

Quality Assurance Project Plan:  
Spokane Regional Wastewater Phosphorus Bio-availability Study

Prepared by

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Spokane, Washington 99260

July 2009

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## 1. Abstract

The hypolimnion of Lake Spokane commonly experiences hypoxia, and it is believed that wastewater treatment plant (WWTP) effluent is contributing to this problem. Therefore, the wastewater treatment plants discharging to the Spokane River upstream of Lake Spokane will be adopting a variety of advanced technologies for phosphorus removal. Different advanced phosphorus removal methods differ considerably in how low they decrease total phosphorus concentrations, as well as the species of residual phosphorus present in their effluent. The bio-availability of phosphorus (BAP) of these various species can vary greatly depending on their source, and BAP is likely to vary with the type of tertiary treatment employed. The objective of the present study will be to determine the percent BAP in effluent from pilot tertiary treatment projects at the main WWTP discharges to the Spokane River. This will be done using the classic algal growth bioassay approach which utilizes algal growth to estimate P availability in unknown samples. This approach is much more labor intensive than chemical approaches to characterize various forms of phosphorus, but this approach is the "gold standard" by which BAP is quantified.

## 2. Background

Dissolved oxygen depletion in the hypolimnion of Lake Spokane is the main driver for the dissolved oxygen total maximum daily load (TMDL) for the Spokane River and Lake Spokane. Using the CE-QUAL W2 model calibrated for the Spokane River, the Department of Ecology (Ecology) determined that the main cause of the observed oxygen depletion results is from the biodegradation of algae in the hypolimnion. Algal production in Lake Spokane is stimulated by phosphorus and other nutrients in the water entering the lake during the summer growing season. As Lake Spokane primary productivity is phosphorus limited, it appears that improving hypolimnetic dissolved oxygen concentrations will require significant reductions in phosphorous loads, and possibly most importantly, bio-available phosphorus loads.

Currently the assumption is that all phosphorus in WWTP effluent is bio-available. That is, during the growing season, all of the phosphorus in effluent discharges is available and used for plant (algae) growth. Phosphorus in WWTP effluent and natural systems is found in a variety of forms including dissolved inorganic molecules of phosphate, dissolved organic compounds, and both organic and inorganic molecules attached to particulate matter. There is considerable debate over which of these forms and conditions of phosphorus is bio-available.

This study is designed to determine the extent to which the various forms of phosphorus in WWTP effluent are available for algal growth in Lake Spokane using an algal bioassay technique. Using the algal bioassay allows the calculation of phosphorus bio-availability using far fewer assumptions than when trying to use wet chemistry approaches to quantify phosphorus forms and infer their bio-availability.

### **3. Previous Work**

In January of 2006 NCASI (National Council for Air and Stream Improvement) undertook a study of effluent from Inland Empire Paper Company's Millwood, WA plant to determine the bio-availability of phosphorus from that plant. The report by researchers from the NCASI Western Research Center in Corvallis, Oregon, concluded that essentially none of the phosphorus in the paper mills effluent was biologically available. (Malmberg, 2006) However, the report points out that the experimental variability of the results was in several cases greater than the phosphorus concentrations in the effluent. Close examination of the study procedures reveals that part of that variability likely stems from changes in the analytical procedures employed during the study. The change in procedure casts considerable doubt on the validity of the study. More importantly, these researchers did not follow a protocol for determining "biologically-available phosphorus" that even remotely corresponds to conventional methods to measure BAP.

Using samples collected in the fall of 2007, Dr. David Yonge (Yonge 2008) of the WSU Department of Civil and Environmental Engineering conducted similar research for Inland Empire Paper. Dr. Yonge reported results which were similar to those of NCASI. However, here again the use of methods different than those employed by NCASI and different from standard practice calls into question their validity.

Dr. Michael Brett of the University of Washington (UW) Department of Civil and Environmental Engineering has used a bioassay approach to determine bio-availability of phosphorus in sediment and other environmental media (Ellison and Brett 2006). This procedure directly measures the algal productivity stimulated by nutrients in a system. By using this approach many of the uncertainties about what the various wet chemistry procedures actually measure and how those parameters relate to biological activity are avoided.

### **4. Project Description**

The Spokane regional wastewater phosphorus bio-availability study has three primary goals:

- 1) Determine the fraction of total phosphorus in effluent from Spokane area WWTP pilot tertiary treatment processes that is biologically available
- 2) Determine how advanced phosphorus removal technology affects the BAP of the effluent
- 3) Determine if the bio-availability of phosphorus from Spokane area wastewater discharges varies seasonally

To accomplish these goals effluent samples from several WWTPs will be evaluated using laboratory bioassay procedures.

The study will focus on effluent from tertiary treatment pilot projects at various WWTPs and samples from several natural stream locations within the watershed. Effluent from WWTPs processed through the following pilot tertiary treatment processes will be used for evaluation in this study (Figure 1):

- City of Spokane
  - Kruger Actiflo sand-ballasted sedimentation
  - Cambridge Water Technology’s CoMag ballasted sedimentation
  - Zenon membrane filtration
  - Corix conventional sedimentation
  - Blue Water continuous upflow filter
  - Corix multi-media granular filtration
- City of Coeur d’Alene
  - Blue Water continuous upflow filter
  - Zenon micro-filtration system
  - Zenon membrane bioreactor system
- Inland Empire Paper
  - Siemens Trident HS system tertiary treatment

Samples will be collected from the pilot projects each month to allow the study to examine effluent from the various treatment processes.

Additionally, a parallel study at Northwestern University is being implemented to conduct detailed phosphorus speciation analysis of effluent samples from the same WWTPs. The Northwestern study is funded in part by the Water Environment Research Foundation. Sampling for both studies will be coordinated, when possible, to help avoid repeated analytical analysis and to allow the two studies to build off the associated results. Both studies are focused on phosphorus in effluent from WWTPs.

## 5. Organization and Schedule

### Organization

Table 1: Project Organization

<b>Role</b>	<b>Name</b>	<b>Organization</b>	<b>Duties</b>
Ecology project manager	David Moore	WA State Dept of Ecology	Contract oversight
UW principal investigator	Dr. Michael Brett	University of Washington	Provide input for QA Project Plan, supervise graduate student, oversee reporting requirements
Technical assistance	Dr. David Stensel	UW and Water Environment Research Foundation	Provide input for QA Project Plan, assist in preparation of final report
Laboratory and data analysis	UW Graduate Student	University of Washington	Conduct BAP bioassays, quantify phosphorus in the effluent samples, prepare draft and final reports.
Field sample collection	Stan Miller	consultant to Spokane County	Prepare QAPP, oversee field sample collection and shipment to UW

## Project Schedule

Table 2: Project Schedule

<b>Activity</b>	<b>Start Date</b>	<b>End Date</b>
Field Work	July 2009	April 2010
Laboratory Work and Analysis	July 2009	April 2010
Data Analysis	July 2009	April 2010
Draft report completed at UW		May 2010
Draft report to Ecology		May 2010
Draft report out for external review		June 2010
Final report		July 2010

## Project Budget

A detailed project budget by task is in Table 3.

Cost Estimate: Personnel Costs: \$ 72,000

UW overhead: \$ 16,000

Supplies and Equipment cost: \$ 16,000

Table 3: Project Budget

<b>Expenditures</b>	
<b>Task</b>	<b>Total</b>
Task 1: Project Management	
Task 1a: UW overhead @ 26%	\$ 16,000.00
Task 1b: UW Contract Admin.	In Kind
Task 1. Total	\$ 16,000.00
Task 2: P Bioavailability Evaluation	
Task 2a: UW Research Team	\$ 44,000.00
Task 2b: UW Research Team	\$ 12,000.00
Laboratory materials and supplies	\$ 16,000.00
Task 2. Total	\$ 72,000.00
Task 4: Project Reporting and Coordination	
Task 4a: UW Research Team	\$ 4,000.00
Task 4b: UW Research Team	\$ 8,000.00
Task 4c: UW Research Team	\$ 2,000.00
Task 4d: UW Research Team	\$ 2,000.00
Task 4. Total	\$ 16,000.00
<b>Total Expenditures</b>	<b>\$ 104,000.00</b>
<b>Revenues</b>	
<b>Source</b>	<b>Total</b>
Ecology	\$ 50,000.00
Spokane County	\$ 10,000.00
City of Spokane	\$ 10,000.00
City of Coeur d'Alene	\$ 10,000.00
Inland Empire Paper Company	\$ 10,000.00
Water Environment Research Foundation	\$ 5,000.00
Kaiser Aluminum	\$ 3,000.00
Liberty Lake Sewer and Water District	\$ 2,000.00
City of Post Falls	\$ 2,000.00
Hayden Area Regional Sewer Board	\$ 2,000.00
<b>Total Revenues</b>	<b>\$ 104,000.00</b>

## 6. Quality Objectives

Due to the sensitivity of algal productivity to nutrient inputs the levels of concern for this study are somewhat lower than normally encountered in wastewater studies. Table 2 summarizes the levels of concern in the TMDL and for general water quality considerations in Lake Spokane.

Table 4: Applicable Water Quality Criteria for Phosphorus

	Approximate Freshwater Criteria	Acute - Chronic
Total P	8.0 µg/L	none
Total Dissolved P	8.0 µg/L	none

Of particular concern in this study is the assurance that the analytical methods employed show what is actually occurring in the test environment. Table 3 summarizes variability allowable in the analytical procedures and the recovery rates for the matrix spikes used in the study.

Table 5: Measurement Quality Objectives

Parameter	Check Standards/ LCS (Recovery)	Duplicate Samples (RPD*)	Matrix Spikes (Recovery)	Matrix Spike Duplicates (RPD*)	Lowest Concentration of Interest
Total P (TP)	85-115%	20%	75-125%	20%	2.0 µg/L
Total Dissolved P (TDP)	85-115%	20%	75-125%	20%	2.0 µg/L
Soluble Reactive P (SRP)	85-115%	20%	75-125%	20%	2.0 µg/L

\*RPD Relative Percent Difference (the difference between samples and duplicates divided by their mean value, expressed as a percentage).

## 7. Sampling Process Design (Experimental Design)

This study will use a bio-assay technique to determine the bio-availability of phosphorus in WWTP effluent and surface water in the Spokane River watershed. Sampling will be conducted over a ten month period to provide a picture of the variability of phosphorus in effluent during several seasons of the year. Once per month sample collection is planned. The WWTP pilot projects are anticipate to produce effluent with low total phosphorus concentration, on the order of 100 µg P/L or less.

Because the bio-assay and other analytical procedures require a fairly tedious set up procedure the study will be conducted in two “Phases.” In Phase I samples from only three sites will be collected and analyzed. After two to three months of work on the limited sample set Phase II, which adds up to four additional samples per month, will be initiated. In addition to providing a chance to perfect the sample set up procedures in the laboratory, the early start of Phase I will provide results that will give an indication of phosphorus bio-availability by the end of 2009. Examining Table 6 reveals that there are a number of high level phosphorus removal pilot projects testing a variety of processes in operation or planned for operation during the study

period. The sampling scheme laid out in Table 6 anticipates collecting samples from these pilot projects and as many as five surface water sites. Due to limitations of time and resources needed for conducting the test procedures, this project does not envision doing more than 45 phosphorus bio-assays during the ten month sampling schedule. Once this project is fully up and running at the Brett UW limnology lab, it may be possible to process more than 5-6 different samples per month. If this is the case, we will decide on which additional samples to add to our list below in consultation with the regional WWTPs and water quality management agencies.

Details of the Field and Laboratory procedures used to accomplish the goals of this study are generally described in the sections below.

Table 6: Proposed Sampling Sites and Schedule

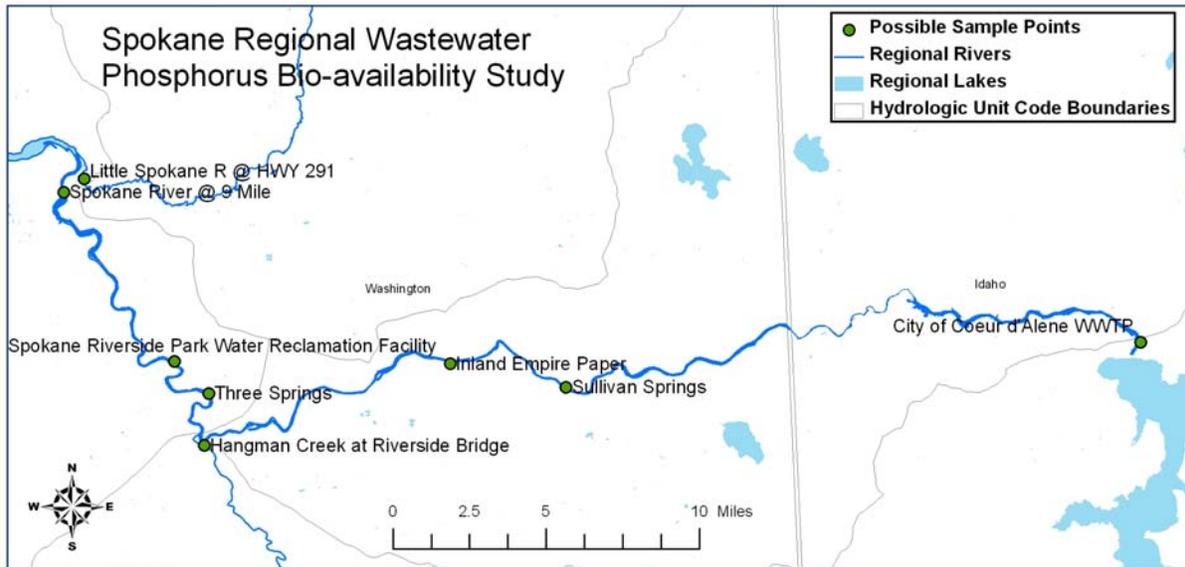
Site	Phase I		Phase II							TOTAL
	8- 09	9- 09	10-09	11- 09	12- 09	1- 10	2- 10	3- 10	4- 10	
<b>Surface Site</b>										
SR 9-mile	X		X			X			X	4
LSR Hwy 291										
Hangman Cr Riverside Ave Br										
Three Springs		X		X			X	X		4
Sullivan Springs										
<b>Discharge Sites</b>										
Spokane Pilot A	X	X	X	X	X	X	X	X	X	8
Spokane Pilot B				X		X		X		3
Spokane Pilot C			X		X		X		X	4
Spokane Pilot D				X		X		X	X	4
Spokane Pilot E										
Spokane Pilot F										
City of C d' A Pilot A		X		X		X		X		4
City of C d' A Pilot B			X		X		X		X	4
City of C d' A Pilot C										
Inland Empire Paper	X		X		X		X		X	5
<b>Total</b>	<b>3</b>	<b>3</b>	<b>5</b>	<b>5</b>	<b>4</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>6</b>	<b>41</b>

The pilot treatment processes to be sampled are listed below:

- City of Spokane
  - Kruger Actiflo sand-ballasted sedimentation
  - Cambridge Water Technology's CoMag ballasted sedimentation
  - Zenon membrane filtration
  - Corix conventional sedimentation
  - Blue Water continuous upflow filter
  - Corix multi-media granular filtration
- City of Coeur d' Alene
  - Blue Water continuous upflow filter
  - Zenon micro-filtration system
  - Zenon membrane bioreactor system
- Inland Empire Paper
  - Siemens Trident HS system tertiary treatment

Samples will be collected from the pilot projects each month to examine effluent from the various treatment processes. The sampling schedule (Table 6) is preliminary at this time and will be finalized during the study. When and where samples will be collected is based on the laboratory capacity to process samples. As the study is conducted, sufficient samples will be included to fill the laboratory capacity.

Figure 1: Study Area



Effluent from at least seven treatment processes and two natural waters will be evaluated for phosphorus bio-availability. The focus of this work will be to evaluate the effluent from the many pilot projects being operated by several of the region's WWTPs. These will include samples from at least one municipal waste stream pilot from Coeur d'Alene, one municipal waste stream pilot from the City of Spokane, and one industrial source, Inland Empire Paper. Table 6 outlines a monitoring framework by which at least three samples for all pilot plants anticipated to be in operation during the study period can be tested. Under this framework an additional five surface water sample sites may be tested up to four times. One sample site is scheduled for monthly testing throughout the study period. While it is probable that six samples per month can be handled by the staffing level presumed for this study, normally only five samples are planned for any given monthly event. Further, only one site is to be tested monthly. All these permutations can be adjusted to emphasize or deemphasize effluent types, sample sites or testing frequency as desired. Only the total number of samples submitted each round is limiting.

All samples, including WWTP effluent and surface water, will be analyzed for total phosphorus and total dissolved phosphorus. This analysis for total phosphorus will allow the determination of the percent bio-availability of TP in the sample. Analysis of TDP will allow for speciation between the dissolved and particulate fraction. Other analysis such as SRP will depend on project funding and coordination with the parallel study at Northwestern University to avoid duplicate analytical procedures.

## 8. Sampling Procedures

### Field Procedures

Samples will be collected in acid washed (HCl) polyethylene bottles that are triple rinsed with deionized water immediately before sample collection. Samples will be collected from as near the final outfall as practical at each of the facilities. Two 1-liter bottles will be collected at each site. Though one liter should provide enough water for the bio-availability and phosphorus analyses, the second sample will be collected as a backup in case of leakage or contamination during transport. Sample bottles will be labeled to identify collection location, date, and time. Samples will be cooled to 4 °C immediately after collection and shipped so they will arrive at the UW laboratory within 24 hours.

### Laboratory Methods

The general procedures followed in this study will involve analysis of the field samples using a combination of bio-assay and wet chemistry procedures. Data collected from this work will be used to determine the bioavailability of phosphorus discharged during the year. These procedures and the rationale for their use are outlined below. Details of the methods are available in the references cited.

Recent research has shown that the determination of soluble reactive phosphorus (SRP), the traditional method of determining bio-available phosphorus, may not always accurately reflect the amount of phosphorus in an effluent source that is available to phytoplankton. The term soluble reactive phosphorus refers to all forms of phosphorus present in a sample following filtration (usually through a 0.45 µm filter) that react to a specific analytical method. The term replaces “dissolved ortho-phosphate,” which research has shown to be an inaccurate descriptor for the species of phosphorus measured by the test procedure. In this study, a more direct approach, actually measuring the amount of algal growth that a water sample can support from the phosphorus it contains, will be used.

As described below, the test procedure for determining phosphorus bio-availability assumes that “raw” that is unfiltered and untreated samples of water will be subjected to the bio-assay. This approach will tell us how much of the total phosphorus in the tested water sample is available to support algal growth. These results will then be coupled with the parallel Northwestern University study conducting detailed phosphorus speciation analysis. Laboratory phosphorus speciation has commonly been used to estimate nutrient bio-availability, but the method lacks the advantage of direct biota growth measurements. The combination of the two studies will allow for an in-depth examination of phosphorus bio-availability and speciation from the pilot treatment processes.

Phosphorus bio-availability will be determined using the bioassay method described in Standard Method 8111. In this method phosphorus-starved *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) and a micronutrient solution are added to the water to be evaluated. Following incubation the amount of algae produced in the test sample is compared to that produced in a series of known phosphorus standards and by back-calculating the amount of bio-

available phosphorus determined. Because the precision of this method is lower than for standard wet chemistry approaches four replicates of each sample will be incubated and the results averaged for the final calculations. In this work four 50 ml aliquots of sample are incubated for 14 days. Five replicates of each of seven standards (e.g., 0, 10, 20, 35, 50, 75 and 100 µg P/L) are incubated simultaneously to establish a “standard curve” for determining phosphorus bio-availability. Samples are incubated at  $24 \pm 2$  °C under continuous fluorescent lighting of 4300 lm  $\pm$  10% for 14 days. The 14 day incubation period is based upon maximum growth potential for the study algae in laboratory conditions. Following incubation, algal cell density in each of the unknown sample and the standard curve solutions will be determined using a particle size analyzer. The average density of the four replicates of each unknown will be compared to a standard curve developed from the densities in the algal biomass standards grown with known phosphorus concentrations to determine the amount of bio-available phosphorus.

The test algae will be deprived of phosphorus prior to incubation in order to stimulate the production of alkaline phosphatase enzymes which are used by algae to convert organic forms of P to inorganic P from the algae’s environment (Reynolds and Davies 2001). This creates a ready supply of enzymes which facilitates the release of all available phosphorus from the incubation media within the 14 day incubation period. This allows an accurate determination of total bio-available phosphorus without long-term incubation of bulk samples. In addition, the samples used for the bio-assay procedures will be analyzed for phosphorus species.

Running the standard phosphorus analyses will provide two benefits. First, the total phosphorus values provide a necessary baseline for calculating the percent bio-available phosphorus. Second, the total phosphorus values will allow observation of the discharge phosphorus composition over the year. Determining soluble reactive phosphorus will provide a base for comparing of the results of the somewhat tedious bio-available phosphorus test with the traditional analytical measure of biologically active phosphorus.

## **9. Measurement Procedures**

The bio-assay procedure described in SM 8111 involves using phosphorus starved algae *Pseudokirchneriella subcapitata* to extract phosphorus from the substrate (effluent). Dividing the concentration of the bio-available phosphorus determined using this technique with the total phosphorus determined by persulfate digestion yields the fraction of bio-available phosphorus.

Total P will be determined using the ascorbic acid method following persulfate digestion of each effluent sample. Total dissolved phosphorus and soluble reactive phosphorus will be determined using the ascorbic acid method on a filtrate (0.45 micron filter) of each effluent sample. The ascorbic acid is described as Standard Method 4500-P.

Table 7 summarizes the analytical procedures employed in this study.

Table 7: Laboratory Measurement Methods

Analyte	Samples [Number/Frequency]	Expected Range of Results	Reporting Limit	Sample Preservation	Sample Preparation Method	Analytical Method
Total P	12 / monthly	2-1000 µg/L	2 µg/L	Cool to 4 °C	Persulfate Digestion	SM 4500-P
TDP	12 / monthly	2-1000 µg/L	2 µg/L	Cool to 4 °C	Filtration (0.45 µ) and Persulfate Digestion	SM 4500-P
SRP	12 / monthly	2 -1000 µg/L	1 µg/L	Cool to 4 °C	Filtration (0.45 µ)	SM 4500-P
BAP	12 / monthly	2 -1000 µg/L	2 µg/L	Cool to 4 °C		SM 8111

## 10. Quality Control Procedures

### Field

Effluent samples will be collected at the primary outfall of the facility being tested. Samples will be collected using an acid washed plastic bucket. The pail will be triple rinsed with deionized water immediately prior to sample collection. Samples for the algal bioassays will be transferred to acid washed and triple rinsed one-liter polyethylene bottles. Two one-liter samples will be collected to ensure that there is adequate sample for the analysis and to provide a backup sample in case of leakage or contamination during transport. Additional samples for other parameters will be drawn and preserved as required. All samples will be preserved for shipment by cooling to 4 °C with ice or in a refrigerator and shipped to the UW on ice in insulated containers. One blind duplicate sample will be collected on each sample collection run. The blind duplicate will be used for complete analysis and bioassay

### Laboratory

Laboratory QC for wet chemistry tests will include check standards/laboratory control samples, method blanks, analytical duplicates, matrix spikes, and matrix spike duplicates. The University research team will select samples for duplicate analysis following their standard practice. At least 10 percent of the samples will be duplicates.

To account for the lowered precision of the bioassay procedure, four aliquots of each effluent sample will be run. The average algal density of the four samples will be used to determine the bio-available phosphorus concentration. The variability of among the four samples will be used to determine sample precision and accuracy. Similar multiple aliquot procedures will be followed for developing the standard curve for each sample run.

As a control for the overall process a laboratory blank and a blind duplicate of one effluent sample will be followed through the incubation process.

## **11. Data Management Procedures**

The field and laboratory data will be entered into Excel spreadsheets. Excursions from the criteria will be identified and evaluated in terms of their impact on the conclusions drawn. These Excel files will be backed up on multiple computers.

## **12. Audits and Reports**

Quarterly progress reports and reimbursement requests will be audited by Ecology and paid according to standard procedures.

Each request for reimbursement will be accompanied by a progress report that tracks progress relative to the overall project schedule. Deviations from the schedule will be explained with comments on impacts for remaining work, timeline, and budget impacts.

A final report detailing the project goals, the methods used and the results of the research conducted shall be submitted according to the agreed upon schedule. While a draft report will be circulated among and reviewed by all funding agencies, final verification of project completion and financial reimbursement will be made by Ecology.

## **13. Data Verification and Validation**

The final report shall include a section on data verification and validation in which the procedures used to collect and record the data used for decision making is described.

Typically this section describes the chain of custody handling of samples between sample collection and data reporting by the lab and any laboratory quality control procedures employed. For this study this will relate only to the initial sample collection and handling. There may be a discussion of "holding times" between removal of aliquots and actual completion of analytical procedures.

## **14. Data Quality Assessment**

The final report shall include a section on data quality assessment in which the data used for decision-making is evaluated in terms of its relationship to expected norms of variability. Deviations from the norms will be explained and any limitations these deviations place on data interpretation or conclusions drawn will be discussed.

Statistical variation (aka "statistical error") in our BAP estimates can come from three sources. First, there will be "error" or uncertainty in our TP estimates for any particular sample. Second, there will be error for the estimated intercepts and standard curves for the regression equations representing the relationship between our actual known phosphate concentrations and algal cell

density in our calibration curves. Third, there will be statistical error in the BAP estimated from the four replicates for any particular samples. In fact, because the BAP bioassays are based on a biological approach, as opposed to the more typical chemical assays used to quantify most nutrient concentrations, the expected variation in typical BAP bioassays are considerably larger.

The variability for the TP analyses will be quantified using the standard deviations for triplicate measurements. The variation in the calibration curve will be represented by the outputs ( $\pm 1$  SD) for statistical software (e.g. SPSS<sup>®</sup>). The variability in the BAP estimates will be quantified as the standard deviation for the four replicate observations. To account for variability from all three sources, Bootstrapping will be used. Bootstrapping is a classic computer intensive technique in statistics (Efron and Gong 1983) that is now easy to do on any desktop computer. This entails randomly selecting one likely TP value, one likely standard curve, and one likely BAP value from the original distributions, and repeating this process 1000 times to come up with a distribution of plausible independent estimates. The Brett group has used this technique in many other applications (e.g. Brett 2004). Uncertainty in the percent BAP estimates will also be estimated as the variation in mean estimates from multiple bioassay experiments.

## 15. References

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