottle. The bottle is then retrieved to the surface where its contents are emptied into a 5-gallon polyethylene carboy. Make sure to rinse the carboy with a little of the sample prior to filling. The procedure is repeated as many times as necessary to obtain enough sample. The discrete sample is thoroughly mixed by shaking before each container is filled. All sample containers are rinsed with sample prior to being filled.

Sample Handling and Treatment

After the samples are collected and the individual containers are filled, those requiring filtration or preservation will be filtered and preserved in the field. The following sample treatments are performed for each analysis:

Chlorophyll-a Samples:
Filtered through GF/C glass fiber filters in the field after being preserved with 3-4 drops of a saturated $MgCO_3$ solution. The filter is folded in quarters and placed in an aluminum foil packet, which is labeled with the date, station, chain of custody number, and volume filtered. The packets are kept on ice until reaching the laboratory. Please refer to procedure guidelines that are stored in the filtering kit.

Dissolved Ortho Phosphorus Samples:
Filtered through 0. 45 □m pore size sterilized, membrane filters in the field. The sample is collected in a 250 mL Nalgene bottle. The sample is kept on ice until reaching the laboratory. Please refer to procedure guidelines that are stored in the filtering kit.

Fecal Coliform, E. coli, and HPC Samples:
Collected in sterilized, preserved 250 mL wide-mouth bottles and kept on ice until reaching the laboratory.

TDS, TSS, Chloride, Nitrite and Nitrate, and Archive Samples:
Collected in a 1 L Cubitainer and kept on ice until reaching the laboratory.

Ammonia, TN and Total Phosphorus Samples:
Collected in a half filled 1 L Cubitainer and preserved with 0.5 mL of concentrated H_2SO_4. The samples are kept on ice until reaching the laboratory.

Zooplankton Samples:
Collected with a 80 micron plankton net and put into a 50 mL vial. The sample is preserved with 10% solution formalin buffered with sucrose and kept on ice until reaching the CLV plant.

All samples except the zooplankton sample are delivered to the Clark County Water Reclamation District Laboratory and received by their personnel. City of Las Vegas Laboratory personnel analyze the zooplankton sample for species identification and enumeration.

<u>FIELD SAMPLING EQUIPMENT</u>
The following equipment is used during the water quality monitoring on Lake Mead:

Hydrolab Surveyor 3/H_2O
Hydrolab Surveyor 4a/H_2O

The Hydrolab is used to measure water temperature, dissolved oxygen, conductivity, turbidity, barometric pressure, depth, and pH. The instrument consists of a deck unit from which readings are taken, a submersible unit with multiple probes for measuring the parameters, and an underwater cable (25 m or 140 m long) connecting the two units.

Hydrolab Surveyor 3/H20

PARAMETER	RANGE	ACCURACY	RESOLUTION
Temperature	-5 to 50°C	±0.15°C	0.01°C
Specific Conductance	0 to 100ms/cm	±1% of range	4 digits
pH	0 to 14 units	±0.2 units	0.01 unit
Dissolved Oxygen	0 to 20 mg/L	±0.2 mg/L	0.01 mg/L

Hydrolab Surveyor 4a/H2O

Parameter	Range	Accuracy	Resolution
Temperature	-5º to 50°C	±0.10°C	0.01°C
Specific Conductance	0 to 100 mS/cm	±1% of reading ±0.001 mS/cm	4 digits
pH	0 to 14 units	±0.2 units	0.01 units
Dissolved Oxygen	0 to 20 mg/L	±0.2mg/L	0.01mg/L
Depth/0-200m	0 to 200m	±0.6m	0.1m
Turbidity (Shuttered)	0 to 100 or 100 to 1000 NTU	±2.6% of range	0.1 or 1 NTU
Barometric Pressure	500 to 850 mmHg	±10 mmHg	0.1 mmHg

Calibration of the instrument is performed prior to each sampling run. The instrument is set in a stand with the probes upright, a calibration cup is attached to the unit and filled with tap water (hard water preferably). It is recommended that the unit be set up at least 1 hour prior to calibration (overnight is preferred). Record all calibration changes on the calibration sheet (Figure 1 and 2).

Measurements are made by lowering the submersible unit from the water surface (0 m) to

the bottom by an underwater cable marked in 1-meter increments. Temperature, pH, conductivity, turbidity and dissolved oxygen readings are taken at each depth (surface to bottom by 1-meter intervals at stations LM 2-5; at station LM 8, LM11 and LM12 readings are taken every meter to 30 m, every 5 m to 100 m, and every 10 m to 140 m or bottom). Dissolved oxygen concentrations are read last to allow time for the probe to equilibrate.

LI-COR Model LI-1000 Data logger

The LI-COR Model LI-1000 Data logger is used to measure photosynthetically active radiation (PAR; 400 to 700 nm) or light transmission. The unit is comprised of a deck unit from which readings are taken, a submersible Quantum sensor attached to an underwater platform, and an underwater cable (30 m long) marked in 1-meter intervals connecting the two. The instrument is capable of giving instantaneous light measurements with a resolution of 25 picoamp on the low scale and a resolution of 25 nanoamp on the high scale. The readings are reported as micromol•second^{-1}•meter^{-2} (□mol•s^{-1}•m^{-2}). The instrument can be used to measure PAR both underwater and in the atmosphere.

Measurements are made at the surface and then every meter until the 1 percent light level (the depth at which the reading equals 1 percent of the surface value) is determined. Make sure to take the measurements on the side of the boat with direct sunlight. The 1 percent light level is the bottom of the euphotic zone, which is where most photosynthetically active algae occur.

Secchi Disk

The secchi disk is used to measure light attenuation or transparency in the water. The disk is a flat black and white painted metal disk approximately 30 cm in diameter which is suspended from a tape measure. The measurement is made by lowering the disk into the water on the shaded side of the boat. Lower the disk until it just disappears and note the depth, then raise it until it is just visible and note this depth. Repeat the process twice to determine an average depth at which the disk is just visible. Report the depth to the nearest 0.1 m.

Integrated Samplers

The integrated samplers are flexible hoses 4.5 cm in diameter and either 2.5 m or 5 m long. They are used to collect water samples, which represent a column of water from surface to 2.5 m or 5 m in depth.

Van Dorn Sampler

The Van Dorn sampler is an opaque, non-metallic, PVC bottle used to collect discrete water samples.

Polyethylene 5 gallon Carboy

The carboys are filled with the water samples after collection. The individual sample containers are filled from the water in the carboy. The carboy is thoroughly shaken prior to filling each container.

Filtering Equipment
The field filtering equipment is comprised of a 12 volt battery powered vacuum pump attached to four filtering flasks, four filtering funnels, filters (GF/C and membrane), forceps, preservatives (saturated $MgCO_3$, concentrated H_2SO_4, and chloroform), disposable 1 mL pipettes, a 1000 mL graduated cylinder, aluminum foil and labels for chlorophyll-a samples, distilled water and 2.4 N HCl to clean the funnels and flasks.

Wisconsin Style Plankton Net
The Wisconsin style plankton net is used to collect zooplankton samples. The net has a top opening of 13 cm and is 23 cm long. The bottom of the net is connected to a brass straining bucket 6 cm long. The size of mesh on both the bucket and the net is 80 microns. The top of the net is attached to a rope marked in 1-meter increments. The net is lowered to 40 m or 1 m above bottom, whichever is shallower, and is slowly retrieved to the boat (approximately 0.33 m/sec). The zooplankton are rinsed from the sides of the net and into the straining bucket with lake water, a 50 mL vial is then placed under the bucket drain and the pin is removed. The zooplankton sample is rinsed into the vial with lake water. Preserve the sample with enough concentrated formalin/sucrose solution to make a 10% concentration (for a 30 mL sample, add 3 mL formalin/sucrose). Place the preserved sample on ice until reaching the laboratory where it should be refrigerated until analyzed.

Combined Anemometer/ Thermometer
The combined unit is used to measure the ambient temperature and average wind velocity.

Ice Chests and Water Cooler
Ice chests are used to keep all the water samples iced. A small cooler is used to keep the zooplankton samples iced. The water cooler holds drinking water for the crew.

Field Sampling Equipment Maintenance
After each sampling run all equipment is rinsed and cleaned prior to being stored.
The Hydrolab probes are rinsed with distilled water and stored in tap water (hard water only) after each run.

The dissolved oxygen membrane is changed monthly or whenever readings are erratic and calibration is not maintained. Fresh electrolyte is added each time the membrane is changed.

The conductivity probe is cleaned with alcohol monthly and the electrodes are polished with emery cloth when they become coated with a film.
The pH reference probe is checked routinely to make sure it is full of solution and that the porous junction has not become plugged. If the junction is plugged, replace it immediately by following the instructions in the Hydrolab manual.
All meters should be kept dry and clean.
Re-charge Hydrolab (refer to Hydrolab SOP for instructions).

The zooplankton net is hung out to dry after each run and it is cleaned monthly in a mild detergent.

All instruments are periodically sent into the manufacturer for servicing and re-calibration.

MONITORING PROCEDURES

The morning of the run the Hydrolab is calibrated and all the field equipment is loaded for the trip to the lake. Bags of ice are put into each of the ice chests and the water cooler is filled with ice and water. Before leaving for the lake a call is placed to the National Weather Service to get the daily weather forecast for the lake.

Upon arriving at the lake the Hydrolab bucket is filled with lake water and the Hydrolab is put together and placed in the bucket (make sure that the probes are in water at all times). The vacuum pump is connected to its battery and the filtering gear is laid out.

We always start with the station farthest away from the incoming effluent, so we go from dilute to concentrated water. This decreases the risk of contamination from one station to the next.

One person does the Hydrolab while a second person measures the secchi depth, light (PAR), wind velocity, and air temperature. The third member of the crew operates the boat and keeps it in position during the sampling. If the boat is anchored they help collect the water samples.

The integrated water samples are collected next and the discrete samples are collected when the appropriate depths are found according to the Hydrolab readings. The metalimnion sample is collected at the mid depth when the lake is destratified and at the thermocline when one is present. The hypolimnion sample is collected at 70 m or bottom; whichever is shallower.

The measurements for each station are recorded on a data sheet along with any comments regarding the lake conditions (Figure 3).

At station CR346.4 and CR350.05SE0.55 the Hydrolab readings are taken every meter to 30 m, then every 5 m to 100 m, and from there every 10 m to 140 m or bottom. At station BB3, the readings are taken each meter to 30 m then at intervals of every 5 m to bottom. At the other stations the readings are made every meter to bottom.

While one person is filtering the water samples the other one is collecting the zooplankton samples and finishing up any of the other measurements. After filtering, the samples are preserved and then iced.

After all the samples have been taken care of, the boat is moved to the next station. The

sampling routine is the same at each station.

As a quality control measure one station's water samples are collected in duplicate every run. A field blank (distilled water) is also used every run for each of the analyses.

STANDARDS and REAGENTS

pH 7.00 su Buffer-(Purchased as stock)

pH 9.18 su Buffer- Dissolve 2.0 g borax buffer (Beckman pre-weighed buffers) in 500 ± 2mL of reagent quality water. Mix well.

pH 10.00 su Buffer-Purchased stock 10.00 su buffer.

Conductivity-
1413 µmhos/cm standard- Dissolve 0.7456 g of KCl in 1000 mL of reagent quality water. Mix well.
2767 µmhos/cm standard- Dissolve 1.491 g of KCl in 1000 mL of reagent quality water. Mix well.

Turbidity-
50NTU standard- Purchased NIST styrene divinylbenzene beads.

Saturated $MgCO_3$ –
Add 1.0 g $MgCO_3$ to 100 mL distilled water.

Formalin/Sucrose Preservative –

Dissolve ½ cup of table sugar in 50 mL of distilled water and combine with 400 mL standard formalin solution (purchased commercially), mix well.

QUALITY CONTROL

Replicate:
Replicate water samples are collected from the same carboy as the initial sample for each analysis at one station every run. BB3 for biweekly and monthly runs, and LVB1.85M for weekly runs. CCWRD sets the qualifying limits.

Readback:
Readback of standards within 24 hours of sampling run.
pH- Readback needs to be within ± 0.10 su of buffer value. Please note: this is a modification for field samples monitored using the Hydrolab, normally field pH samples do not have a pH readback check (see Aqueous pH SOP for additional information).
Conductivity- Readback needs to be within 2% of standard value.
Turbidity- Readback needs to be within 10% of standard value.

QA ACTION

If any of the samples fall outside of the acceptable limits, report the data, and reference the field notes report. The discrepancies need to be qualified in the field notes area under the date of the sampling.

City of Las Vegas

Environmental Division Laboratory
Standard Operating Procedure
Ammonia - Nitrogen

Ref: Standard Methods18th ed 4500-NH3-H (Automated Phenate)

The automated procedure for the determination of Ammonia is based on the modified Berthelot reaction: alkaline phenol and hypochlorite react with ammonia to form indophenol blue at a concentration that is proportional to the ammonia concentration.

APPLICATION

Ammonia-nitrogen is a permit-required parameter. In that, the City holds discharge or other permit(s) that require ammonia-nitrogen to be determined on certain samples. Currently, those samples are:

Site	Permit-Required Frequency	Typical Frequency
WPCF INF-N	Quarterly	Weekly
WPCF EFF-C	Daily	Daily
NWEFF	Monthly	Weekly
Bonneville	Quarterly	Quarterly
Neonopolis	Quarterly	Quarterly
City Center	Quarterly	Quarterly

Ammonia-nitrogen is also routinely determined in numerous process control, collection system, industrial waste, surface water, groundwater, and other special samples. Ammonia-nitrogen is determined in process control samples mainly to assess the efficiency of nitrification systems.

MATRICES AND REPORTING LIMITS

Ammonia - nitrogen is determined in waters and wastewaters over a maximum range of 0.02 to 2.0 mg N/L using a photometer at 630 to 660 nm in a 15 or 50 mm tubular flow cell. Higher concentration samples outside of the standard range are analyzed by diluting the sample. The reporting limit is 0.1 mg N/L under normal operating conditions. The normal operating range is 0.1 to 1.0 mg N/L.

SAMPLE COLLECTION, PRESERVATION AND STORAGE

Samples are preserved after sample receipt with concentrated sulfuric acid to assure a pH between 1.5 and 2 by adding 0.8-1.0 mL H_2SO_4/L and store preserved samples under refrigeration (nominally 4 °C). Some wastewaters may require more concentrated H_2SO_4 to achieve this pH. It is important to eliminate marked variations in acidity or alkalinity among samples. Check all effluents or any special samples for residual chlorine by using the SOP for dechlorination (routine samples—such as plant effluents--are submitted to the BOD analyst for dechlorination). Recheck to assure residual chlorine has been eliminated as needed.

INTERFERENCES AND LIMITATIONS

Eliminate any marked variation in acidity or alkalinity among samples because intensity of measured color is pH dependent. Likewise, insure that wash water and standard ammonia solutions approximates that of the samples. If needed, remove interfering turbidity by filtration. If color in the samples that absorbs in the photometric range used for analysis interferes, another method may be of better choice.

SAFETY

Phenol and sodium nitroprusside are TOXIC and should be handled as hazardous. Any hazardous waste material generated are collected in a designated container and labeled as such. This waste can be mixed with waste generated from the CFA TKN procedure. Refer to Conditionally Exempt Small Quantity Generator Waste Program SOP for details.

REAGENTS

Log the preparation of all reagents in the Reagent Log Book (rinsing liquid lot numbers and their preparation are recorded on benchsheet daily). For all reagents prepared, the use of ammonia-free water is essential**.** Use Milli-Q water if available. Use equivalent quality water otherwise.

Buffer Solution

Dissolve 33 g potassium sodium tartrate in ± 500 mL ammonia free water. Add 24 g sodium citrate and dissolve. Adjust the pH to 5.0 with sulfuric acid. Fill up to 1 L, add 0.5 mL to 1 mL Brij 35 and mix.

Phenolate solution

Dissolve 16 g sodium hydroxide in ± 150 mL ammonia free water. Add 41.5 g Phenol. Fill up to 500 mL.

Hypochlorite solution

Dilute 50 mL hypochlorite solution (commercial bleach) to 250 mL with ammonia free water.
Discard solution after 2 weeks.

Sodium Nitroprusside
Dissolve 0.5 g sodium nitroprusside in ± 400 mL ammonia-free water. Fill up to 1000 mL.

Rinsing liquid
Add 1 mL sulfuric acid/L Milli-Q water. Mix well. Keep covered when not in use since the rinsing liquid tends to absorb ammonia from the atmosphere.

Dechlorinating agent
Please refer to the Dechlorination SOP.

Stock standard solution: 100 mg NH_3-N/L
Dilute 0.3819 g dried ammonium chloride in ± 800 mL distilled water. Add 1 mL concentrated sulfuric acid. Fill up to 1 L and mix.

EQUIPMENT

Skalar San System Continuous Flow Analyzer.

STANDARDS

Use micropipettes, the 100 mg NH_3-N/L stock standard, and 100 mL volumetric flasks to prepare calibration standards as follows:

1.0 mg NH_3-N/L: 1000 µL of 100 mg NH_3-N/L stock to a 100 mL final volume with Rinsing liquid
0.8 mg NH_3-N/L: 800 µL of 100 mg NH_3-N/L stock to a 100 mL final volume with Rinsing liquid
0.6 mg NH_3-N/L: 600 µL of 100 mg NH_3-N/L stock to a 100 mL final volume with Rinsing liquid
0.4 mg NH_3-N/L: 400 µL of 100 mg NH_3-N/L stock to a 100 mL final volume with Rinsing liquid
0.2 mg NH_3-N/L: 200 µL of 100 mg NH_3-N/L stock to a 100 mL final volume with Rinsing liquid
0.1 mg NH_3-N/L: 100 µL of 100 mg NH_3-N/L stock to a 100 mL final volume with Rinsing liquid

CALIBRATION AND STANDARDIZATION

Please see below

CALCULATIONS

Not Applicable – Instrument Printout of results, however QC performance data (please see below) are calculated and recorded on the benchsheet by the analyst.

PROCEDURES

The Continuous Flow Analyzer manifold is setup according to general procedures described by the manufacturer. Attached is a layout of the recommended specifications for the Skalar SAN system.

Prepare reagents
Prepare the Sodium Potassium Tartrate buffer, Phenolate, Sodium Hypochlorite, Sodium Nitroprusside reagents, if necessary the Rinsing Liquid. Check that sufficient volumes are available to complete the run.

Prepare Benchsheet
Record all reagents prepared in the Reagent prep logbook and record the reference numbers on the benchsheet.

Turn on all equipment
Turn on the computer first. Then switch on the auto sampler, analyzer (san system) and the interface with green buttons. Log on and open the Flow Access software, click Active System icon on left then open System 1. Use remote control to turn on equipment through software.

Start reagents pumping
See Skalar CFA Ammonia SOP for diagram of pump tubing (copy attached) and the manifold layout specifications. Check all the pump tubes for wear and replace or switch to other side if necessary. Record any pump tubing changes made on the benchsheet. Insure that the pump tubes are hooked up to their appropriate reagent lines. The pump tubes must run across the pump deck with shoulders behind clamps and there must be sufficient pump grease on the pump decks. Close the pump decks on the analyzer making sure module holder is closed and locked. Check that the ammonia sample tube is connected to the sampler. Check if sufficient waste container storage is available). Use the remote control to click the pump on and let the baseline stabilize. Be sure the water bath is set to 50 °C and sufficient water is in water bath. Wait for stabilization.
Make/Edit the sample table before starting the analysis. From the software interface, click the table icon. To load a table for editing, remove current table first. Then load an existing table. Then you can edit the table. The system table is set up to run a drift/wash every 5 samples. Be sure to save the edited file.

Prepare sample tray
Pour samples/diluted samples and standards into the sample cups and load the tray with sample cups according to the sample table that has just been set up.

Start Analysis
Click the 'check mark' icon then click the analysis you are running. Check the baseline by clicking the graph icon. Check for baseline stability and when you are satisfied with the baseline, click the start sign icon. To monitor the peaks, click on the graph icon. To see the results as they are calculated, the calibration curve and the peaks, click on the calculator icon.

Shut down procedures
Use the automated shutdown procedure or alternately: An automated shut down procedure is available. Turn off the heater and the detector. Put the weighted ends of the reagent lines in a beaker of DI water and let rinse. Continue pumping until all water has rinsed and the coils no longer have any water remaining in them. Switch off the pump, open the pump decks. Disconnect the pump tubing from clamps. Neutralize and discard standards, sample dilutions and samples remaining in sample cups. All glassware used is cleaned following the Glassware Cleaning SOP.

QUALITY CONTROL

After completing the analysis, calculate the necessary quality control responses and record these calculations on the benchsheet.

Summary of Quality Control Measures

QC Check	Frequency	Acceptance Criteria	Corrective Action
Calibration (5 Stds.)	Each batch	R>0.995	Correct problem, recalibrate
Low Standard Reread	Each batch	Check for MDL	Not applicable
Method Blank	Each batch	<0.1 mg N/L	Reanalyze, investigate
External Control Rec.	1 per batch	± 15% TV	Reanalyze batch
Internal Control Rec.	1 per batch	± 15% TV	Reanalyze batch
Blank Spike Recovery	1 per batch	± 15% TV	Flag, investigate
Matrix Dup. Precision	1 per batch	± 15% RPD	Flag, investigate
Matrix Spike Recovery	1 per batch	± 15% TV	Flag, investigate
Matrix Spike Dup. Rec.	1 per batch	± 15% TV	Flag, investigate
MS/MSD Precision	1 per batch	± 15% RPD	Flag, investigate

± 15% TV is the same as 85 – 115% recovery

If any QC deficiencies or anomalies occurred during the run, document these on a QA Action form and consult the Quality Assurance Officer.

Drift/Wash
A drift is part of the system table and is analyzed every 5 – 8 samples followed by a wash each time. Data between the "drift/wash" is recalculated to adjust for any drift during the run.

Out of Range
Any sample result higher than 110% of the high standard is considered out of range. The sample shall be reanalyzed using an appropriate dilution.

Calculation of Matrix Duplicate Precision

$$\text{Relative Percent Difference (RPD)} = \frac{(\text{sample result, mg N/L} - \text{duplicate result, mg N/L})(100\%)}{(\text{sample result, mg/L} + \text{duplicate result, mg/L})/2}$$

Calculation of Matrix Spike (MS) or Matrix Spike Duplicate (MSD) Recovery

$$\text{Percent Recovery} = \frac{(\text{spiked sample result, mg N/L} - \text{original sample result, mg N/L})(100\%)}{\text{spike added, mg/L}}$$

Calculation of Matrix Spike/Matrix Spike Duplicate Precision

$$\text{MS/MSD RPD} = \frac{(\text{MS recovery, \%} - \text{MSD recovery, \%})(100\%)}{(\text{MS recovery, \%} + \text{MSD recovery, \%})/2}$$

DATA ENTRY

Submit completed data packages to the Data Entry Person for processing.

POLLUTION PREVENTION AND HAZARDOUS WASTE MANAGEMENT

Please see above under SAFETY Subsection.

ADDITIONAL REFERENCES

Please see attached.

City of Las Vegas

Environmental Division Laboratory
Standard Operating Procedure

Total Oxidized Nitrogen

Ref: Standard Methods 18th Ed 4500-NO3 F (Automated Cd Reduction)

The automated procedure for the determination of Nitrate+Nitrite is based on the cadmium reduction method; the sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (any originally present, plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with a-naphthyl-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured at 540 nm.

APPLICATION AND PERMIT LIMITS

This procedure is applicable to potable water, surface water, ground water, and domestic and industrial wastewaters. Total Oxidized Nitrogen (TON) values are monitored weekly on the WPCF, NWWRC and BOMO effluents. Monitoring wells are analyzed quarterly.

SAMPLE STORAGE/PREPARATION

Samples can be preserved with refrigeration if stored for less than 24 hours, preserve the samples with sulfuric acid to a pH of <2 su. The maximum holding time of preserved samples is 28 days.

INTERFERENCE'S AND LIMITATIONS

Sample turbidity may interfere. Remove as needed by filtration before analysis. Sample color that absorbs in the photometric range used for analysis also will interfere. Iron, copper and other metals present in the sample lower reduction efficiency. Add disodium-EDTA to sample if interference occurs. Check samples when necessary for residual chlorine. If samples results are expected to be above the applicable range, dilute the samples before analysis. Low Cd column reduction efficency may result in reduced sensitivity in the procedure, however all permit required samples typically are detected in concentrations far above limits of detection and are unlikely to be affected.

SAFETY

Use precaution when handling wastewater samples, especially if they are acidified. Prepare the copper-cadmium column under a fume hood. Use gloves when handling cadmium. Cadmium waste (including rinsates) must be treated as hazardous waste. Some acid waste generated from the TON analysis may be disposed of in the sink after neutralization in sodium bicarbonate. Refer to MSDS and also Equipment and Materials section of this SOP for more information.

PREPARATION OF REAGENTS. use Milli-Q (MQ) water or the equivalent.

A. Buffer solution

Preparation: Dissolve 85 g ammonium chloride in -800 mL Milli Q water. Add 0.1 g

ethylenediamine tetra-acetic acid disodium salt and dissolve. Fill to about 998 mL. Adjust the pH to 8.5 with ammonium hydroxide. Add Brij 35 (30%) and mix well. If problems with bubbles occur, degass before adding Brij.

B. Color reagent

Dilute 150 mL of o-phosphoric acid carefully in ≈ 800mL Milli-Q water. Add 10 g sulfanilamide and 0.5 g a-naphthylethyldiamine dihydrochloride (NED) and dissolve. Fill up to 1 L, add 3 mL Brij 35 (30%).

C. Rinsing Liquid /sampler liquid /sample dilution

Sulfuric acid preparation: Dilute 1 mL sulfuric acid per L of Milli-Q water.

STANDARDS

Stock solution 100 mg N/L

Dissolve 0.7218 g sodium nitrate in ≈800 mL distilled water. Add 1 mL concentrated H_2SO_4. Fill up to 1 L and mix well. The stock may be made in 1000 mg N/L concentration (use 7.218 g/L of MQ water with no acid addition. Dilute 100 mL of this stock to 1,000 mL for a 100 mg N/L standard (add 1 mL H_2SO_4).

Internal Control

Prepare an Internal Control Sample (ICS) from an alternate reagent source from the source of the calibration standards. The ICS concentration must be within the calibration range. Suggestion: In a 1000 mL volumetric flask, add 1.4436 g of sodium nitrate to about 800 mL of water. Add 1 mL of concentrated sulfuric acid and dilute to volume (stock 200 mg N/L nitrate). Dilute 1 mL of this stock ICS to 100 mL using the rinsing liquid to prepare the working ICS (2.0 mg N/L).

Preparation of Cd Column

Mix Cu coated Cd with Copper granules (about 5-10% Cu). Put the Cu-Cd into a funnel inserted into the tubing (purple-purple-purple CFA skalar tubing, approx. 6 inches in length). A funnel may be made from a 1 mL disposable pipet with last segment of tip and the bulb end cut off. Fill tube to the outside purple segments. If copper coated granules are not available, purchase 40-60 mesh cadmium granules. Rinse with 6 N HCl. Rinse with tap water. Swirl about 25 g Cd granules in 100 mL of 2% $CuSO_4$ solution (20 g $CuSO_4$/L water). When blue color partially fades or after five minutes, decant and repeat with fresh $CuSO_4$ until a brown colloidal precipitate develops. Gently flush with de-ionized water until all precipitated copper is removed. Let dry prior to mixing with copper granules.

Starting the Instrument

Turn on the Interface, monitor and lastly the computer. The Skalar software program will automatically come up.

Put the corresponding flow cell and filters in the appropriate detection head. Check that the corresponding photometer wire is connected to the right spot in the interface channel.

Check the water level of the bath (if required) and turn it on to the method specific temperature.
Rinse off the weighted ends of the reagent lines and immerse them in their respective reagent bottles (each of the reagent lines are labeled), do the same with the rinsing liquid line. The rinsing liquid composition is dependent on the analysis.

Check the pump tubes for wear and replace or switch to the other side as needed. Record any changes on the bench sheet.

Close the pump decks on the analyzer making sure the module holder is closed and locked. The pump tubes must run across the pump deck with the shoulders behind the clamps and having sufficient pump grease on the pump decks.

Check that the sample tube is connected for the proper analysis.
Check to see if sufficient waste facilities are available. Hazardous waste is collected separately from non hazardous wastes and disposed of properly (see Hazardous Waste SOP for more information).

A cadmium column is used for total oxidized nitrogen analysis (TON), make sure the column valve is off prior to starting up, it may be open as soon as a normal bubble pattern is established.

Press the Alt-S key combination to obtain the channel panel menu for activating the analyzer components where the pumps, water bath and detection system are turned on. Use system 1 for all analyses.

While still in the channel panel, arrow over to the selection and use spacebar to activate the pump (the analyzer and sampler will both go on).

Press the escape key to backup to the control panel, then arrow down to analysis panel. Press the enter key and toggle to the proper analyses settings then tab over to the channel panel for the select analysis.

Select the graphics heading and view the analysis in real time while the system is stabilizing.
Wait for stabilization of baseline before beginning the analysis. It should be fairly (but not completely) flat. If it is perfectly flat, check to see if everything is hooked up correctly including photometer to interface, that appropriate cell is in place.

Bubbles are used as spacing and should only be injected only at the proper place and of uniform size and spacing. If additional bubbles are being added, follow the bad bubble back to the source and fix the problem.

Start up of TON analysis requires that all reagents are flowing before opening the cadmium column and also requires additional time to condition the column. Typical CFA settings are as follows:

CFA Settings
Baseline approxamately 300, A filter=540, B filter=620, 10 mm flow cell, Channel 5, Analysis panel 2, time sequence 70/60, drift/tracer in C4, no water bath, no heater.

System Table
System tables are set up for each analysis with tracer, drift, wash, standards and unknowns. Changes can be made under **Options, Assign Group, Table, Edit** (see CFA SOP for examples). Drift and wash are set to analyze every 5 samples.

Sample Table
Pre-made sample tables are available for all for methods and can be modified once a run has begun to include other changes.
After an analysis has begun, to identify the samples: add the dates, sample code and Chain of Custody ID (as space allows). Find sample table under **File, Table, Edit, File name (hit enter).**

Adjust Baseline
After allowing the system to stabilize, check baseline value and adjust if necessary at the top of the Real Time screen.

The baseline is adjusted with the coarse and fine dials on the photometer for the corresponding detector head.

Put the selector on B and use the coarse and fine dials for B to obtain a value on the photometer display of about 50**.** Then put the selector on A-B and adjust the coarse and fine dials for A to obtain a positive value on the photometer display (optional). The baseline should be fairly stable with little upward or downward drift and have an M Raw Value on the Real Time Display of 250-450 depending on analysis. The baseline may be slightly noisy even after it has stabilized. If the baseline is very unstable or noisy after much longer, then look for such things as leaking tubing, problem with reagents, poor bubble pattern. Always have damping (switch on back of photometer) in off position when making adjustments.

Check gain on high standard
If necessary, check the sensitivity (gain) of the high standard, by sampling it for at about 3 minutes. This can be done manually by carefully removing the sample needle assembly from its holder and putting it directly into a sample cup (usually the high standard). After about 3 minutes, carefully move the sample needle back into its holder as it was before. After the standard appears on the screen (two to six more minutes depending on the analysis) and gives a fairly steady peak value, make adjustments to the corresponding interface adjustment screw on the face of the interface with a small screwdriver to get M

Raw Value to be **3600-3900.** If interface adjustments were necessary, then check baseline again and adjust as before.
Check possible leakage.

Prepare sample tray
Pour samples into sample cups. Load tray with sample cups according to sample table.

Start Analysis
With both **Control Panels** highlighted and using left or right arrow key to display drop down menu. Enter operators **name**, **sample table**, yes to **System table**, then press the **"ENTER"** key to begin the run.

Shut down procedures
After the "run-out wash", switch off heater and detector (if the system is in the Relative mode, this will happen automatically). Put weighted ends of reagent lines in beaker of DI water and let rinse. Continue pumping until all water has rinsed and the coils no longer have any water remaining in them. Switch off pump, and open the pump decks. Disconnect the pump tubing from the clamps. All glassware used is cleaned following the **Glassware Cleaning SOP.**

MAINTENANCE
Make a note on the benchsheet of any maintenance performed during the analysis. Change the position of the chemistry unit tubing as needed. Once both sides appear worn, replace them with new pump tube. Change the sampler unit tubing as needed

QUALITY CONTROL
Blank: Analyze a blank for at least 5% of the samples with each run.
Out of Range: Any samples over range need to be reanalyzed using the appropriate dilution.
Standards: Run a minimum of 5 standards.
Replicate: Run 10% of permit samples in replicate.

$$\text{Relative Percent Difference} = \frac{(\text{Original, mg/L} - \text{Replicate, mg/L}) \times 100\%}{(\text{Original, mg/L} + \text{Replicate, mg/L})/2}$$

Matrix Spike: Run a fortified sample and fortified duplicate (MS/MSD) at a frequency of 10%. It is recommended to fortify samples between 5 and 50 times the MDL or between 1 and 10 times the ambient level. It is also recommended to use concentrated solutions so that any volume change in samples is negligible. Calculate the relative percent difference (RPD).

$$\text{Percent Recovery} = \frac{(\text{spiked sample result, mg/L}) - \text{original result, mg/L}) \times 100\%}{\text{spike added, mg/L}}$$

$$\text{Relative Percent Difference} = \frac{(\%\text{ rec MS} - \%\text{ rec MSD}) \times 100\%}{(\%\text{ rec MS} + \%\text{ rec MSD})/2}$$

Internal Control: A 2.0 mg NO_3-N/L standard is used as an internal control.

External Control: One is done with each run. Usually an ERA.

Nitrite Reduction Efficiency Check: A NO_2-N/L standard is run to confirm column reduction efficiency. Note the Cd column reduction efficiency on the benchsheet (typically 60% – 80%). If the column reduction efficiency drops below about 75%, samples with elevated nitrite concentrations relative to the nitrate concentration may yield inaccurate results for Nitrite + Nitrate. None of the permit-required effluent samples are expected to have any appreciable nitrite concentrations as all of these samples are subject to nitrification, however in the event of any permit-required samples to be analyzed were obtained under conditions of elevated nitrite concentrations (such as nitrite lock conditions--or are suspected to contain elevated nitrite relative to the nitrate concentration), if the reduction efficiency for the column is less than 75%, confirm the result by reanalysis.

Document QC any deficiencies on a QA Action form and submit with results of analyses to the Quality Assurance Officer. If there are no QC deficiencies, submit the package to the Data Entry Person directly.

CLV ENVIRONMENTAL DIVISION - STANDARD OPERATING PROCEDURE
NITRITE /020117/SOPN02 USGS I-4540-85

PRINCIPLE

The automated procedure for the determination of Nitrite is based on the following reaction; the diazonium compound formed be diazotation of sulphanilamide by nitrite in water under acid conditions is coupled with a-naphthylethylene dihydrochloride to produce a reddish-purple color which is measured at 540 nm using a 1 cm path length.

SAFETY

Use precaution when handling wastewater samples.

APPLICATION AND PERMIT LIMITS

This method may be used to analyze surface, domestic and industrial water from 0.01-1.0 mg/L of nitrite-nitrogen. Higher concentrations can be determined by diluting a sample.

INTERFERENCES AND LIMITATIONS

Oxidizing agents interfere by oxidizing nitrite to nitrate. The following ions interfere because of precipitation under test conditions and should be absent: Sb^{3+}, Au^{3+}, Bi^{3+}, Fe^{2+}, Pb^{2+}, Hg^{3+}, and Ag^{+}. Cupric ions may cause low results by catalyzing decomposition of the diazonium salt. Colored ions that alter the color system also should be absent. Remove suspended solids by filtration.

SAMPLE STORAGE/PREPARATION

Never use acid preservation for samples to be analyzed for NO_2^-. Samples must be analyzed within 24 hours. For short-term preservation for 1 to 2 days, freeze at –20 °C or store in refrigerator set at 1-4 °C.

Preparation of Reagents

Log reagents in Reagent Log Book (Rinsing liquid lot numbers and prep are recorded on benchsheet daily) For all reagents prepared, **use of ammonia-free water is essential.** Use Milli Q water if available. Use equivalent quality water otherwise.

Distilled water + Brij 35

Dilute 3 mL Brij 35 in 1 L of distilled water or MQ water and mix.

Color reagent

Dilute the 100 mL o-phosphoric acid carefully in ± 700 mL distilled water. Add 10 g sulfanilamide and 0.5 g a-naphthylethyldiamine dihydrochloride and dissolved. Fill up to 1 L and add 3 mL Brij 35 and mix well,

Rinsing Liquid

Distilled water

Stock NO2 Standard solution: 100 mg/L N

Dissolve 0.4926 g Sodium Nitrite in ± 800 mL distilled water. Fill up to 1 L and mix well.

Using Micopipets make a 10. mg/L intermediate NO_2^- STD
0.2 mg/L N: 2.0 mL of 10.0 mg/L NO_2^- stock STD with 100 mL final volume with Milli Q water.
0.15 mg/L N: 1.5 mL of 10.0 mg/L NO_2^- stock STD with 100 mL final volume with Milli Q water.
0.07 mg/L N: 0.70 mL of 10.0 mg/L NO_2^- stock STD with 100 mL final volume with Milli Q water.
0.05 mg/L N: 0.50 mL of 10.0 mg/L NO_2^- stock STD with 100 mL final volume with Milli Q water.
0.02 mg/L N: 0.20 mL of 10.0 mg/L NO_2^- stock STD with 100 mL final volume with Milli Q water.

Interferences:

Sample turbidity may interfere with this method, when no dialyzer is used. Remove by filtration before analysis. Sample color (that absorbs in the photometric range used for analysis) will also interfere.

Start up Procedure for Nitrite CFA method

Prepare Reagents:

Color reagent, Distilled water with Brij and Rinsing Liquid if necessary. Check for sufficient volumes to complete the analysis.

Prepare Benchsheet:

Record all reagent batch #'s onto benchsheet for those that apply.

Turn on all equipment:

Switch on sampler, analyzer (san system), photometer, interface with green buttons, always in this order. Switch on computer along with screen and printer last. The Skalar software program will automatically begin.

Start reagents Pumping

See Skalar CFA books for diagram of pump tubing and manifold layout. Place the weighted ends of the reagent lines in the proper reagent bottles. Immerse the weighted end of the rinsing liquid line into the rinsing liquid. Check all pump tubes for wear and replace or switch to other side if necessary. Record all pump tubing changes on benchsheet. Assure pump tubes are hooked up to the appropriate reagent lines. The pump decks must run across the pump deck with shoulders behind clamps and sufficient pump grease on the pump decks. Close pump decks on the analyzer making sure module holder is closed and locked. Check that the nitrite sample tube is connected to the sampler. Check if sufficient waste facilities are available. Press ALT S to obtain a menu for activating the analyzer components. Arrow over to the selection and use spacebar to toggle ON/OFF the activation the Pumps (analyzer) and Detection in System 1.

Prepare standards

See Standards in reagent prep section.

Prepare system for Nitrite analysis

Put the nitrite 10 mm flow cell, spacer and filter in the appropriate detection head. The A filter is 540 nm and the B filter is 620 nm. Check that the corresponding photometer channel is connected to the interface channel for nitrite, channel #4. Select the 60/60-time sequence under **Analysis Panel** and Nitrite chemistry in the **Channel Panel**. Then select Graphics heading and view the chemistry in Real Time while the system is stabilizing.

Prepare System table:

Use System table with tracer, drift, wash and then standards and unknowns under **Options, Assign Group, Table, Edit**. (see CFA SOP for examples)

Prepare Sample Table

Use Chain of Custody, sample codes and dates to identify samples. Also use pre and post dilution information under **File, Table, Edit**.

Adjust Baseline:

After allowing the system to stabilize, check baseline value and adjust if necessary at the top of the **Real Time** screen. **Always have damping in off position when making adjustments.** The baseline is adjusted with the coarse and fine dials on the photometer for the corresponding detector head. First put the selector on **B** and use the coarse and fine dials for B to obtain a value on the photometer display of about **50.** Then put the selector on **A-B** and adjust the coarse and fine dials for A to obtain a positive value on the photometer display. The baseline should be fairly stable with little upward or downward drift and have a M Raw Value on the Real Time Display around 300 (a stable value from 150 - 450 is recommended). The baseline may be slightly noisy even after it has stabilized. The damping should be set on high. If the baseline is very unstable or noisy after an hour on reagents, then use the decontamination/cleaning procedure. If baseline persists to be extremely bumpy, see CFA SOP for further procedures to eliminate the bumpy baseline.

Check gain on high standard:

If necessary, check the sensitivity (gain) of the high standard, by sampling it for at least **3 minutes.** This can be done manually by carefully removing the sample needle from its holder and putting it directly into the highest standard cup. After about **3 minutes**, carefully move the sample needle back into its holder as it was before. After the standard appears on the screen and gives a steady state raw value make adjustments to the #4 interface with a small screwdriver to get **M Raw Value** to be **3200-3600.** If interface adjustments were necessary, then check baseline again and adjust as before. If interface adjustments were necessary, then check and adjust the baseline as before. Check possible leakage. Pour samples into

sample cups and put parafilm over the top to avoid any contamination. Load tray with sample cups according to sample table.

Start Analysis

With **Control Panels** highlighted and using left or right arrow key to display drop down menu. Enter operators **name**, **sample table**, yes to **System table**, then press **"ENTER"** to begin the run.

Shut down procedures

After the "run out wash", switch off the detector (if the system is in the Relative mode, this will happen automatically). Put weighted ends of reagent lines in beaker of DI water and let rinse. Continue pumping until all water has rinsed and the coils no loner have any water remaining in them. Switch off pump, open pump decks. Disconnect pump tubing from clamps. Discard standards, sample dilutions and samples remaining in sample cups. All glassware used is cleaned following the **Glassware Cleaning SOP and Pipet Cleaning SOP.**

Maintenance

Make a note on the benchsheet of any maintenance performed. The system can be decontaminated by rinsing with 1% hypochlorite solution for 20 minutes then rinse with distilled water for 30 minutes afterwards. As needed, change the position of the reagent and sampler pump tubing. Degas reagents if air bubbles spontaneously appear by filtering with degasser unit. Avoid any turbidity in reagents and filter if necessary.

QUALITY CONTROL

After completing the analysis, calculate the necessary quality control and enter the results into the computer. Document QC deficiencies on the benchsheet and have the lead analyst sign.

Blank:

A blank is analyzed at the beginning of each run and rerun from the same cup at the end of every run.

Drift:

A drift is part of the system table and is analyzed every 5 samples followed by a wash each time. Data between the "drift/wash" is recalculated to adjust for any drift during the run.

Wash:

A wash is part of the system table and is analyzed every 5 samples following the drift.

Internal Control:

An internal control is prepared in the lab using a different stock standard than the standard used for making the calibration solutions. It is then analyzed with each run.

Continuing calibration verification:

A low standard analyzed as part of the calibration curve is rerun from the same cup at the end of each run and the data is used as the continuing calibration verification. The drift data can also be used as the "continuing calibration verification".

Control chart:

Control chart are generated and monitored by the QAO. Control charts are used to monitor the blank, external control, internal control, CCV. Warning limits and tolerance limits are established by data generated. If any items are out of control, corrective action must be taken. Where insufficient data has been generated to establish a control chart, Standard Methods 18th ed. is followed to regulate the acceptable range of replicate percent difference

Out of range:

Any samples out of range and the sample directly following it will automatically be rerun by the instrument using the next higher dilution.

Replicate: Analyze all non-routine samples and at least 10% of all routine samples in replicate.

Relative Percent Difference = [Original Result (mg/L)Replicate Result (mg/L)] * [Original Result (mg/L) + Replicate Result (mg/L)]/2

Matrix Spike:

A matrix spike and matrix spike duplicate is run on all non-routine samples and 10% of all routine samples. Spike samples between 5 and 50 times the MDL or between 1 and 10 times the ambient level. It is required to calculate a relative percent difference between the matrix spike and matrix spike duplicate.

Percent Recovery =(Fortified result (mg/L) - Original result (mg/L) x 100% / Spike added (mg/L)

Relative Percent Difference = [(% recovery MS - % recovery MSD)] x 100% / (% recovery MS + % recovery MSD)/2

Standards: Run a minimum of 3 dilutions of standards.

City of Las Vegas

Environmental Division Laboratory

Standard Operating Procedures

Total Phosphorus

Ref: Standard Methods 20th ed. 4500-P E.
Ascorbic Acid, with Persulfate Digestion (modified)

Summary of Method:

A sample is heated in acidic conditions in the presence of persulfate to convert all forms of phosphorus to orthophosphate. After cooling, the steps for orthophosphate phosphorus analysis are then followed. Ammonium molybdate and potassium antimony tartrate react in an acid medium with orthophosphate to form phosphomolybdic acid that is reduced to intensely colored molybdenum blue by ascorbic acid which can be quantitated colorimetrically at 880 nm (Beckman DU 640 or other spectrophotometer).

Application:

Monitoring of permit required and process control samples. Also, industrial pretreatment and various miscellaneous samples as requested.

Permit Limit:

Permit monitoring daily on 24-hour influent and effluent composites.
Effluent loading limit is 130 lb/day (monthly average March 1 to November 1).

Detection Limit: PQL 0.015 mg P/L.

Hazards and Precautions:

See MSDS for reagents. Use normal precautions for handling heated acids. Digest in a properly operating fume hood.

Sampling and Storage:

Analyze samples immediately after receipt in the laboratory or preserve with 1 mL H_2SO_4/1 L. Sample preserved to pH < 2 with H_2SO_4 , and kept at 4 °C, maximum hold time is 28 days.

Interferences:

Do not store samples containing low concentration of phosphorus in plastic bottles (cubitainer is acceptable) unless kept in a frozen state due to adsorption of phosphates on the walls of plastic bottles. Concentration as low as 0.1 mg/L of arsenic reacts with the

molybdate reagent to produce a blue color, which interferes with the phosphorus determination. Hexavalent chromium and nitrite also interfere, refer to Standard Methods 18 Ed for further information. Wash all glassware according to the Glassware Cleaning SOP.

Reagents:

Sulfuric Acid Solution 10 N: Add approximately 500 mL of Milli-Q water to the Pyrex bottle used for this purpose. Place the container on a magnetic stir plate under a fume hood. Add a stir bar, and slowly add 300 mL concentrated sulfuric acid while stirring. Bring to volume to the 1L mark with Milli-Q water. Mix well.

Sodium Hydroxide Solution 2 N: Fill a container with approximately 3000 mL of Milli-Q water. Place the container on a magnetic stir plate under a fume hood and add a stir bar. Slowly add 320 g of NaOH pellets to the container while stirring on a magnetic stir plate. Bring the volume to the 1gallon mark with Milli-Q water. Mix well.

Phenolphthalein indicator solution: Commercially purchased.

Ascorbic acid: Weigh out 0.53 g of ascorbic acid per 30 mL of Milli-Q water. Prepare an appropriate amount according to number of samples being analyzed. Prepare daily.

Ammonium Molybdate/Mixed Reagent: Fill the 1 gallon bottle used for this purpose approximately half full with Milli-Q water. Place the container on a magnetic stir plate under a fume hood. Add a stir bar. Slowly add 380 mL concentrated sulfuric acid while stirring. Add 0.7444 g of antimony potassium tartrate and 35.571 g of ammonium molybdate to the solution. Bring up to the 1-gallon mark with Milli-Q water. Mix well.

Stock Phosphorus Standard (100 mg/L): Dry approximately 5 g of potassium dihydrogen phosphate at 105 °C for 1 hour. Quickly weigh 0.4393 g of the cooled, dry KH_2PO_4 and dissolve in Milli-Q water and dilute to 1000 mL in a class 'A' volumetric flask. Mix well.

Working Phosphorus Standard (5 mg/L): Using a class 'A' volumetric pipette, pipette 50 mL of the stock standard into a 1000 mL volumetric flask. Bring to volume with Milli- Q water and mix well.

Stock Phosphorous Control (Internal control): Dry approximately 5 g of potassium dihydrogen phosphate at 105 °C for 1 hour. Quickly weigh 0.3582 g of the cooled, dry KH_2PO_4. Dissolve in Mill-Q water and dilute to 250 mL with Milli-Q water in a class 'A' volumetric flask. Mix well.

Working Phosphorus Internal Control (IC): Pipette 3.0 mL of the stock phosphorus control into a 1000 mL volumetric flask. Bring up to volume with Milli-Q water and mix well. (True value = 0.98 mg/L)

Ammonium Persulfate: Purchased as a reagent grade solid. May be substituted with potassium persulfate.

External control: Usually an ERA

Procedure:

Note: The Analyst performing phosphorus analysis is usually responsible for sulfur dioxide analysis, which must be done within 30 minute of sample collection time (or at least a Chlorine HACH Test Kit).

Sample Preparation:

1) Pour out, as needed, adequate standard and all controls for the day into labeled beakers.

2) Preheat the hot plate on high setting under the fume hood.

3) Turn on DU 640 monitor and toggle vis lamp on.

4) To each labeled Erlenmeyer flask, pipette a well mixed sample (do not use graduated cylinder). You may use the previous days' data to determine dilution volumes.

5) If the receipt pH is greater than 9.0, add one drop of phenolphthalein to the sample flask. If the sample turns pink/red then add 10 N sulfuric acid until the color disappears, then proceed to the next step. Record on the bench sheet if any pH adjustment was performed.

6) Add 1 mL of 10 N sulfuric acid to each flask.

7) Add 1/2 a scoop (approximately 0.4 g) of ammonium persulfate using the small white scoop to each flask. (Alternately, use Potassium persulfate)

8) Bring the total volume to approximately 50 mL with Milli-Q water

9) Boil gently on a preheated hot plate under a fume hood for 30-40 min, or until a final volume of 10 mL is reached. Let the samples cool down to near room temperature.

10) Bring the volume to about 15-20 mL (using Milli-Q water rinse off the inside of the flask from the top down).

11) Add 0.05 mL or 1 drop of phenolphthalein to each flask.

12) Neutralize to a faint pink color with 2 N NaOH solution. Swirl the samples while adding NaOH. If you overshoot the endpoint, use 10 N sulfuric acid to bring the solution to just slightly pink.

13) Pour each sample into a 50 mL volumetric flask. Be careful not to spill the sample. Bring to volume with Milli-Q water

14) Pour the contents of the volumetric flask back into the Erlenmeyer flask.

15) Add 5.6 mL of ammonium molybdate mixed reagent solution to each flask and swirl.

16) Add 2.5 mL of ascorbic acid solution to each flask. Swirl each sample and let it sit in order for the blue color to develop at least 10 minute before reading (but no more than 20 minutes).

17) Using the mouse select 'Single Component Analysis' on the DU640 screen. Select the method Total P method and verify standard concentrations you are using (if you are changing the standard concentrations).

Calibration:

1) Remove any residual air bubbles or contaminants as follows: Put the sipper in the Milli-Q water rinse flask. Hold down the flush button to flush the sample tubing for about one minute.

2) Put the sipper in the prepared blank sample. Hold down the flush button until the absorbance reading steadies, then press the Fill blank button a few times until you are sure the absorbance reading is steady at ± 0.0000.

3) Next, read all the standards. First flush with the low standard, continue flushing until a steady reading is obtained. Click the mouse on " Read 1".

4) Repeat for the replicate reading of the first standard (by flushing sample and rereading that sample).

5) Read the standards in the order indicated on the screen. Press the flush button with the next standard until a stabilized reading is obtained prior to each reading or replicate. For any replicate reading that varies more than 5% from the original reading, reread the standard.

6) When finished, click 'DispStdCurve' on the top of the screen. The correlation coefficient (r-coefficient) must be greater or equal 0.9998 for the curve to be acceptable. If the r-coefficient is less than 0.9995, prepare a new standard series.

7) Click ‘print’ and record the r-coefficient on the bench sheet.

8) When printing is completed, click ‘exit’ to go to the standards screen.

9) Click ‘print’ to print the standards page.

Sample Analysis:

1) After printing, click ‘Samples’ on the left top of the screen. Start reading each run with the blank, then follow with the IC (Internal control) or the EC (External control, ERA). Then follow with the standard 0.2 mL. (daily detection limit standard)

2) On the left side of screen, select the sample ID box and change the ID to the sample code include the volume used and C of C# (for example: INFC 3 mL at the first line and 36703A at the second line). Then click on the right side of the screen where the dilution factor is and enter the appropriate dilution factor.

3) Flush with the sample to be read until a steady reading is obtained.

4) Press the fill read button for the first reading of the sample.

5) Enter the C of C #, then press the fill read button again. This will give a replicate reading for the sample. Whenever the replicate reading varies more than 5% from the original reading, reread the sample and make a notation on the printout (with your initials and the date).

6) Repeat steps 2-5 for each sample.

7) Read an EC or IC at least every 10 samples.

8) Reread the blank solution and at least two standards (which bracket the concentrations of the samples), and whichever EC/IC still needs to be run (one is chosen at the start of the run).

9) Print the results by clicking ‘print’.

10) Save the sample data by clicking ‘save/clear’. Click the letter A to switch to the B drive. Click ‘Work-res’ to get the typewriter screen and save the data by entering ‘TP’ for total phosphorus and current date as TPMMDDYY (for example: TP072601) Click on ‘OK’ to return to the save screen. If file name is OK, then click OK.

11) To save the standard data, return to the standard screen by clicking 'standard' at the top of the screen and save the standard data just like the sample data in #10.

12) Flush the flow cell with Milli-Q water. Never leave anything but Milli-Q water in the cell. If air bubbles have been a problem a flush with ethyl alcohol may be needed, followed by a flushing with Milli-Q water. Record any such problems in the maintenance log book.

13) Turn off the vis lamp, turn off the monitor.

14) Total phosphorus is calculated by using a regression of the standard curve and reported as mg P/L.

Quality Control and Documentation

Acceptance criteria for QC parameters are specified in the QA plan. If any QC response falls outside these limits consult the QAO on appropriate actions.

Replicate Calculation:

$$\text{Relative Percent Difference} = \frac{(\text{Original mg/L - Replicate mg/L})(100\ \%)}{(\text{Replicate average}}$$

Fortified Samples Calculation:

$$\%\ \text{Recovery} = \frac{(\text{spike conc.})}{(\text{spike dil. factor})} - \frac{(\text{replicate average})(\text{vol. of sample in spike})}{(\text{orig. sample dil. factor})(\text{vol. of orig. sample})} \times 100\%$$

0.2(expected recovery of spike if use 2.0 mL)

$$\%\ \text{RPD} = \frac{(\text{MS\% recovery} - \text{MSD\% recovery})(100\ \%)}{(\text{MS\% recovery} + \text{MSD\% recovery})/2}$$

Anomalies: Record any abnormality observed in the sample. Consult the QAO (or the Laboratory Superintendent in the absence of the QAO) for evaluation. If the sample is being used as a QC check sample and it is known that the sample is non-homogenous or unusual (such as containing unusually large chunks) analyze a triplicate for confirmation.

QA Action Form. Some data may be deemed unacceptable -- e.g. poor spike recoveries for a sample having unknown matrix interference or in a case where no more sample is available, etc. Fill out Comment/QA Action Section on the benchsheet. Consult the QAO (or the Laboratory Superintendent in the absence of the QAO) for evaluation.

Rerun. Be sure that the next analyst is informed when there are reruns. If a sample is to be rerun for Total Phosphorus, be sure that there is sufficient sample preserved with 1 mL of conc. H_2SO_4/L of sample.

City of Las Vegas

Environmental Division Laboratory

Standard Operating Procedures

Orthophosphate-phosphorus

Ref: SM 18th Ed. 4500-P E

Summary of Method:

Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form phosphomolybdic acid that is reduced to intensely colored molybdenum blue by ascorbic acid which can be quantified colorimetrically at 880 nm on Beckman DU 640 or other spectrophotometer.

Application:

Daily monitoring of WPCF Effluent plus other samples as requested.

Permit Limit:

Daily permit monitoring is required on 24- hour WPCF effluent composites.

Hazards and Precautions:

See MSDS for individual reagents and precautions when handling acids.

Sampling and Storage:

Maximum hold time for storage is 48 hours under refrigeration at 0-4 °C.

Interferences:

Do not store samples containing low concentrations of phosphorous in plastic bottles (cubitainer is acceptable) unless kept in a frozen state due to absorption of phosphates on the walls of plastic bottles. Concentrations as low as 0.1 mg/L of arsenic reacts with the molybdate reagent to produce a blue color, which interferes with phosphate determination. Hexavalent chromium and nitrite also interfere, refer to Standard Methods for further information. Wash all glassware according to the Glassware Cleaning SOP.

Reagents:

Ascorbic acid: Weigh out 0.53 g ascorbic acid per 30 mL Milli-Q water. Prepare an appropriate amount according to number of samples being analyzed. Prepare daily.

Ammonium Molybdate/Mixed Reagent: Fill the 1 gallon bottle used for this purpose approximately half full with Milli-Q water. Place the container on a magnetic stir plate under a fume hood. Add a stir bar. Slowly add 380 mL concentrated sulfuric acid while stirring. Add 0.7444 g antimony potassium tartrate and 35.571 g ammonium molybdate to the solution. Bring up to the 1-gallon mark with Milli-Q water. Mix well.

Stock Phosphorous Standard (100 mg/L): Dry approximately 5 g of potassium dihydrogen phosphate at 105 °C for 1 hour. Quickly weigh 0.4393 g of the cooled, dry KH_2PO_4 and dissolve in Milli-Q water and dilute to 1000 mL in a class 'A' volumetric flask. Mix well.

Working Phosphorous Standard (5 mg/L): Pipette 50 mL stock standard into a 1000 mL Class 'A' volumetric flask. Bring up to volume with Milli-Q water. Mix well.

Stock Phosphorous Control (Internal control): Dry approximately 5 g of potassium dihydrogen phosphate at 105°C for 1 hour. Quickly weigh 0.3582 g of the cooled, dry KH_2PO_4 and dissolve in Milli-Q water and dilute to 250 mL in a Class 'A' volumetric flask Mix well.

Working Phosphorous Control: (Internal control) Pipette 3.0 mL stock phosphorus control into a 1000 mL volumetric flask. Bring up to volume with Milli-Q water and mix well.

External Control: usually an ERA

Sample Preparation:

1) Pour out, as needed, adequate standard and all the controls for the day into labeled beakers.

2) Turn on the DU 640 monitor and toggle the vis lamp on.

3) Pipette well mixed samples, standards and all controls (do not use graduated cylinder) into labeled 50 mL volumetric flasks. You may use the previous days' data to determine dilution volumes.

4) Bring flasks up to the 50 mL volume with Milli-Q water.

5) Pour contents of the volumetric flask back into the Erlenmeyer flasks.

6) Add 5.6 mL ammonium molybdate mixed reagent solution to each flask and swirl.

7) Add 2.5 mL ascorbic acid solution to each flask. Swirl each sample and let it sit in order for the blue color to develop (at least 10 minutes) before reading (the sample must be read within the next 20 minutes).

8) Using mouse select 'Single Component Analysis' on the DU640 screen. Select Ortho P method and verify the standard concentrations you are using (if you are changing the standard concentrations).

Calibration:

1) Remove any residual air bubbles or contaminations as follow: Put the sipper in the Milli-Q water rinse flask. Hold down the flush button to flush the sample tubing for about one minute.

2) Put the sipper in the prepared blank sample. Hold down the flush button until the absorbance reading steadies, then press on the Fill blank button a few times until you are sure the absorbance reading is steady at ± 0.0000.

3) Next, read all the standards. Flush with the low standard first, and then continue flushing until a steady reading is obtained. Click the mouse on 'Read 1'.

4) Repeat for the replicate reading of first standard (by flushing and rereading the sample).

5) Read the standards in the order indicated on the screen. Press on the flush button with the next standard until a stabilized reading is obtained prior to each reading or replicate. For any replicate reading that varies more than 5% from the original reading, reread the standard.

6) When finished, click 'DispStdCurve' on the top of the screen. The correlation coefficient (r-coefficient) must be greater or equal 0.9995 for the curve to be acceptable. If the r-coefficient is less than 0.9995, prepare a new standard series.

7) Click on 'print' and record the r-coefficient on the bench sheet.

8) When printing is completed, click 'exit' to go to the standard screen.

9) Click 'print' to print the standard page.

SAMPLE ANALYSIS:

1) After printing, click 'Samples' on the left top of the screen. Start reading each run with the blank, then follow with the IC (Internal control) or the EC (external control, ERA). Then follow with the standard 0.2 mL (the daily detection limit standard).

2) On the left side of screen, select the sample ID box and change the ID to the sample code include the volume used and C of C# (for example: INFC 3mL 36703A). Then click on right side of the screen where the dilution factor is and enter the appropriate dilution factor.

3) Flush with the sample to be read until a steady reading is obtained.

4) Press the fill read button for the first reading of the sample.

5) Enter C of C#, then press the fill read button again. This will give a replicate reading for the sample. Whenever the replicate reading varies more than 5% from the original reading, reread the sample and make a notation on the printout (with your initials and the date).

6) Repeat steps 2-5 for each sample.

7) Read an external (EC) or internal control (IC) at least every 10 samples.

8) Reread the blank solution and at least two standards (which bracket the concentrations of the samples), and whichever EC/IC still needs to be run (one is chosen at the start of the run—may have duplicate readings as in step 9 for more than 10 samples).

9) Print the results by clicking 'print'.

10) Save the sample data by clicking 'save/clear'. Click the letter A to switch to the B drive. Click 'work-res' to get the typewriter screen and save the data by entering ' OP' for orthophosphate phosphorus and current date as OPMMDDYY. Click on 'OK' to return to the save screen. If file name is OK, then click OK.

11) To save the standard data, return to the standards screen by clicking 'standard' at the top of the screen and save the standard data just like the sample data in #10.

12) Flush the flow cell with Mill-Q water. Never leave anything but Milli-Q water in the cell. If air bubbles have been a problem a flush with ethanol may be needed, followed by a flushing with Milli-Q water. Record any such problem in the maintenance logbook.

13) Turn off the vis lamp, turn off the monitor.

14) Orthophosphate phosphorus is calculated by regression of the standard curve and reported as mg P/L.

Quality Control and Documentation

Acceptance criteria for QC parameters are specified in the QA plan. If any QC response falls outside these limits consult the QAO on appropriate actions.

Replicate Calculation of RPD:

$$\text{Relative Percent Difference (RPD)} = \frac{(\text{Original mg/L} - \text{Replicate mg/L})(100\%)}{(\text{Replicate average})}$$

Fortified Samples (spike) Calculation:

% Recovery =

$$\left[\frac{(\text{spike conc.})}{(\text{spike dil. factor})} - \frac{(\text{replicate average})\,(\text{vol. of sample spike})}{(\text{orig. sample dil. factor})\,(\text{vol. of orig. sample})} \right] \times 100\%$$

0.2(expected recovery of spike if use 2.0 mL)

$$\%\ \text{RPD} = \frac{(\text{MS\% recovery} - \text{MSD\% recovery})(100\%)}{(\text{MS\% recovery} + \text{MSD\% recovery})/2}$$

(spike conc.) _ (replicate average)(vol. of sample spike)

Anomalies: Record any abnormality observed in the sample. Consult the QAO for evaluation of QC exceedances. If the sample is being used as a QC check sample and it is known that the sample is non-homogenous or unusual (such as containing unusually large chunks) analyze a triplicate for confirmation.

QA Action Form: Some data may be deemed unacceptable -- e.g. poor spike recoveries for a sample having unknown matrix interference or in a case where no more sample is available, etc. Attach a QA Action Form and notify the QAO for evaluation.

Rerun. Be sure that the next analyst is informed when there are rerun needed.

Appendix F

QA/QC Information for Sample Collection and Sample Analysis

Phycotech Standard Operating Procedures

PhycoTech, Inc. General Technical Approach

PhycoTech is the only commercial lab in North America to utilize a unique proprietary permanent mounting technique for archiving and preparing samples for enumeration. These mounts allow you to get further data at a later date, as well as maintain a permanent archive of the sample that is easily stored, maintains fluorescence, and does not degrade with time (100+ years). Permanent algal mounts allow archiving of diatoms AND soft algae. All periphyton samples to species include both HPMA mounts for the whole sample and Naphrax, acid cleaned mounts, for diatom identification to species level. Zooplankton samples are also permanently mounted using a slightly different process. Our algal taxonomist, Dr. Ann St. Amand, has over 20 years of experience and has processed over 13,000 periphyton, phytoplankton, bacteria and zooplankton samples from both freshwater and marine systems. Dr. St. Amand is the only person who enumerates algal and zooplankton samples at PhycoTech, ensuring data integrity and consistency. Our In-house key and publication library numbers in the thousands, including the most current references. We have processed several state wide surveys in the Mid-West, West and Florida for phytoplankton and periphyton, each comprised of several hundreds of samples. PhycoTech also consults with Federal and State Agencies, including the Corps of Engineers, on experimental design and QA/QC issues. We process samples for general water quality, as well as the determination of exotic, toxic or taste and odor producing blue-green and chrysophyte algae. PhycoTech has extensive experience enumerating Prymesium parvum, a difficult to identify, toxic haptophyte from the Southwestern United States.

There are two state of the art microscopes used to process algal and zooplankton samples: an Olympus BX60, research-grade compound microscope equipped with Nomarski optics (40x, 100x, 200x, 400x, and 1000x), Phase Optics (400x, 1000x), a 1.25-2X multiplier, epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a Microfire digital camera attached, and an Olympus BHT, research-grade compound microscope equipped with Nomarski optics (100x, 200x, 400x, and 1000x), Phase Optics (400x), epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a Ricoh Camera Back attached using traditional slide and print film. For larger material PhycoTech also has a dissecting microscope. We have access to Notre Dame's SEM facility as well. Our computer network is a newly installed Novel Network connected to 6 new 2.4+ Gig workstations, with special adaptations for graphics and extra memory for efficiently handling our new data management system, ASA. All count related data is backed up daily to different media, with on-site and off-site copies weekly.

PhycoTech is now utilizing its proprietary data management software, ASA. This unique, powerful program not only tracks samples from receipt to data delivery within

the same software program (every processing step is documented with initials and date, from Login to Analysis), but also provides significantly more information for each sample. With ASA, we are able to provide not only biovolume estimates, but volume and surface area estimates as well. Our biovolume, volume and area formulas are the most complete set available commercially, drawn from a variety of sources including current primary literature (See our Technical Approach), custom calculations designed in-house for complicated morphologies (e.g. Ceratium) and independently derived calculations from an Outside Engineering Firm that specializes in volumetric studies (e.g. area of a prolate, oblate ellipsoid). We also now provide data summaries on phyla, division, class down to taxa level automatically, depending on the analysis requested. In addition, our new program has the capability to calculate over 72 different diversity indices and summary statistics, including Shannon, Maragalef, Alpha and Berger_Parker Diversity measures, Species Richness and Evenness, Pollution Tolerance for diatoms, Environmental Tolerance for algae, Siltation Index for diatoms, Pollution Tolerance for diatoms, Palmer Index, ACC:CMN for diatoms, in addition to others. All taxonomic information from organism down to coloniality and structure is provided in the data set. All indices are calculated on an abundance (both Natural units/mL and Cells/mL) and total biovolume, biomass, volume and area basis, if biovolume/biomass is measured. QA/QC reports are generated from within the program, comparing dominant taxa, reporting distribution checks and doing similarity calculations between the original sample and QA/QC sample. All slides are labeled with a unique Tracking ID code that appears on every report, data file and database generated within the laboratory.

Reports are provided in pdf format with summary graphics by group for each sample. Data files are provided in Excel format or other spreadsheet or database formats requested by the customer.

Algae

The HPMA method for producing algal sample slides provides an optically clear background while permanently infiltrating and preserving the sample for archival purposes (See references). Mounting distortion is minimal and the method provides the advantage of being able to go 100x to 1000x on the same specimen. Wet sample is always maintained in case clarification of identification is necessary. We strongly encourage our customers to use glutaraldehyde (final concentration of 0.25-0.50 %) for preservation of algal samples. It offers minimal distortion and allows the use of epifluorescence on algal samples while counting, which can dramatically improve the final results.

1) GENERAL PROTOCOL FOR MAKING PERMANENT ALGAL MOUNTS USING HPMA

EQUIPMENT:
Bunsen burner
Beaker tongs
Ice bath

Pyrex beakers (150 ML)
2 Dropper bottles
Mixed ester nitrocellulose filters (0.45 µm, 25 mm, plain)
Glass slides (25 mm x 75 mm)
Avery Laser Labels: #2181
Glass coverslips (25 mm x 25 mm, #1 or #1.5)
Full view series support/drying racks (102 pin)
Graduate cylinders
Dumont forceps
Glass filter towers (25 mL)
Rubber stoppers (#2, #10)
Filtration Manifold (6 station)
Vacuum pump (plus appropriate plumbing, 25-50 mm Hg)
Drying oven (60"C, not forced air)
Hood

REAGENTS:
HPMA (2-hydroxypropyl methacrylate)
Catalyst (azo-bis-iso-butyronitrile)
Iodine
Glutaraldehyde (25%, general grade)
Distilled water

CAUTION: Store HPMA and catalyst in refrigerator. Keep glutaraldehyde under hood.
METHOD:

SAMPLE:

1. Add enough glutaraldehyde to bring the final concentration to approximately 0.25% to 0.5% (for periphyton samples or "bloom tows", increase the final concentration to approximately 0.5%-1%). Keep the sample dark and refrigerate if possible.

2. Remove the sample from the refrigerator and let it warm to room temperature before mounting.

RESIN:

1. Prepare ice bath in plastic tub.

2. Measure 25 mL of HPMA and 0.025 g of catalyst into a 150 mL beaker.

3. Deal with HPMA under hood and use gloves for both HPMA and catalyst.

4. Under hood, light Bunsen burner and set to high flame.

Heat HPMA (with catalyst added) until you see density currents starting to form. Cool mixture by swirling in ice bath, and return to flame. **DO NOT LET MIXTURE**

BOIL!!!!!. Keep heating and cooling, alternately, until the mixture is approximately the thickness of Kayro syrup. Make sure the mixture is cool when it reaches this point or it will polymerize further. Transfer to a clean, glass jar for storage until usage. The entire procedure takes 2 to 1 hour, depending on how brave you are.
CAUTION!! THIS REACTION IS EXOTHERMIC ONCE IT REACHES A CERTAIN TEMPERATURE AND WILL TAKE PLACE ALMOST EXPLOSIVELY IF YOU LET IT GET TOO HOT. THE FUMES ARE TOXIC. KEEP WATER OUT OF THE PRE-POLYMER.
NOTE: Wash beakers in ethanol by letting them soak for 24 to 48 hours twice; wash with soap and rinse with distilled water. Be careful to keep dust out of the beakers when making the resin.
Fill 2 amber dropper bottles with resin. Add crystalline iodine to one of the bottles until the resin is nearly opaque. The iodine-resin will be slightly thicker than normal resin. (Resin is light sensitive -- be sure to cover the extra resin with foil.)

SLIDES:

MAKE THREE SLIDES FOR EACH SAMPLE -- SHAKE SAMPLE WELL (100 TIMES-phyt. or 200 TIMES-peri.). Use Millipore 6-place stainless steel manifold and Millipore Filtration Towers.

1. Put membrane filters onto filtration bases and wet with distilled water. Drain excess water through filter. If filter has any opaque areas (very white when wet), replace with another filter.
2. Assemble filter towers.
3. Measure out phytoplankton sample using micropipetor or macropipetor (use graduate cylinder for very dilute samples, e.g. 30+ mL). For periphyton samples, remove sample with micropipetor (usually from 0.05-0.5 mL) and dilute to 10 mL in a graduated cylinder with distilled water. Agitate to mix. Choose sample volume so that each field at 200x contains approximately 20-30 cells.
4. Add sample to the tower and open valve. For periphyton samples or large phytoplankton samples using cylinders, rinse graduate cylinder into tower. Filter sample until water <u>just</u> clears the filter surface. Close valve and remove filtration tower just after the water disappears from the inner edge of the tower.
5. Place filter, <u>FACE</u> down, on a cover slip (# 1.5). Be careful to avoid bubbles under the filter.
6. Samples:
    1. <u>Samples preserved in glutaraldehyde:</u>
       <u>3 slides</u>: Add 1-2 drops of clear resin to the back of the filter, and rotate the cover slip until the resin covers the back of the filter.

    2. <u>Samples preserved in lugols:</u>
       <u>3 slides</u>: Add 1-2 drops of the iodine-resin to the back of the filter, and rotate the cover slip until the resin covers the back of the filter.

7. Place cover slips on the drying rack and place in drying oven for 12 to 24 hours.

8. Remove cover slips from oven. Add 1 drop of resin to the filter side of the cover slip and attach to a labeled slide. Add as little resin as possible to cover the filter surface!!!! The less resin, the faster it will polymerize and the better the prep.
9. Put slides in the oven and let polymerize for approximately 24 hours. If the resin is not completely polymerized, replace and heat for as long as 2-3 days. Make sure that the slides are completely polymerized before you store them or they will run and/or evaporate!!!! And believe me, its a mess!!!!
10. Label slides with ASA generated labels. All slides are labeled with the Tracking ID, which appears on all reports, data files and in all databases associated with that sample bottle and associated data.

ACID CLEANING AND MAKING NAPHRAX SLIDES FOR DIATOMS

Phytoplankton/Periphyton:

If species identifications for diatoms are required or unknown diatom taxa are present, acid cleaned mounts in Naphrax are prepared according to the following procedure:

1) Take 5-20 mL of sample and transfer to a clean, 250 mL Pyrex beaker in the hood. Add room-temperature nitric acid to a total volume of 40-60 mL.
2) Cover with a watchglass.
3) After at least 24 hours has elapsed, carefully siphon off acid using glass siphon. Dilute acid and discard down drain with lots of extra water (Let water run for a minimum of 30 minutes after discarding acid).
4) A. Transfer remaining sample to a centrifuge tube and bring volume up to 14 mL with distilled water. Cap tube, mix well, and centrifuge at 3000 RPM for 5 minutes. Remove tube and carefully remove supernatant to the 2 mL volume marker with a micropipetor. Bring volume back up to 14 mL with distilled water, mix well, and repeat process. Complete a minimum of 6 centrifuge cycles. Check pH. If pH is lower than 7, repeat centrifuging process until the pH reaches 7.

4) B. On the final cycle, remove supernatant to the 1 mL volume marker and bring volume back to 5 mL. Mix well to suspend pellet and decant into the storage bottle. Rinse the centrifuge tube 2 more times with 5 mL of distilled water and decant into the storage bottle. The total volume of the cleaned sample should be 15 mL. If the sample is very sparse, lower final volume.
5) Using a pasture pipette, transfer enough sample to a cover slip (#1, 22mm square) to cover the entire area and place in a vibration-free area until dry.
6) Add 1 small drop of Naphrax to the cover slip and invert onto a slide. Compress the coverslip with a clean object and place in an oven (60oC) for 1-3 hours, or finish on a hot plate.
7) Ring cover slip with fingernail polish and store.

8) Identify taxa at 1000x under oil immersion. Reference taxa are identified using a diamond scribing objective and permanent ink labels.

Zooplankton

The HPMA method for producing zooplankton sample slides provides an optically clear background while permanently infiltrating and preserving the sample for archival purposes (See references). Mounting distortion is minimal. Wet sample is always maintained in case clarification of identification is necessary. Lignin Pink Double Stain allows for better visualization of animals and highlights critical morphological structures necessary for identification. Preferred preservative for zooplankton is EtOH. Lugol's iodine can also be used, but sometimes interferes with staining and obscures structures.

2) GENERAL PROTOCOL FOR MAKING PERMANENT ZOOPLANKTON MOUNTS USING HPMA

EQUIPMENT:
Bunsen burner
Beaker tongs
Ice bath
Pyrex beakers (150 ML)
2 Dropper bottles
Mixed ester nitrocellulose filters (5.0 µm, 47 mm, plain)
Analyslide (47 mm)
Laser Labels: 1½ x ¾ inch
Full view series support/drying racks (102 pin)
Graduate cylinders
Dumont forceps
Lignin Pink Double Stain
Glass filter tower (250 mL)
Filter Flask
Rubber stopper (#8)
Glass Microanalysis Filter Holder 47 mm disc
Vacuum hand pump
Drying oven (43°C, not forced air)
Hood

REAGENTS:
HPMA (2-hydroxypropyl methacrylate)
Catalyst (azo-bis-iso-butyronitrile)
Iodine
Alcohol (70% ETOH)
Distilled water

CAUTION: Store HPMA and catalyst in refrigerator.
METHOD:

SAMPLE:

1. Add enough alcohol to bring the final concentration to approximately 70%, or Lugols until a dark tea color.

RESIN:

1. Prepare ice bath in plastic tub.

2. Measure 25 mL of HPMA and 0.025 g of catalyst into a 150 mL beaker.

3. Deal with HPMA under hood and use gloves for both HPMA and catalyst.

4. Under hood, light Bunsen burner and set to high flame.

Heat HPMA (with catalyst added) until you see density currents starting to form. Cool mixture by swirling in ice bath, and return to flame. **DO NOT LET MIXTURE BOIL!!!!!.** Keep heating and cooling, alternately, until the mixture is approximately the thickness of Kayro syrup. Make sure the mixture is cool when it reaches this point or it will polymerize further. Transfer to a clean, glass jar for storage until usage. The entire procedure takes 2 to 1 hour, depending on how brave you are.

CAUTION!! THIS REACTION IS EXOTHERMIC ONCE IT REACHES A CERTAIN TEMPERATURE AND WILL TAKE PLACE ALMOST EXPLOSIVELY IF YOU LET IT GET TOO HOT. THE FUMES ARE TOXIC. KEEP WATER OUT OF THE PRE-POLYMER.

NOTE: Wash beakers in ethanol by letting them soak for 24 to 48 hours twice; wash with soap and rinse with distilled water. Be careful to keep dust out of the beakers when making the resin.

Fill 2 amber dropper bottles with resin. Add crystalline iodine to one of the bottles until the resin is nearly opaque. The iodine-resin will be slightly thicker than normal resin. (Resin is light sensitive -- be sure to cover the extra resin with foil.)

SLIDES:

MAKE ONE SLIDE FOR EACH SAMPLE -- SHAKE SAMPLE GENTLY 50 TIMES, if necessary, split sample with a Folsom plankton splitter.

1. Put membrane filter onto filtration base and wet with distilled water. Drain excess water through filter. If filter has any opaque areas (very white when wet), replace with another filter.
2. Assemble filter tower.
3. Measure out zooplankton sample using graduate cylinder. Choose sample volume so that each field at 100x contains approximately 5-10 animals.
4. Add one drop of Lignin Pink to graduated cylinder for every 5mL of sample. Let sample sit for 15 minutes.
5. Place entire contents of graduated cylinder into filter tower. Rinse graduate cylinder into tower twice. Filter sample (using vacuum hand pump) until water

just clears the filter surface. Remove filtration tower just after the water disappears from the inner edge of the tower.

6. Place filter, FACE up, on analyslide (47 mm). Be careful to avoid bubbles under the filter.
7. Add 8-10 drops of clear resin to the filter, and rotate the analyslide until the resin covers the whole filter.
8. Place analyslide on the drying rack and place in drying oven for 12 to 24 hours.
9. Remove analyslide from oven. Add just enough resin to the filter to cover the filter surface!!!! The less resin, the faster it will polymerize and the better the prep.
10. Put slides in the oven and let polymerize for approximately 24 hours. If the resin is not completely polymerized, replace and heat for as long as 2-3 days. Make sure that the slides are completely polymerized before you store them or they will run and/or evaporate!!!! And believe me, it's a mess!!!!
11. Label slides with ASA generated labels. All slides are labeled with the Tracking ID, which appears on all reports, data files and in all databases associated with that sample bottle and associated data.

Quality Assurance Plan

Taxonomic Accuracy

Dr. Ann St. Amand, a senior level phycologist and taxonomic expert, will perform all phytoplankton, periphyton, and zooplankton identifications, enumerations, and biovolume/biomass measurements. Dr. St. Amand has published extensively in the area of algal ecology and has processed over thirteen-thousand algal and bacterial samples, and is qualified to analyze zooplankton and macroinvertebrates. Outside taxonomists will be utilized for taxonomic verifications when necessary.

All samples are initially test mounted for counting density before final mounting. Any major questionable IDs are noted in the database during counting, and indicated on the report as uncertain for taxonomic clarity. If enough sample is present, samples are sent out to other taxonomists for taxonomic confirmation. Distribution is checked on approximately every tenth sample, during the counting process. All biovolume calculations have been verified by comparing with current literature, and by comparing calculations using outside mathematical consultations.

Sample Custody

The chain-of-custody requirements for all laboratory operations for each sample (broadly interpreted to include procedures for the preparation of reagents or supplies which become an integral part of the sample, record keeping associated with sample acquisition, documentation of sample preservation, sample labeling, sample tracking to establish chain-of-custody, and shipping and packing) and laboratory analysis (i.e.,

laboratory coding, storage, check-out, and documentation of sample movement) will be fully documented in our data management software. Each sample received will be assigned an individual tracking number. The sample bottle, chain-of-custody, and sample log sheet which accompanies each sample sent are then used in conjunction with one another, to enter the samples individual tracking number and all available sample information, into our sample database, ASA. The database allows for quick and accurate tracking of each sample received by PhycoTech. Dated and initialed entries by appropriate personnel on all worksheets and in the log database are required for data validation. All information entered into ASA is fully QA/QC'd for content and accuracy. Sample receipt is confirmed with each customer. All slides are labeled with the Tracking ID, which appears on all reports, data files and in all databases associated with that sample bottle and associated data.

COUNTING

Microscope:

There are two microscopes used to process algal samples: an Olympus BX60, research-grade compound microscope equipped with Nomarski optics (40x, 100x, 200x, 400x, and 1000x), Phase Optics (400x, 1000x), a 1.25-2X multiplier, epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a Microfire digital camera attached. For larger material PhycoTech also has a dissecting microscope. The BX60 is the primary microscope used for algal and zooplankton identification. There is also an Olympus BHT, research-grade compound microscope equipped with Nomarski optics (100x, 200x, 400x, and 1000x), Phase Optics (400x), epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a Ricoh Camera Back attached using traditional slide and print film.

Data Entry:

Samples are enumerated within ASA directly. ASA is a database driven program with an integrated virtual TallyMeter module, containing over 130 databases. Up to 400 taxa can be enumerated within any one sample, and the entire database currently contains over 33,000 taxa, including algae, zooplankton, macroinvertebrates and bacteria. All calculations are completed within ASA, including concentrations, biovolumes, biomasses and diversity indices. Data files are also generated by ASA and saved in Excel format, while reports are formatted and saved to pdf format utilizing Microsoft Access, including summary graphics on a per sample basis. PhycoTech can then format data files in any format required by the customer, either horizontally or vertically oriented. QA/QC on counting is a recount done on approximately every 10th sample. ASA produces a QA/QC report comparing the original sample and the recount sample (quantitatively and qualitatively), including the distribution check. Samples pass that are within 10% of the QA/QC recount, quantitatively. Percent similarity may vary up to 20% on exceptionally diverse or sparse samples.

Phytoplankton:

The magnification used will depend on the size of the dominant taxa and the size and number of particulates. The goal is to count at multiple magnifications in order to correctly enumerate and identify taxa present that may vary by several orders of magnitude in size. If the sample is dominated by cells below 10-20 µm or the cells are fragile and difficult to identify, the majority of counting will be completed at 400x-1000x. Measuring for biovolume includes measuring GALD and additional measurements including length, width and depth of different aspects of the colony or cell. ASA allows up to 28 separate measurements per taxa. Cell and colony shapes are approximated to a geometric figure and or figures and the appropriate calculations made. Currently, ASA has over 44 different shapes defined. From 10 up to a total of 30 natural units (sometimes higher on exceptionally variable taxa) are measured for each taxa depending on variability and number encountered.

1. Use ONE of the following methods depending on sample composition:

A. DOMINATED BY SOFT ALGAE: If the sample is dominated by soft algae greater than 10-20 µm in GALD, count a minimum of 300 natural units and 15 fields at 200x (when possible, maximum of 100 fields). In addition, count taxa below 10 µm or fragile, difficult to identify taxa at 400x (minimum of 100 natural units and 10 fields). Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ µm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

B. DOMINATED BY SOFT ALGAE: If the sample is dominated by soft algae less than 10-20 µm in GALD or is dominated by fragile, difficult to identify taxa, count a minimum of 400 natural units and 15 fields at 400x (when possible, maximum of 100 fields). In addition, count taxa above 20-30 µm in GALD at 200x (minimum of 15 fields). Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ µm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

C. DOMINATED BY DIATOMS: If the sample is dominated by diatoms other than large, easily identified taxa (e.g. Asterionella), count a minimum of 15 fields at 1000x, and a minimum of 400 natural units total (when possible, maximum of 100 fields). In addition, count soft algae according to size distribution (see A or B above) for a minimum of 15 fields at either 200x or 400x. Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per

slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ µm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

*** NOTE: The goal, regardless of magnification, is to enumerate and identify a minimum of 400 natural units per sample exclusive of misc. microflagellates.***

Periphyton:

The magnification used will depend on the dominant taxa. If the sample is dominated by diatoms, the majority of counting will be completed at 1000x. If the sample is dominated by soft algae, the majority of counting will be completed at 200-400x, whichever is appropriate considering cell size and particulates. The goal is to count at multiple magnifications in order to correctly enumerate and identify taxa present that may vary by several orders of magnitude in size.

The general counting method is as follows:

1. Use ONE of the following methods depending on sample composition:

A. DOMINATED BY SOFT ALGAE: If the sample is dominated by soft algae greater than 10-20 µm in GALD, count a minimum of 300 natural units and 15 fields at 200x (when possible, maximum of 100 fields). In addition, count taxa below 10 µm or fragile, difficult to identify taxa at 400x (minimum of 100 natural units and 10 fields). Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ µm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

B. DOMINATED BY SOFT ALGAE: If the sample is dominated by soft algae less than 10-20 µm in GALD or is dominated by fragile, difficult to identify taxa, count a minimum of 400 natural units and 15 fields at 400x (when possible, maximum of 100 fields). In addition, count taxa above 10-20 µm GALD at 200x (minimum of 15 fields). Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ µm): scan at least one whole slide at 100x. This tiered counting method should
yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

C. DOMINATED BY DIATOMS: If the sample is dominated by diatoms, count a minimum of 15 fields at 1000x, and a minimum of 400 natural units total (when possible, maximum of 100 fields). In addition, count soft algae according to size distribution (see A or B above) for a minimum of 15 fields at either 200x or 400x. Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ µm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.
*** NOTE: The goal, regardless of magnification, is to enumerate and identify a minimum of 400 natural units per sample exclusive of misc. microflagellates.***

Zooplankton:

Zooplankton are enumerated at 100x to 200x, depending on the average size of animal present (structures can be viewed at 400x, if necessary). Counting procedure is consistent with Standard Methods, with the target being 200 animals. Studies requiring greater precision or focusing on diversity require a higher counting threshold. Generally, when the sample is sparse, the entire slide is counted. Measurements for biomass include length, width and depth. ASA calculates biomass on crustaceans using published length/weight regressions, and on rotifers using biovolume formulae where biovolume is then converted to biomass. ASA can also use constant weights. If requested, customers may provide custom biomass calculations for ASA to use as well.

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St. Amand, A. 1990. Mechanisms controlling metalimnetic communities and the importance of metalimnetic phytoplankton to whole lake primary productivity. Ph.D. Dissertation. University of Notre Dame, Notre Dame, Indiana, USA.

Olrik, K., et. al. 1998. Methods for Quantitative Assessment of Phytoplankton in Freshwaters, part I. Naturvårdsverket, Stockholm.

Hillebrand,H., et. al. 1999. Biovolume Calculation for Pelagic and Benthic Microalgae. Journal of Phycology. 35: 403-424.

Due to the steady increase in shipping and handling expenses, PhycoTech, Inc. charges a $25.00 fee for the return shipment of each cooler sent to us. If you do not want your cooler/s to be returned to you and wish to avoid the $25.00 shipping and handling fee, please write "disposable cooler" on the exterior surface of the cooler or enclose a memo with each cooler stating that the cooler is disposable.

Shipment Information for Samples:

1. **Samples should still be shipped to us via overnight service.**
2. **All plastic coolers will be automatically returned with a $25.00 shipping and handling fee.**
3. **PhycoTech cannot accept any weekend deliveries.**

Alternate methods of packaging samples (either method will be more economical than shipping an actual cooler):

Securely tape each container lid closed. Place each sample container into a sealed bag for either shipping method. Dark plastic sample containers are highly recommended. All containers (plastic and glass) should be firmly packed with Styrofoam, paper, or vermiculite (to avoid breakage), and shipped with freezer packs or ice (sealed in airtight bags).

1. **Place bagged samples into a disposable Styrofoam cooler and put the cooler into a corrugated box.**

OR

Place bagged samples into a corrugated box lined with Styrofoam sheeting.

Appendix G

QA/QC Information for Sample Collection and Sample Analysis

Southern Nevada Water Authority Laboratory Division Standard Operating Procedures

SOUTHERN NEVADA WATER SYSTEM
STANDARD OPERATING PROCEDURE

Subject:	CHLOROPHYLL a: ANALYSIS USING THE VARIAN SPECTROPHOTOMETER	Number: L565	Page 1 of 3
Division: LABORATORY	**Approved:** Signed by Linda R. Blish — Laboratory Manager; **7/09/04** — Date	Reviewed & Revised By: **ABS 6/04**	**Issue: 2**

PURPOSE

Once chlorophyll a samples have been extracted (see SOP #L567, Chlorophyll a: Extraction Analysis Using The Grinding Method) they should be analyzed using a spectrophotometer. This SOP is designed to allow you to analyze chlorophyll extracts using a Varian-Cary 1E, UV-visible spectrophotometer operated by a personal computer.

MATERIALS

- Varian-Cary 1E, UV-visible spectrophotometer
- Grid, IBM compatible, computer
- Varian, Cary chlorophyll a program
- Printer
- Surge protector

PROCEDURE

A chlorophyll abc analysis program has already been prepared and this SOP will only describe how to enter the computer and program, run analysis, and exit. However, it is suggested that you read the Getting Started section of the Varian-Cary 1E Operation Manual to become familiar with the other parameters that will also be displayed.

Note: In this SOP, all words in capital letters denote what will be displayed on the computer screen; a *** means press the enter key; and words in italics indicate actual words you are to type after a prompt.

1. Turn on the surge protector power switch. The computer, screen, and printer should then operate.

2. The computer boots-up and at C:\> type *Cary*

3. CARY APPLICATIONS AVAILABLE appears and move cursor with arrows (right side of keyboard) to BASE SYSTEMS and ***.

4. After PLEASE WAIT located at the top left of screen disappears, turn on spectrophotometer (spectrophotometer is plugged into a separate 115 v electric outlet). The system will now be RESETTING, make sure the spectrophotometer cuvette holders are empty and the lid is closed; wait until this is finished before proceeding (see top left corner of screen).

   At the bottom of the screen are basic instructions and parameters, which read: PARAMETERS DISPLAY REPORTS ACCESSORIES APPLICATION FILING MORE. You can tag any parameter by moving the cursor with arrows to a word and pressing *** or by typing the capital letter (generally the first) in the word required. Esc will allow you to return to the INDEX screen.

5. To use the chlorophyll a program move the cursor to FILING and *** or type *F*. This will list all the programs that have been created. Program Number 3 is the Chlorophyll Analysis program and must be the only program tagged (number should be in a yellow box) to use. See appendix at the end of this SOP to change the tagged program.

6. To start the Chlorophyll Analysis program, move cursor to new USE/VIEW and ***, then press F8 (START). This will engage the chlorophyll program specifically designed for SNWS.

7. At the bottom of the screen, the following questions will be asked. (Note: when a question is asked and a wrong key is pressed you can backspace to correct and then retype. However, one you press *** the mistake cannot be corrected). Most of the information required to answer the chlorophyll program questions are recorded on SNWS Form #478 (completed by monitoring staff).

   a. ENTER NUMBER OF SAMPLES: do not include replicates in this number ***

   b. ENTER NUMBER OF REPLICATES PER SAMPLE: generally 3 ***

   c. ENTER THE NAME TO STORE REPORT UNDER: generally write *Lake Mead Chlorophyll* and date collected and date analyzed ***

   d. Now you must zero the spectrophotometer using acetone blanks. Place separate cuvettes filled with 90% acetone in front and rear cuvette holders and close spectrophotometer lid. The sentence presented at the bottom of the screen will state PRESS ENTER TO START A BASELINE COLLECT: Once the blanks are in place, you ***. The screen will show the zeroing process. Wait for next prompt.

   e. ENTER STATION NAME: write station name, number and depth ***

f. ENTER SAMPLE DATE: enter month, day, and year ***

g. ENTER ML EXTRACTED: 10 generally or whatever volume ***

h. ENTERE ML FILTERED: 500 to 1000 generally but may be variable (see information recorded on Form 478 or sample label) ***

i. INSERT SAMPLE, PRESS THE ENTERE KEY TO READ REP #1: Remove blank cuvette from front holder (the rear cuvette with acetone remains throughout the analysis) and place sample #1 in the front holder. Close spectrophotometer lid and ***. The wavelength in nm is shown at top right of the screen and absorbance is shown at top left of the screen. Wait for next prompt.

j. ADD THE ACID, PRESS THE ENTER KEY TO READ SAMPLE AGAIN: Remove front cuvette and add 0.1 mL of 0.1 N HCl to sample, gently shake, place cuvette back in front holder, and wait until 90 seconds have passed since acidification before pressing ***. Wait.

8. Once absorbencies from all wavelengths are measured for a sample, the information is automatically stored and printed out. On the printer, make sure the ON LINE light and POWER light are on.

9. Finish remainder of samples at prompts and when all samples are completely analyzed, "HAVE A NICE DAY!" will appear.

10. To exit the program and computer, press Esc two times. Computer will then ask EXIT TO SELECT DOS/ APPLICATIONS (Y/N). Press Y and *** and this brings you back to CARY APPLICATIONS AVAILABLE. Press Esc and this brings you back to DOS at the C:\> prompt.

11. Turn off the spectrophotometer and turn off the main surge protector switch.

APPENDIX

While in FILING, if another program number is tagged, to tag Program #3 (Chlorophyll Analysis) press F2 (EDIT), which will allow you to edit. With the arrows, move cursor to the program number hat you want tagged. The cursor that is on that untagged program number will be shown in a green box. To tag this program press *** and the box will change from green to yellow. You also must un-tag the previously tagged program; move the cursor with the arrows to the program number in the yellow box and press *** to un-tag this program. When the correct program you need is tagged and all others untagged, press Esc to exit from edit mode.

Now view the specific spectrophotometer parameters used for the Chlorophyll Analysis program. While still in FILING, move cursor to USE/VIEW (at bottom of screen) and ***. The program should read as follows:

Method Name	
Chlorophyll Analysis	
Photometric Mode	ABS
Abscissa Mode	NM
Ordinate (Y) Min/Max	-
0.1000/5.000	
Abscissa (X) Min/Max	
625.00/755.00	
SBW (nm)	1.0
Signal Average Time (sec)	0.500
Data Interval	1.000
Scan Rate (nm/min)	120.000
Lamps On	VIS
Baseline Correct	ON
Auto Scale	NO
Auto Store Data	NO
Auto Store Report	NO

SOUTHERN NEVADA WATER SYSTEM
STANDARD OPERATING PROCEDURE

Subject:	CHLOROPHYLL a: COLLECTION, FILTRATION AND STORAGE		Number: L566	Page 1 of 2
Division: LABORATORY	Approved: Signed by Linda R. Blish Laboratory Manager	7/09/04 Date	Reviewed & Revised By: ABS 6/04	Issue: 5

PURPOSE

The concentration of chlorophyll a can be used to give an estimate of phytoplankton biomass and is used for detecting potential water quality issues. The objective of this SOP is to describe collection, filtration, and storage of chlorophyll samples.

EQUIPMENT

- 6 m flex hose
- 2 m flex hose or van Dorn
- Large carboy (10 to 20 L)
- Graduated cylinders (1000mL)
- Filter funnel(s)
- Filter flask
- Wash bottle
- Rinse water (preferably reagent, deionized, distilled)
- Vacuum source with no greater than 250 mm Hg suction pressure
- Whatman GF/C (glass fiber filter) with 0.45 µm mean pore size and 47 mm diameter
- Saturated magnesium carbonate ($MgCO_3$) solution (make by adding 1 g of powder $MgCO_3$ to 100 mL distilled water)
- Forceps for handling filters
- Aluminum foil cut into squares about 8 x 8 cm
- Labels
- Water tight container
- Ice chest or refrigerator to chill samples

METHOD

A. Collection

1. Before collecting a new sample, always rinse sampling and collection equipment (flex hose, van Dorn, collection carboy, graduated cylinder, filter

funnel) either with water collected at the required depth or station or rinse water.

2. Use a 6 m flex hose for collecting surface samples in clear-water areas (most open water areas of Lake Mead) and use a 2 m flex hose in areas where surface waters are high in phytoplankton abundance (especially, Las Vegas Wash inflow). If required, van Dorn is used to collect water at discreet depths in the water column.
3. A minimum of about 10 L of water should be collected and thoroughly mixed in a carboy. When sampling with any water-collecting apparatus (flex hose, van Dorn, etc.) the entire contents should be emptied into the carboy.

B. Filtration

1. Set up filtering apparatus with GF/C filter. Be cautious not to touch filter (use forceps) or inside of funnel and base.

2. Three replicates are filtered for each site and or depth. Sample volume varies depending on time of year and location; however, always filter the greatest volume of water that can be filtered. Generally, filter a maximum of 1000 mL in most clearwater areas and 250-500 mL at Las Vegas Wash inflow.

3. Completely mix the sample in the carboy and pour the required amount, to be used for all replicates, into a graduated cylinder.

4. Before filtering the entire volume of sample, pour about 50 mL of the sample into the filter funnel and add three (3) drops of $MgCO_3$. Completely filter this volume and then filter the remainder of the sample. **NOTE** If all of the required amount of measured sample in the graduated cylinder will not filter completely, discard sample. Begin again by thoroughly mixing water in carboy and measuring a smaller volume.

5. Once the entire volume of sample is filtered, rinse funnel with about 30 mL of reagent water.

6. Use forceps to fold filter into quarters, place in foil, and fold foil so that filter is completely sealed from light.

7. Label each sample with date, location, depth, and volume filtered.

C. Storage

1. Place completed samples into a sealed watertight container and keep in an ice chest or refrigerator approximately 4°C. Bring back to the laboratory and place in freezer section of a refrigerator.

NOTE: It is important to record weather information at each sampling station on Data Form #478. For consistency, use descriptions listed below, however, any additional detail is appreciated.

Wind Direction- N, S, E, W, NW, NE, etc.
Wind Strength- calm, light breeze, breeze, strong
Wave Action- calm, ripple, 0.3 m (1 ft) waves, 0.7 m (2 ft) waves, etc.
Sky Conditions- sunny, partly cloudy (indicate by how much- 25%, 50%, 75%), overcast, rain.

SOUTHERN NEVADA WATER SYSTEM
STANDARD OPERATING PROCEDURE

Subject:	CHLOROPHYLL a: EXTRACTION ANALYSIS USING THE GRINDING METHOD		**Number: L567**	**Page 1 of 3**
Division: LABORATORY	**Approved:** Signed by Linda R. Blish Laboratory Manager	**7/09/04** Date	Reviewed & Revised By: ABS 6/04	**Issue: 3**

PURPOSE

The purpose of this SOP is to describe chlorophyll a extraction analysis and the procedure for spectrophotometric analysis. The method used at Southern Nevada Water System (SNWS) is the grinding method using 90% acetone as the extraction solvent. Note: This procedure requires one long day or an afternoon and early morning for sample preparation and for spectrophotometric analysis.

MATERIALS

- Chlorophyll samples (see Chlorophyll a: Collection, Filtration, and Storage, SOP #L566)
- An area with subdued lighting in which to work
- Three 15 mL polypropylene centrifuge tubes for each sample (as the sample is in triplicate) and two additional tubes for acetone blanks
- Centrifuge
- Forceps
- 90% acetone (minimum quality of spectrophotometric grade); make by adding 10 mL of distilled water to every 90 mL of acetone. **CAUTION**: Read MSDS before using acetone. Work under a fume hood to avoid breathing acetone fumes.
- Tissue culture grinder
- Tissue culture flasks
- Teflon pestle
- Minimum of two optical glass cuvettes having a width of 10 mm
- Automatic pipettes (5 mL)
- Spectrophotometer with a visible light source and a band width not greater than 2.0 nm
- 0.1 N hydrochloric acid (make by adding 8.3 mL of concentrated HCl to a final volume of 1000 mL with distilled water). Follow MSDS safety precautions.
- 0.15 N NH_4OH (make by adding 5 mL concentrated NH_4OH to a final volume of 500 mL with distilled water). Follow MSDS safety precautions.

- 90 second timer
- 3 mL micropipetter and tips
- 0.1 mL micropipetter and tips

METHODS

NOTE: The chlorophyll molecule is very sensitive to ambient light intensities and heat, either of which can cause it to degrade rapidly. Therefore, when doing analysis it is important to keep samples (filters and acetone extracts) protected from direct light and heat in addition to working in a cool area with subdued lighting.

A. EXTRACTION

1. Prepare 15 mL polypropylene tubes by removing lids and labeling each with the station number and replicate number. Label two extra tubes as blanks.
2. Remove samples from freezer (see Chlorophyll a: Collection, Filtration, and Storage, SOP #L566) in a dark area allowing only sufficient light to work in.

B. GRINDING

1. Set up tissue culture grinder on ring stand and insert Teflon pestle into attachment holder.
2. Remove filter from aluminum foil.
3. With forceps, separate filter into 2-4 pieces to facilitate grinding.
4. Place pieces of filter in clear tissue culture flasks, pre-rinsed in deionized water, and add 5 mL of 90% acetone to flask.
5. Using an appropriate speed to minimize excessive heating and/or sample deterioration, place the pestle in the tissue culture flask and grind the filter for 30-45 seconds.
6. Pour acetone in appropriately labeled 15 mL centrifuge tube. With forceps remove filter and place in centrifuge tube. Add 5 mL of 90% acetone (10 total mLs of extractant) and cap centrifuge tube.
7. Clean pestle with Kim Wipe wetted with 100% Acetone,
8. Obtain new sample, another tissue culture flask and repeat above procedure until all samples are processed.
9. Place all samples in cold environment for at least 6 hours to allow for chlorophyll to be extracted into acetone solution.
10. After extraction, place tubes in centrifuge and spin at 2800 RPM at 8°C for 8 minutes.

11. Place tubes in racks in the dark and allow to warm to room temperature.

C. ANALYSIS

NOTE: Chlorophyll analysis at SNWS is done using a Varian, Cary 1E, UV-visible spectrophotometer that is operated using a personal computer. See SOP #L565 (Chlorophyll a: Analysis Using the Varian Spectrophotometer) to operate this particular spectrophotometer. The analysis section in this SOP will describe spectrophotometer specifications and the procedure of analysis.

1. While samples are in centrifuge, warm-up spectrophotometer and perform a baseline zero by using acetone blanks according to the manufacturer's specifications. When using 10 mm cuvettes, add 3 mL of sample to determine absorbance.
2. When baseline zeroing is complete, remove the front acetone blank from cuvette holder and place a cuvette containing 3 mL of the chlorophyll extract sample in the front cuvette holder. Read optical densities at 750 nm, 664 nm, 647 nm, and 630 nm and record the amount of absorbance.
3. Acidify the same chlorophyll sample (front cuvette) by adding 0.1 mL of 0.1 N HCl and gently shaking. After 90 seconds of acidification, read optical densities of acidified sample at 750 nm and 665 nm and record absorbance.
4. Between each sample rinse cuvette twice with distilled water, once with 0.15 N NH_4OH, twice again with distilled water, and a final rinse with acetone.
5. Complete the remainder of samples and when finished, rinse cuvettes as you would between samples.

D. CALCULATIONS

1. SNWS only requires the use of the United States Environmental Protection Agency (USEPA) monochromatic equation that corrects for pheophyton when determining chlorophyll a. This equation is calculated on the Varian spectrophotometer computer automatically.

$$\text{Chlorophyll a } (\mu g/L) = \frac{26.7\ [(664-750)-(665-750)] \times V_1 \times 1000}{V_2 \times L}$$

750 = absorbance used to correct for turbidity
664 = absorbance before adicidification
V_1 = volume of 90% acetone extract (mL)

V_2 = volume of sample filtered (mL)
L = width of cuvetter (cm)

2. Absorbencies measured at wavelengths 647 nm and 630 nm are used to determine chlorophyll a using the USEPA trichromatic equation that does not correct for degradation products. If needed, the equation is presented below.

$$\text{Chlorophyll a } (\mu g/L) = \frac{11.85\,(664-750)-1.54(647-750)-0.08(630-750)}{V_2} \times V_1 \times 1000$$

3. The concentration of chlorophyll a degradation product, pheophyton, using a USEPA equation also may be useful.

$$\text{Pheophyton a } (\mu g/L) = \frac{26.7\,[1.7\,(667-750)-(664-750)]}{V_2 \times L} \times V_1 \times 1000$$

QUALITY CONTROL AND ASSURANCE

Multiple volumes of a sample are analyzed. This involves taking a sample and filtering known volumes, also in triplicate, to be analyzed. The relative difference of the sample triplicates are compared to their average.

Appendix H

QA/QC Information for Sample Collection and Sample Analysis

Southern Nevada Water Authority Research and Development
Laboratory Standard Operating Procedures

Southern Nevada Water Authority
Water Quality Research and Development

Algal Toxin Analysis Using Liquid Chromatography with Tandem Mass Spectrometry Detection
Version 1.0

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1-METHOD SUMMARY

A small portion of an aqueous sample is injected into a liquid chromatograph (LC). The sample merges with a formic acid/water/methanol eluent and is pumped through the LC system using an eluent gradient. Six algal toxins -- Cylindrospermopsin toxin, Anatoxin-a, Nodularin, and Microcystins LR, YR and RR -- are separated on the basis of their affinity for the active sites of the column packing material. As each compound elutes from the LC, it is ionized in the ion source of the mass spectrometer. During the analysis, each ionized compound's molecular transition is monitored by the mass spectrometer. As each compound elutes from the LC, it is detected by the mass spectrometer and the intensity of the peak is measured. The intensity of the peak is used to calculate the concentration of algal toxin in the sample based on the intensity of known concentrations of previously injected standards.

2-ANALYTICAL WORKING RANGE

A calibration curve for each analyte of interest is constructed to cover the range of concentrations expected for samples. Using a tandem mass spectrometer, the calibration range for the algal toxins is 0.50 μg/L to 100 μg/L.

3-SAMPLE COLLECTION, PRESERVATION AND STORAGE

3.1-Samples should be collected in pre-cleaned 250 mL amber glass bottles. The volume collected should be sufficient to insure a representative sample, allowing for all quality control analysis.

3.2-Samples should be preserved in the field with ice to maintain a temperature of 4°C.

3.3-If a sample is to be analyzed for total algal toxins (including algal toxins contained within algal cells), an aliquot of the sample should be transferred to a plastic bottle and frozen at -80°C when it is received by the laboratory.

3.4-If the samples are to be analyzed for only free algal toxins, they must be immediately filtered through a 0.45 micron filter to remove all algal cells and turbidity. The filter paper can then be stored at -80°C for potential future analyses. The filtrate can then be stored at 4°C until analysis.

4-STANDARDS AND REAGENTS

4.1-*Preservative*

4.1.1-No chemical reagents are necessary to preserve the samples.

4.2-Ultrapure water

4.2.1-Ultrapure™ water used for the eluent and dilutions of stock solutions is obtained from a Barnstead NANOpure Infinity water purification system.

4.3-Eluent

4.3.1-Two solutions are used during the gradient elution: A: 0.1% (v/v) formic acid in ultrapure water and B: 100% methanol. 0.1% (v/v) formic acid is prepared by diluting 1 mL of pure formic acid into 1 L ultrapure water.

4.4-Stock Solutions

4.4.1-Cylindrospermopsin toxin was obtained from Wayne Carmichael at Wright State University and James Sinclair of the US EPA in Cincinnati, Ohio. One mg of toxin was dissolved into 1 mL of methanol to produce a 1000 mg/L stock solution.

4.4.2-Nodularin toxin was obtained from Calbiochem. 250 μg of toxin was dissolved into 1 mL of methanol to produce a 250 mg/L stock solution.

4.4.3-Microcystin YR was obtained from Calbiochem. 250 μg of toxin was dissolved into 1 mL of methanol to produce a 250 mg/L stock solution.

4.4.4-Microcystin LR was obtained from Sigma Chemical. 500 μg of toxin was dissolved into 2 mL of methanol to produce a 250 mg/L stock solution.

4.4.5-Microcystin RR was obtained from Sigma Chemical. 250 μg of toxin was dissolved in 2 mL of methanol to produce a 125 mg/L stock solution.

4.4.6-Anatoxin-a was obtained from Sigma Chemical. 250 μg of toxin was dissolved in 5 mL methanol to produce a 50 mg/L stock solution.

4.5-Working Standards

4.5.1-A 5.0 mg/L intermediate standard was prepared by diluting appropriate amounts of each of the six stock standards in 10 mL methanol. This intermediate stock was then diluted in water to make a standard calibration curve from 0.5 μg/L to 100 μg/L.

5-SAMPLE PREPARATION

5.1-Total Algal Toxins

5.1.1-Samples for total algal toxins should have been frozen at -80°C after sample collection in a plastic bottle. To lyse the algal cells and release the algal toxins contained within the cells for analysis, a series of freeze/thaw events are necessary. Samples previously frozen at -80°C should be thawed at room temperature until the sample has reached room temperature. This freeze/thaw procedure should be repeated a total of three times. After this is complete, the sample should be filtered with a 0.45 micron filter to remove all algal cell debris along with any turbidity remaining in the sample. The filtrate should then be stored at 4°C until analysis.

6-ANALYTICAL

6.1-Instrument Configuration

6.1.1-All analyses are performed using an Agilent 1100 LC chromatographic system coupled with an Applied Biosystems API 4000 triple quadrupole mass spectrometer.

6.2-Liquid Chromatograph Parameters

6.2.1-An injection volume of 50 μL (20μL injection loop) is used for all standards and samples.

6.2.2-A Synergi Max-RP 250mm x 4.6mm column is used for all analyses. A flow rate of 700 μL/min is used throughout. The gradient that is used to elute the algal toxins of interest is shown in Table 1. (A: 0.1% (v/v) formic acid in water and B: 100% methanol)

Table 1. Elution Gradient for Algal Toxin Analysis

Time	A	B
0.01	95%	5%
3.50	95%	5%
10.00	20%	80%
13.00	20%	80%
13.01	0%	100%
17.00	0%	100%
17.01	95%	5%
26.00	95%	5%

6.3-Compound-Specific Parameters of Interest (Table 2)

Table 2. Compound-Specific Parameters

Compound	Retention Time (min)	Precursor Ion (m/z)	Product Ion (m/z)
ESI Positive			
Anatoxin-a Transition #1	9.2	166.26	149.15
Anatoxin-a Transition #2	9.2	166.26	91.05
Anatoxin-a Transition #3	9.2	166.26	43.15
Microcystin RR	13.3	523.98	514.75
Microcystin YR	14.7	534.48	135.05
Nodularin	14.7	825.48	134.95
ESI Negative			
Cylindrospermopsin toxin	10.1	414.24	95.95
Microcystin LR	14.9	993.71	975.55

6.4-Quality control

6.4.1-Established calibration curves must be calculated using either linear or quadratic regression and be weighted by a factor of at least 1/x. Calculated curves must display a correlation coefficient of 0.99 or greater.

6.4.2- Continuing calibration verifications (CCV) using a mid-level standard are carried out at a frequency of no less than every six samples, including a final calibration verification at the end of the run. The definition of a failure of calibration will be left to the judgment of the analyst. A recovery of 70-130% will be used as a guideline. If a CCV is deemed to be out of control, the analyst will determine what corrective actions need to be taken to ensure the quality of the data.

6.4.3-At least one matrix spike will be performed on each set of samples collected during a sampling event. The definition of a failure of calibration will be left to the judgment of the analyst. If a matrix spike is deemed to be out of compliance, the analyst will determine what corrective actions need to be taken to ensure the quality of the data.

6.4.4-A blank is analyzed following calibration, before and after each set of six samples, and before and after each CCV to detect possible carryover.

6.4.5-If any sample tests positive for any algal toxin, it will be reanalyzed to confirm the positive result. If the reanalysis is again positive for the algal toxin, it will be considered valid. If the reanalysis is negative, the sample will be analyzed a third time to confirm or deny the presence of the algal toxin.

6.4.6-When data quality fails to meet established criteria, samples are re-analyzed and/or the entire run repeated if analyst suspects poor data quality.

6.5-Reporting

6.5.1-All algal toxins are reported in μg/L.

6.5.2-The Reporting Limit (RL) for all six algal toxin species is 0.500 μg/L.

6.5.3-Non-detect (ND) is reported if a concentration is less than the reporting limit.

6.5.4-Target analyte concentrations are reported to two (2) significant figures.