Quality Assurance Project Plan

for

Indiana Clean Lakes Program (2015-2018)

ARN # 4-216

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Prepared for:

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Section 1: Study Description

Historical Information

The Indiana Clean Lakes Program (Program) was created in 1989 as a program within the Indiana Department of Environmental Management's (IDEM) Office of Water Management. The program is administered through a grant to Indiana University's School of Public and Environmental Affairs (SPEA) in Bloomington. Faculty, staff and graduate students associated with SPEA's Enviromnental Science Program implement the tasks associated with the Program. The Indiana Clean Lakes Program is a comprehensive, statewide public lake management program having five components:

1. Public information and education
2. Technical assistance
3. Volunteer lake monitoring
4. Lake water quality assessment
5. Coordination with other state and federal lake programs.

Water sampling and analysis activities that require QAPPs are #3 and #4 above. The QAPP for the Volunteer Lake Monitoring Program is a separate document. This document is the QAPP for the lake water quality assessment componant of the Indiana Clean Lakes Program.

Study Goals

Approximately 70-80 public lakes each year are selected randomly for detailed analysis designed to provide a statistical analysis for all Indiana public lakes that are accessable to trailered boats, totally 320 lakes during the whole 4 year period. This assessment work has the following goals:

Goal 1: Determine the condition of Indiana lakes and identify improving or declining water quality trends.

Goal 2: Update the Indiana Lake Classification System and Management Plan.

Goal 3: Compile information collected for inclusion in the Section 305(b) reports to U.S. EPA.

Goal 4: Identify impairments that would affect the Section 303(d) listing of state impaired waterbodies.

Goal 5: Analyze lake samples using internal quality controls to ensure accuracy and precision following methods stated in the Standard Methods for the Examination of Water And Wastewater, 21st ed.

Study Site

The area of study of the Program includes the entire State of Indiana. Only public lakes having an accessible boat launching area will be sampled, unless directed otherwise by IDEM.

Sampling Design

For the first time beginning in 2010, lakes to be sampled will be randomly selected over a two-year period designed to coincide with the two-year 305(b) reporting cycle. The capacity of the survey crew is approximately 80 lakes per summer. Thus, 160 lakes will be randomly drawn from approximately 400 candidate lakes that: a) are greater than 5 acres in surface area and b) have a public boat launch suitable for our 16-foot Carolina Skiff. The first 80 lakes in the draw will be sampled during the first summer and the second 80 lakes will be sampled the second summer. The July-August period is used because this is the time of year when worst-case and stable conditions (warm temperatures, thermal stratification, hypolimnetic anoxia, algal blooms) are expected. Samples collected during this period of relative summer stability may be compared from one year to the next. Field sampling generally occurs on Mondays, Tuesdays, and Wednesdays of each week. The lakes in a particular summer draw will not be sampled in random order because that will uncessarily waste energy and money. Instead, lakes will be sampled according to transects originating in Bloomington and spread around the State. For example, on any given Monday-Wednesday sampling period, we might sample lakes from Steuben County to Bloomington one week and from Evansville to Bloomington on the next week. The alternating

transect design is intended to help account for changing weather patterns that could otherwise bias a strictly geographic sampling design.

The lake sampling site is choosen by using a depth meter to locate the deepest water in the lake (index site). This index site location is recorded using a GPS unit. Samples of epilimnetic (an integrated sample from the surface to 2-meter depth) and hypolimnetic water (~2 meters above the lake bottom) will be taken. The parameters choosen for in-field sampling and for lab analyis collection are essential to calculate the Carlson Trophic State Index for each lake. These samples will be collected and poured into appropriate sampling bottles with an appropriate preservative if necessary. Samples will be returned to SPEA's limnology laboratory in Bloomington, Indiana and analyzed for: (1) alkalinity, (2) pH, (3) total phosphorus, (4) soluble reactive phosphorus, (5) total Kjeldahl nitrogen, (6) nitrate-nitrogen, (7) ammonia-nitrogen, and (8) chlorophyll a. While on the lake, (9) temperature and (10) dissolved oxygen profiles will be made at one-meter intervals, and (11) Secchi disk transparency, (12) percent light transmission, and (13) conductivity will be measured at the sampling point. Also while on the lake, an integrated surface to 2-meter water wample will be collected for plankton analysis. This sample will be preserved and returned to the lab where a total plankton count and species distribution will be determined (14).

This sampling design allows us to meet our goals to identify and analyze the condition of Indiana lakes.

Study Schedule

Table 1: Study Schedule Sample. This is repeated for each sampling season, 2015-2018.

Activity	Start Date	End Date
CLP Training	1st week of June	Last week of June
CLP sampling starts	On or about the 1st week of July	Last week of August
CLP Sampling Trip #1	1st week of July, Monday	Wednesday
CLP Sampling Trip #2	2nd week of July, Monday	Wednesday
CLP Sampling Trip #3	3rd week of July, Monday	Wednesday
CLP Sampling Trip #4	4th week of July, Monday	Wednesday
CLP Sampling Trip #5	1st week of August, Monday	Wednesday
CLP Sampling Trip #6	2nd week of August, Monday	Wednesday
CLP Sampling Trip #7	3rd week of August, Monday	Wednesday
CLP Sampling Trip #8	4th week of August, Monday	Wednesday

Once returning from the field, lake samples are analyzed and recorded within the laboratory database within the holding times specified for each sample parameter (see Sample Procedures).

Section 2: Study Organization and Responsibility

Key Personnel

Melissa Clark, Senior Lecturer and Program Director
SPEA, 1315 East Tenth Street, Bloomington, IN 47405; 812-855-4556
mlaney@indiana.edu
Works with IDEM Project Officer to select lakes for sampling; hires all personnel; sets employee tasks and work schedules; coordinates employee training; prepares quarterly progress reports

Sarah Powers, Laboratory Manager and Quality Control Officer
SPEA, 1315 East Tenth Street, Bloomington, IN 47405; 812-855-1600; sarellis@indiana.edu
Maintains and operates analytical instruments and insures laboratory Quality Assurance/Quality Control; assists in CLP field and laboratory training; prepares QC samples; compiles QA./QC results; responsible for performing corrective action when necessary. Trains the other Lab Staff appointed to run the Autoanalyzer. Also assists in all laboratory analytical procedures.

Lab and Field Staff, Field Preparation

Responsible for preparing all field equipment prior to departure for the field sampling; performs day to day maintenance on all field equipment replacing any materials/parts necessary; prepares all sample bottles for lake samples with proper labeling, sample storage, and an “extras” box with duplicates for each parameter and materials for in-field repairs; updates the Sampling Lake File for each sampling trip including Indiana road maps, USGS topographic maps, bathymetric maps for each lake, and additional information relevant to the trip. Also assists in all laboratory analytical procedures, including blind analyses of QC samples.

Lab and Field Staff, Data Management

Responsible for data entry following sample analysis. Also assists in all laboratory analytical procedures, including blind analysis of QC samples.

Lab and Field Staff, Autoanalyzer Operator

Responsible for processing lake samples for determination of organic nitrogen, nitrate-nitrogen, and ammonia-nitrogen concentrations via the Alpkem Autoanalyzer. Also conducts blind analysis of QC samples.

Project Organization

Melissa Clark, Senior Lecturer of Environmental Science at Indiana University's School of Pubic and Environmental Affairs' (SPEA) is the Project Manager. Sarah Powers, a professional staff member and adjunct faculty of Indiana University's School of Public and Environmental Affairs is the Laboratory Manager and Quality Control Officer.

Qualified graduate students enrolled in SPEA’s Masters of Science in Environmental Science Program will implement much of this plan, following extensive training under the direction of Mrs. Clark.

Section 3: Data Quality Indicators

Precision

There are several procedures used to ensure precision with field sampling and laboratory analysis. (1) Duplicates are two separate samples collected or measurements made from the same location and depth. For example, a duplicate nitrate sample at the 1 meter depth would require two separate sample retrievals with the Kemmerer sampler from 1 meter deep. Duplicates evaluate variation in sampling technique, bottle preparation, and laboratory technique. Duplicate values are accepted if they fall within +/- 2 standard deviations of values obtained from previous laboratory runs of the same parameter. (2) Replicate samples are two samples drawn off from the same Kemmerer or integrated sampler. In this case, field sampling technique is the same and the replicates evaluate bottle preparation and laboratory tecnique. One duplicate and replicate are evaluated for every ten field samples. Replicate values are accepted if they fall within +/- 2 standard deviations of values obtained from previous laboratory runs of the same parameter. (3) Standard curve - Analyses for total phosphorus, soluble phosphorus, nitrate-nitrogen, ammonia-nitrogen and total Kjeldahl nitrogen all require preparation of standards and running a standard curve. The fit ot each standard curve (regression coefficient) is evaluated against the lower warning limit of the control chart. Standard curve regression coefficients are accepted if they fall within +/- 2 standard deviations of values obtained from previous laboratory runs of the same parameter (see Section 8) .

Regular, scheduled maintenance, according to manufacturer’s instructions, is used to insure the precision of all electrical analytical instruments.

Accuracy

Proper maintenance, calibration, and use of field and laboratory meters will insure their accuracy. The accuracy of each instrument is given by the manufacturer and is included in Table 2. The main procedure used to ensure accuracy in field samples are spikes. Spikes are samples of known concentration that are run through the analytical techniques. Spike samples are purchased from outside sources with documented reliability. One spike sample is evaluated for every ten field samples. The spike analysis must result i n +/- 10% recovery. If outside this range, the analytical run is rejected.

% Recovery = found value/true value x 100%

Field blanks are analyzed against the method detection limits as a test of accuracy by insuring that no outside contamination occurs during the process of cleaning sample bottles in the lab or filling sample bottles in the field. Measured values of field blanks should theoretically be 0, however since some contamination is inevitable, we will compare the field blank values with the method detection limits for each chemical parameter tested. Field blank values should not exceed these method detection limits (see Section 8).

Table 2: Data Quality Objectives

Parameter	Precision (Std. Dev.)	Accuracy	Sensitivity
Temperature	**± 2 S.D.**	±0.1°C	0 °C
Conductivity	**± 2 S.D.**	± 5% full scale	0 uS
Dissolved oxygen	**± 2 S.D.**	± 2% Air Saturation ± 0.3% mg/L	0 mg/L
Secchi disk transparency	**± 2 S.D.**	No QA Standards are available	n/a
Percent light transmission	**± 2 S.D.**	No QA Standards are available	0 PAR
pH	**± 2 S.D.**	± **0.**5% full scale	n/a
Total plankton	**± 2 S.D.**	No QA Standards are available	n/a
Alkalinity	**± 2 S.D.**	± 10%	n/a
Nitrate-nitrogen	**± 2 S.D.**	± 10%	0.008 mg/L
Ammonia-nitrogen	**± 2 S.D.**	± 10%	0.014 mg/L
Total Kjeldhal nitrogen	**± 2 S.D.**	± 10%	0.230 mg/L
Total Nitrogen	**± 2 S.D.**	± 10%	0.100 mg/L
Total phosphorus	**± 2 S.D.**	± 10%	0.002 mg.L
Soluble reactive phosphorus	**± 2 S.D.**	± 10%	0.002 mg/L
Chlorophyll *a*	**± 2 S.D.**	No QA Standards available	0.5 mg/L

Completeness

Study objectives are to sample between 70 and 80 lakes each summer. Bad weather or mechanical breakdowns could reduce the number of lakes sampled. However, the data quality objectives can be met even if the number of lakes sampled during one year falls below the 70-80 lake total because this is an ongoing program…more lakes can always be sampled the following year. For the purposes of this section, DQO can be met with 90% completeness.

Representativeness

Refer to Section 1, Sampling Design

Comparability

Methods are common and EPA approved as recommended in Standard Methods, 21st Edition (APHA, 2005).

Sensitivity

The sensitivity is the reporting limit or the method detection limit. Table 2 lists the reporting limit for each parameter.

Section 4: Sampling Procedures

In-situ Measurements:

In-situ measurements of temperature, conductivity and dissolved oxygen are taken using a YSI Model 85 Oxygen, Conductivity, Salinity, and Temperature probe (manufactured by Yellow Spring Instrument, Co., Inc., Yellow Springs, Ohio). The probe is slowly lowered into the lake and measurements are taken according to manufacturer's instructions at one-meter intervals.

Other in-situ measurements include Secchi disk transparancy and percent light transmission.

Secchi disk transparency is measured using a conventional black and white eight-inch diameter disk attached to a rope graduated in tenths of meters. The disk is lowered into the water until it is no longer visible. It is then lowered some more and raised until it again becomes visible. The midpoint between these two measurements is recorded as the Secchi disk depth.

Percentage of light transmitted is determined by the use of a Li-Cor LI-193 Underwater Sperical Quantum Sensor attached to a LI-189 Quantum Photometer. A measurement will be taken at three feet (as specified by the IDEM Eutrophication Index) and the depth at which one percent transmittance is reached will also be recorded.

Appendix B contains the field sampling form for lake activities.

Water Samples:

Water samples are collected in the field using a two-meter integrated sampler for epilimnetic samples and a Kemmerer Sampler for hypolimnetic samples for laboratory analysis of alkalinity, total Kjeldahl nitrogen, ammonia-nitrogen, nitrate-nitrogen and chlorophyll a. Samples are collected in appropriately sized high-density polyethylene (HDPE) bottles (Table 3). Soluble reactive phosphorus and total phosphorus samples will be collected in acid washed glassware. Ammonia-nitrogen, nitrate+nitrogen, total Kjeldahl nitrogen and total phosphorus samples will be preserved by acidifying each sample to a pH<2 by addition of a $H2SO_4$ solution and stored at 4 degrees C in an ice chest in the field and later in the laboratory refrigerator until analysis is begun. Soluble reactive phosphorus and chlorophyll a samples are filtered in the field.

A 250-ml sample will be taken for alkalinity and pH in a HDPE sampling bottle. Care will be taken to minimize sample agitation during collection. Bottles will be completely filled to minimize air trapped beneath the lid. Samples will be taken at both hypolimnetic and epilimnetic levels. Each sample will be placed in an ice chest in the fiield and then stored at 4 degrees C in the laboratory refrigerator until analysis, which is preferably within 1 day.

A 125-ml sample in a HDPE bottle will be collected for analysis of ammonia-nitrogen and nitrate-nitrogen. The samples will be acidified at the sampling site to a pH<2, placed in an ice chest in the field, and then stored at 4 degrees C in the laboratory refrigerator. Samples will be taken at both the hypolimnetic and epilimnetic levels of each lake. The analysis will be completed within 28 days after the samples are collected.

A 125-ml sample in a HDPE bottle will be collected for analysis of total Kjeldahl nitrogen. The samples will be acidified at the sampling site to a pH<2, placed in an ice chest in the field, and then stored at 4 degrees C in the laboratory refrigerator. Samples will be taken at both the hypolimnetic and epilimnetic levels of each lake. The analysis will be completed within 28 days after the samples are collected.

A 125-ml sample in an acid-washed, glass sampling bottle will be taken for analysis of total phosphorus. The sample will be acidified, placed in an ice chest in the field, and stored at 4 degrees C in the laboratory refrigerator. The samples will be collected at both the hypolimnetic and epilimnetic levels of the lake. The analysis will be completed within 28 days after the sample is collected.

A filtered sample of 125 ml in an acid-washed glass bottle will be taken for analysis of soluble phosphorus. The sample will be filtered on site (using Whatman GF/C filters in a Buchner funnel, side-arm flask, and a Nalgene hand-operated vacuum pump) and then placed in an ice chest in the field, and stored at 4 degrees C in the laboratory refrigerator. Samples will be taken at both the hypolimnetic and epilimnetic levels of each lake tested. The analysis will be completed within 48 hours after the samples are collected.

For chlorophyll a analysis, a specific amount of lake water will be filtered through a Whatman GF/F filter using a Nalgene PSF filter holder with receiver and a Nalgene hand-operated vacuum pump. The minimum amount of water to be filtered will be determined by the Secchi disk measurement as follows:

Secchi depth (m)	Volume of water (ml)
< 0.3	50
> 0.3 to 0.5	100
> 0.5 to 0.75	200
> 0.75 to 1.0	300
> 1.0 to 2.0	500
> 2.0 to 3.0	800
> 3.0 to 5.0	1000
> 5.0	1500

After filtration, the chlorophyll filter will be folded and placed in a 50-ml opaque HDPE bottle with forceps. The sample will be placed in an opaque ice chest in the field and later stored in the dark at <0 degrees C in the laboratory freezer until laboratory analysis - up to three weeks.

Algae will be measured by lowering a two-meter integrated sampler into the water at a slow rate allowing it to fill as it is lowered. The ball valve at the bottom is then closed and the collected sample discharged into an opaque plastic pitcher. Approximately 200 mls of the sample will be poured into a 250-ml opaque, high-density polyethylene (HDPE) bottle and preserved with glutaraldehyde. Preserved samples will be placed in an ice chest in the field and later transferred to a laboratory refridgerator until analysis. The glutaraldehyde preservative provides excellent preservation of plankton cells for at least one year and does not stain the specimens as does Lugol's solution. Our algae samples will be counted within one year of collection.

Zooplankton will be collected by lowering a Wisconsin-style tow net (243 micron mesh) to the 1% light level and slowly raising the net to the water surface. The collection bucket will be placeed in a pail filled half full with lake water to which 2 CO_2 (alka-seltzer) tablets will be added. The CO_2 narcotizes the zooplankton to relax their external structure prior to preservation in 95% ethanol.The concentrated sample will be poured into a 60-ml opaque, high-density polyethylene (HDPE) bottle and preserved with 95% ethanol (Section 10200 B in Standard Methods for the Examination of Water and Wastewater, 21st ed). Preserved samples will be placed into an ice chest in the field and later transferred to a laboratory refridgerator until analysis.

In 2018 samples for phytoplankton will be concentrated using membrane filtration then permanent mounts for all sampled. This process is detailed in Standard Methods for Examination of Water and Wastewater, 22nd ed Section 10200 C and D.

Table 3: Sampling Procedures

Parameter	Sampling Frequency	Sampling Method	Sample Container	Sample Volume	Holding Time
Temperature	1 measurement for each 1-meter depth per lake	YSI Model 85 Oxygen, Conductivity, Salinity, and Temperature probe (manufactured by Yellow Spring Instrument, Co., Inc., Yellow Springs, Ohio). Standard Method 2550 B-2000 The probe is slowly lowered into the lake and measurements are taken according to manufacturer's instructions at one-meter intervals.	NA	NA	NA
Conductivity	1 epilimnetic & 1 hypolimnetic measurement per lake	YSI Model 85 Oxygen, Conductivity, Salinity, and Temperature probe (manufactured by Yellow Spring Instrument, Co., Inc., Yellow Springs, Ohio). Standard Method 2510 B-1997 EPA Method 120.1 The probe is slowly lowered into the lake and measurements are taken according to manufacturer's instructions for an epilimnetic and hypolimnetic sample.	NA	NA	NA
Dissolved Oxygen	1 measurement for each 1-meter depth per lake	YSI Model 85 Oxygen, Conductivity, Salinity, and Temperature probe (manufactured by Yellow Spring Instrument, Co., Inc., Yellow Springs, Ohio). Standard Method 4500-O G-2001 USGS Method I–1576–78 The probe is slowly lowered into the lake and measurements are taken according to manufacturer's instructions at one-meter intervals.	NA	NA	NA
Transparency	1 measurement per lake	Secchi disk transparency is measured using a conventional black and white eight-inch diameter disk attached to a rope graduated in tenths of meters. The disk is lowered into the water until it is no longer visible. It is then lowered some more and raised until it again becomes visible. The midpoint between these two measurements is recorded as the Secchi disk depth.	NA	NA	NA
% Light transmission	2 measurements per lake	Percentage of light transmitted is determined by the use of a Li-Cor LI-193 Underwater Spherical Qualtum Sensor. A measurement will be taken at three feet (as specified by the Indiana Trophic State Index) and the depth at which one percent transmittance is reached will also be recorded.	NA	NA	NA
Alkalinity/pH	1 epilimnetic & 1 hypolimnetic sample per lake	Water samples are collected using integrated and Kemmerer samplers	HDPE Nalgene	250 ml	48 hours
Ammonia-nitrogen	1 epilimnetic & 1 hypolimnetic sample per lake	Water samples are collected using integrated and Kemmerer samplers	HDPE Nalgene	125 ml	28 days
Nitrate-nitrogen	1 epilimnetic & 1 hypolimnetic sample per lake	Water samples are collected using integrated and Kemmerer samplers	HDPE Nalgene	125 ml	28 days
Total Kjeldahl nitrogen	1 epilimnetic & 1 hypolimnetic sample per lake	Water samples are collected using integrated and Kemmerer samplers	HDPE Nalgene	125 ml	28 days
Total phosphorous	1 epilimnetic & 1 hypolimnetic sample per lake	Water samples are collected using integrated and Kemmerer samplers	Glass Media/Lab bottles	125 ml	28 days
Soluble phosphorus	1 epilimnetic & 1 hypolimnetic sample per lake	Water samples are collected using integrated and Kemmerer samplers. The sample will be filtered on site (using Whatman GF/C filters in a Buchner funnel, side-arm flask, and a Nalgene hand-operated vacuum pump)	Glass Media/Lab bottles	125 ml	48 hours
Chlorophyll a	1 epilimnetic sample per lake	Water samples are collected using an integrated sampler. A specific amount of lake water will be filtered through a Whatman GF/F filter using a Nalgene PSF filter holder with receiver and a Nalgene hand-operated vacuum pump.	HDPE Nalgene-opaque	30 ml	3 weeks
Algae	1 sample per lake	Algae are collected using an integrated sampler.	HDPE Nalgene-opaque	250 ml	One year

Zooplankton	1 sample per lake	Zooplankton are collected using a Wisconsin-style tow net (243 micron mesh) towed from the 1% light level to the water surface	HDPE Nalgene-opaque	60 ml	One year

Section 5: Custody Procedures

After samples are collected at the sampling site they will be returned to the SPEA limnology laboratory in Bloomington, Indiana for analyses. The samples will remain in the custody of the samplers until they reach the labs and will be placed in storage in an iced cooler by the samplers or will be immediately analyzed. For two-day sampling trips, the samples are placed in an iced cooler overnight until they reach the laboratory. Once in the lab, those samples stored for later analysis are sorted by their respestive parameter and stored in the laboratory refrigerater. Those samples immediately analyzed are sorted by their respective parameter and placed at the appropriate laboratory station. Samples that have longer storage are constantly moved forward for priority analysis, giving priority to older samples.

Section 6: Calibration Procedures and Frequency

All equipment used to perform the tests for this study will be operated according to manufacturer's instructions. Calibrations of field equipment will take place at each sampling site for the in-situ tests. The YSI Model 85 Oxygen, Conductivity, Salinity, and Temperature probe is auto-calibrated based on the altitude and salinity of the sample site just prior to the time of use. Periodically, the temperature measurement shall be checked against a standard thermometer.

Analyses performed in the lab will be calibrated against manufacturer recommended standards and blanks prior to the performance of each test. Recalibrations of equipment for each test will be accomplished as addressed in Section 7 of this plan.

Equipment Calibration Procedures:

Equipment: Orion model 407A selective ion meter and an Orion 91-02 pH probe

Calibration: Fisher calibration buffer (7.0 and 4.0) or equivalent prior to each two measurements.

Parameters: pH, alkalinity

Equipment: Li-Cor LI-193 Underwater Quantum Sensor with LI-189 Quantum Photometer

Calibration: Sensor is factory calibrated

Parameters: % surface light at 3 feet; depth of 1% light level

Equipment: Alpkem FLOW Solution Autoanalyzer Model 3570

Calibration: Calibration will be with at least five serial dilutions of a standard solution (respective of the analyzed parameter, i.e., standard nitrate stock solution for nitrate nitrogen) and a solution blank. Calibration is performed for each parameter prior to each run.

Parameters: nitrate-nitrogen, ammonia-nitrogen, total nitrogen, total phosphorus, orthophosphate

Equipment: Thermo Sientific Evolution 220 UV-Visible Spectrophotometer

Calibration: Calibration will be with at least five serial dilutions of a standard phosphate solution and a solution blank.

Parameters: Soluble reactive phosphorus, total phosphorus

Equipment: Thermo Scientific Evolution 220 UV-Visible Spectrophotometer

Calibration: Calibration will be with a 9:1 acetone/water solution in both cuvettes at 750nm. The spectrophotometer is checked for calibration every 3 samples at each of the three wavelengths (664 nm, 665 nm, 750 nm).

Parameters: chlorophyll-a

Equipment: Eppendorf Centrifuge 5804R – Parameter Cholorophyll-a

Section 7: Sample Analysis Procedures

Each alkalinity sample will be analyzed according to the potentiometric titration to a preselected pH method (Section 2320 B in Standard Methods for the Examination of Water and Wastewater, 21st ed). An Orion model 407A selective ion meter and an Orion 91-02 pH probe will be used. The meter and probe will be calibrated against a Fisher calibration buffer (7.0 and 4.0) or equivalent prior to each two measurements.

Temperature, dissolved oxygen, and conductivity are measured in situ using a YSI Model 85 meter, Quanta Hydrolab, or a In-Situ Troll 9500 (Standard Method 2550 B-2000, Standard Method 4500-O G-2001, USGS Method I-1576-78, Standard Method 2510 B-1997, EPA Method 120.1, respectivitely).

Each nitrate-nitrogen sample will be analyzed by the cadmium reduction method (USEPA Method 353.2) using segmented flow analysis on an Alpkem FLOW Solution Autoanalyzer Model 3570 (OI Analytical. 2000. Methology: Nitrate plus Nitrate Nitrogen and Nitrite Nitroge, USEPA by Segmented Flow Analysis, Publication 14900500, College Station, TX). Calibration will be with at least five serial dilutions of a standard nitrate-nitrogen solution and a solution blank.

Each ammonia-nitrogen sample will be analyzed by in-line gas siffusion on ammonia, by gas diffusion segmentd flow analysis (SFA) on an Alpkem FLOW Solution Autoanlyzer Model 3570 (OI Analyticall. 2012. Methodology Part 136.3 Table 1b Parameter 4 Ammonia for Method Modification and Analytical Requirments. (EPA method 350.1) Calibration will be with at least five serial dillutions of a standard ammonia-nitrogen solution and solution blank

Each total nitrogen and total phosphorus sample will be ananlyzed by digestion. A 25-ml aliquot of sample will be digested for total nitrogen and total phosphorus in test tubes in autoclave at 120°C for 30 minutes using alkaline persulfate methods (Standard Methods for the Examination of Water and Wastewter, 22nd ed. Section 4500-P J.)

The final dissolved phosphorus measurment will be determind by the ascorbic acid methodology of colorimetry listed in Standard Methods for the Examination of Water and Wastewater, 22nd ed (Section 4500-P J.) Analysis will be don on the Alpkem Flow Solution Autoanlyzer Model 3570 (OI Analytical. 2000. Methodology: Ttoal Phophorus USEPA Method 3651 (Reference15.4). This method can and will also be used for analysis of soluble reactive phosphorus samples without digestion. Callibration will be with at least five serical dillutions of a standard nitrate-nitrogen solution and solution blank.

The final total nitrogen values is measured from the nitrogen compounds as they are oxidized by persulfate to nitrate under lakaline conditons. Nitrate is measured by EPA 353.2 methodology the same as the nitrate-nitrate methods listed above for the Alpkem FLOW Solution Autoanalyzer Model 3570. Calibration will be with at least five serial dillutions of a standard phosphorus solution and solution blank.

Soluble reactive phosphorus samples will be analyzed by use of the ascorbic acid method of colorimetry listed in Standard Methods for the Examination of Water and Wastewater, 21st ed (Section 4500-P E). An Thermo Scientific Evolution 220 UV-Visible spectrophotometer will be used in this analysis or analysis will take place on the Alpkem FLOW solution Autoanlyzer Model 3570. Calibration will be with at least five serial dilutions of a standard phosphate solution and a solution blank.

Chlorophyll a concentration will be determined using the acetone extraction, grinding, and spectrophotometric method in Standard Methods for the Examination of Water and Wastewater, 21st ed (Section 10200 H). An Thermo Scientific Evolution 220 UV-Visible spectrophotometer will be used for this analysis

Algal genera will be concentrated using Utermoehl Sedimentation Chambers and identified and enumerated using a PhytoTech nannoplankton counting chamber. For each sample, all algae within the 0.08 ml chamber will be counted at 400x magnification. Identification and genera names will be according to Wehr and Sheath (2003), Prescott (1982), or Whitford and Schumacher (1984).

Zooplankton genera will be counted with a standard Sedgewick-Rafter counting chamber. All zooplankton within the 1-ml chamber will be identified and counted at 40x magnification. Identification and genera names will be according to Wehr and Sheath (2003) or Whitford and Schumacher (1984).

Flags, similar to those in Table 4, will be used to identify problems with samples analyzed.

Table 4: Suggested Flags for Verification and Validation of NPS Sample Concentration

FLAGS and Purpose	**Description & Examples**
R: Rejected	Data not used in any evaluations
J: Estimated	Small errors in QC found, can use in any evaluations
Q: Quality Control Checks	One or more of the QC checks or criteria was out-or-control.
H: Holding Time	The results will be estimated or rejected on the basis listed below: **1.** If the analysis was performed between the holding time and 1½ times the holding time window, the result will be estimated. (**HJ)** 2. If the analysis was performed outside the 1½ times the holding time window, the result will be rejected. **(HR)**
D (RPD) or **D** (SS)	Duplicate samples for either Relative Percent Difference or split samples The Relative Present Difference (RPD) for this parameter was above the acceptable control limits, the established control limits the sample RPD will be Identified in brackets. Example: **(D, 45**) The RPD for the split Sample for this parameter was above the acceptable control limits. The parameter will be considered estimated or rejected on the basis listed below: 1. If the SS RPD is between the established control limits and two times the established control limits then the sample will be estimated. **(DJ)** 2. If the SS RPD is twice the established control limits then the sample will be rejected. **(DR)**
B: Blank Contamination	This parameter was found in field or lab blank. Whether the result is accepted, estimated, or rejected will be based upon the level of contamination listed below. 1. If the result of the sample is greater than the reporting limit but less than five times the blank contamination the result will be rejected. **(BR)** 2. If the result of the sample is between five and ten times the blank contamination the result will be estimated. **(BJ)** **3.** If the result of the sample is less than the reporting limit or greater than ten times the blank contamination the result will be accepted with the concentration identified. Example: **(B, 45)**

U: Less than Reporting Limit	The result of the parameter is above the Method Detection Limit (MDL) but below the reporting limit and shall be estimated.

Section 8: Quality Control Procedures

A variety of techniques will be employed to maintain accuracy and precision for the measurements obtained in this study.

Precision:

Ten percent of the samples for pH/alkalinity, total phosphorus, soluble reactive phosphorus, nitrate-nitrogen, ammonia-nitrogen, total Kjeldahl nitrogen, microcystin and chlorophyll a will be collected in duplicate and analyzed to obtain a measure of the variability of the analyses and as a measure of the heterogeneiety of the lake.

Ten percent of the samples for pH/alkalinity, total phosphorus, soluble reactive phosphorus, nitrate-nitrogen, ammonia-nitrogen, total and Kjeldahl nitrogen, and microcystin will be processed as replicates from the same sample bottles for each parameter. Replicates assess the precision of the laboratory procedure. Chlorophyll a is the only parameter that only offers an option for duplicate analysis and not a replicate sample, since it is the filter that is processed and not lake water. Replicate samples of plankton will be quantified by the same skilled analyst to assess precision.

Analyses for total phosphorus, soluble phosphorus, nitrate-nitrogen, ammonia-nitrogen and total Kjeldahl nitrogen all require preparation of standards and running a standard curve. The fit ot each standard curve (regression coefficient) is evaluated against the lower warning limit of the control chart as described below.

Control charts will be used to evaluate precision of laboratory procedures. Control charts will be generated from data produced over the course of normal laboratory workload. The center of the chart will be the baseline mean of the average deviation and percent recovery of 10 samples of the specific analyte of interest. The upper and lower warning limits will be defined as +/- 2 standard deviation units. If the sample value approaches these limits it is suspect but can still be used. It is a warning to the analyst that something is wrong with the analytical procedure and must be corrected.

The upper and lower control limits are defined as +/- 3 standard deviation units. If a result falls outside the control limits on the control chart, the analysis is "out of control." The result cannot be trusted and immediate action to determine the cause of the outlying result must be taken.

Accuracy:

An entire set of field blanks (all parameters) will be analyzed for 10% of each test's samples to check sampling procedures, bottle preparation, and laboratory reagents. Lab blanks will be analyzed with each sample run. Field blank results exceeding the upper warning limit are a warning to the analyst that something is wrong with the analytical procedure and steps will be taken to identify and correct the problem. The analytical results can be used as long as the results do not exceed the upper control limit. If a result is determined to be "out of control", the result cannot be trusted and immediate action to determine the cause of the outlying result must be taken. The analysis will be re-run after the problem is corrected.

Lab spikes prepared from certified solutions by the QA Officer will be used to test the accuracy of lab and field operations for the following parameters on each sample analysis: alkalinity, ph, soluable reactive phosphorus, total phosphorus, nitrate-nitrogen, ammonia-nitrogen, total Kjeldahl nitrogen, and microcystin. The research assistants will process all spikes as blind analyses. Spike samples are purchased from outside sources with documented reliability. One spike sample is evaluated for every ten field samples. The spike analysis must result i n +/- 10% recovery. If outside this range, the analytical run is rejected.

% Recovery = found value/true value x 100%

Standard curves, with concentration ranges consistent with the expected concentrations of the samples, will be analyzed with the samples each day in the lab. Charts will be maintained to assess the consistency of the standard curves and to monitor lab procedures. Standard curve correlation

coefficients are acceptable if they do not exceed the upper control limit. If the standard curve correlation coefficient is "out of control" the analysis is halted and the problem is identified and corrected. The analysis is then run again. If the standard curve correlation coefficient exceeds the upper warning limit, but is less than the upper control limit, the analysis can proceed but steps will be taken to identify and correct the problem.

Proper maintenance and calibration of electronic meters will insure their performance at published instrument accuracy levels.

Section 9: Data Reduction, Analysis, Review, and Reporting

Data Reduction

Total phosphorus, soluble reactive phosphorus, nitrate-nitrogen, ammonia-nitrogen, and total nitrogen will be reported in milligrams per liter. Alkalinity will be reported in milligrams per liter of calcium carbonate. Chlorophyll a will be reported in milligrams per cubic meter. Any equations used to ascertain the values for the above tests can be found in the sections for the respective tests in Standard Methods for the Examination of Water and Wastewater, 21st ed and in Methodolgy: USEPA by Segmented Flow Analysis. Dissolved oxygen will be reported in parts per million as read off the meter at each depth. Temperature will be reported in Celsius degrees as read off the meter at each depth. Light transmission will be reported as percent transmission as read off the meter. The Secchi disk measurement will be reported in meters as discerned from the field reading. Conductivity will be expressed in micro Siemens per centimeter as read off the meter. pH measurements are reported as pH units as read off the meter. Zooplankton will be reported in number of organisms (genus level) per liter and algae will be reported as both number of cells per liter and as number of natural units per liter.

Data obtained from field and lab measurements will be collected into a database (Microsoft Access) for ease of analysis. Lake and lab data sheets will be maintained in permanent files.

Data Analysis

Results from the analyses will be used to calculate the Carlson Trophic State Index (TSI) for each lake and to better understand the limnology of the sampled lakes. Carlson TSI scores are calculated for total phsophorus, chlorophyll a, and Secchi disk. Eutrophy points are assigned to each parameter, and can be looked at individually. The data will be used to identify changes that may have occurred in the lakes since other analyses were done. These results may also be incorporated into the Section 305(b) reports that the State submits to U.S. EPA.

Data Review

Review of analysis results are checked after each processing by the Quality Control Officer and/or Laboratory Manager. Particular attention is devoted to reviewing duplicates, replicates, and field and lab blanks, which is set up for quality assurance checks.

Field data are reviewed before weighing anchor on each lake by the boat operator, who also measures *in situ* parameters with field instruments. This person's initials are at the bottom of each field data sheet.

Data Reporting

All raw data and data analysis results will be entered in a Microsoft ACCESS database, consructed specifically for this project. Additionally, sample date and time, sample site, latitude and longitude of the sample site will also be included for each result.

All raw data and data analysis results generated as part of this grant project will be submitted in an electronic format with the Final Report to the IDEM Project Manager, according to the reporting schedule specified in our contract. The data spreadsheet will be submitted to IDEM in a spreadsheet format for AIMS. Summary reports are prepared periodically according to schedules specified by IDEM. Because these data are considered public record, data are transmitted electronically to anyone who requests it.

Section 10: Performance and System Audits

Instrument performance is evaluated by the laboratory manager or analyst prior to any analysis of samples to ensure that all equipment is performing adequately. In addition, standard curve preparation for sample analysis will also check performance.

No specific field audits, other than instrument checks, field blanks, duplicates and replicates, are conducted.

IDEM reserves the right to conduct external performance and/or systems audits of any component of this study.

Section 11: Preventative Maintenance

Preventative maintenance on the various equipment that will be used in these analyses will be performed as required by manufacturer's schedules. Sampling personnel will perform day to day maintenance. Maintenance is logged in laboratory log books.

Section 12: Data Quality Assessment

Precision

Control charts of precision, accuracy, lab blanks, and standard curve correlation coefficients, will be kept for each analytical method. The control charts will be generated from data produced over the course of normal laboratory workload. The upper and lower warning limits will be defined as +/- 2 standard deviation units (see Appendix C). If the value approaches these limits it is suspect but can still be used. It is a warning to the analyst that something is wrong with the analytical procedure and must be corrected. We will assess the QC results for field measurements by comparing duplicate results with DQOs to determine whether data are precise enough to meet our goals.

The upper and lower control limits are defined as +/- 3 standard deviation units. If a result falls outside the control limits on the control chart, the analysis is "out of control." The result cannot be trusted and is not reported. Immediate action to determine the cause of the outlying result must be taken.

Accuracy

If recovery of spike samples is +/- 10% of the true value, the parameter run for which the spike sample was analyzed is accepted as being accurate. If recovery is greater than +/- 10%, the run is determined to be inaccurate and is rejected. The run is then analyzed for problems, corrections made, and the samples are run again.

Completeness

All accurate and precise data are considered valid and are reported even if completeness objectives are not met.

Section 13: Corrective Action

The QA Officer will be responsible for performing corrective action when a data point is found to be out of control limits. The QA officer will go through the procedure step by step with the laboratory analyst to assess where corrective action is necessary. The method, reagents, glassware, technique, and instruments will all be checked to ascertain the problem. Following this procedure, the samples are run again. These steps are repeated until data fall within the control limits.

Field blanks are taken after each sampling sequence, so if it is determined that the field blanks are contaminated ("out of control"), then the sample water within that sampling sequence is also considered contaminated. The method, reagents, glassware, technique, and instruments will all be checked to ascertain the problem before the analysis resumes.

Once the data points are again within control limits, all analyses done after the system went out of control will be repeated. Remaining sample water will be used to reassess the sample value. If all sample water has been used an attempt will be made to repeat the sampling sequence. For this reason, any remaining sample will not be disposed of until the analyses have been shown to be within the control limits.

Section 14: Quality Assurance Reports

Quality Assurance (QA) reports will be submitted to IDEM's Watershed Management Section once a year as part of the Quarterly Progress Report and/or Final Report.

These reports will be given to the project manager by the QA/QC manager and will include an assessment of measurement data, accuracy, precision, and completeness, as well as the results of any performance audits and/or system audits, and any significant QA problems.

References

APHA. 2005. Standard Methods for the Examination of Water and Wastewater, 21st Edition. American Public Health Association, American Water Works Asssociation and the Water Environment Federation, Washington, D.C.

Metertech Inc., 63-2, ChengGong Rd., Sec 1, NanGang, Taipei, Taiwan, ROC.

O I Analytical. 2000. The FLOW Solution Operations Manual, for Segmented Flow Analysis, College Station, TX.

Prescott, G. W. 1982. Algae of the Western Great Lakes Area. Otto Koeltz Science Publishers, Koenigstein, West Germany.

Ward, H.B. and G.C. Whipple. 1959. Freshwater Biology, Second Edition. W.T. Edmondson, editor. John Wiley & Sons, Inc.; New York.

Wehr, J.D. and R.G. Sheath. 2003. Freshwater Algae of North America, Ecology and Classification. Academic Press, San Diego.

Whitford, L.A. and G.J. Schumacher. 1984. A Manual of Frsh-Water Algae. Sparks Press; Raleigh, N.C.

Appendix A

Candidate Lake List – 2015

Lake Name	County	Latitude	Longitude	Lake_ID
Atwood	LaGrange	41.53951	85.41325	1068
Bass	Sullivan	39.06179	87.32563	1226
Bass (N.Chain)	St. Joseph	41.69724	86.37123	1085
Bear	Noble	41.32215	85.51785	823
Big Otter	Steuben	41.72386	85.01025	1131
Big Turkey	LaGrange	41.59153	85.19404	608
Blackman	LaGrange	41.55098	85.28574	609
Bobcat	Greene	39.05545	87.35114	1478
Boones Pond	Boone	39.98509	86.39558	52
Brush Creek Reservoir	Jennings	39.05644	85.52431	443
Cagles Mill (Cataract)	Putnam	39.47140	86.90068	1038
Canada	Porter	41.52760	87.04632	997
Carr	Kosciusko	41.15805	85.86346	524
Cedar	Lake	41.37201	87.43360	675
Center	Kosciusko	41.24513	85.85658	525
Clear (LaPorte)	LaPorte	41.61716	86.72249	708
Crane	Noble	41.27769	85.48248	836
Crystal	Greene	39.11840	87.23438	316
Failing	Steuben	41.70535	84.99847	1149
Fish (Lower)	LaPorte	41.55928	86.5491	711
Fish (Upper)	LaPorte	41.57161	86.54636	712
Gambill	Sullivan	39.04676	87.25204	1254
Geist Reservoir	Marion	39.90733	85.98307	750
Goldeneye	Kosciusko	41.33526	85.66468	535
Goodman	Greene	39.01283	57.23573	323
Goose	Kosciusko	41.18962	85.88158	536
Green	Steuben	41.72711	84.99894	1161
Griffy	Monroe	39.20458	86.52659	782
Grouse Ridge	Bartholomew	39.10950	86.04458	35
Hackberry	Sullivan	38.97644	87.24823	1259
Hale	Sullivan	38.97150	87.24646	1260
Hartz	Starke	41.17739	86.49512	1117
Hindman	Noble	41.565278	85.649444	862
Hog	LaPorte	41.70546	86.62885	715
Huntingburg City	Dubois	38.29699	86.98180	219
James	Kosciusko	41.32317	85.73151	546
John Hay	Washington	38.68971	86.14996	1414
Kickapoo	Sullivan	39.16338	87.24595	1270
King	Fulton	41.12896	86.42290	267
Larwill	Whitley	41.17223	85.62251	1448

Lake Name	County	Latitude	Longitude	Lake_ID
Little Bause	Noble	41.33712	85.60307	881
Long	Porter	41.52561	87.04883	1009
Loon	Steuben	41.65047	85.04865	1185
Loon	Whitley	41.26936	85.54880	1454
Manitou	Fulton	41.05051	86.17236	273
Mansfield Reservoir (Hardin)	Parke	39.71887	87.07245	969
Marsh	Steuben	41.721020	84.98585	1186
Martin	LaGrange	41.56466	85.38535	637
Mud (Chain of Lakes)	Noble	41.33462	85.40325	900
Nauvoo	LaGrange	41.52833	85.33222	644
North Little	Kosciusko	41.08622	85.90172	561
Oliver	LaGrange	41.57034	85.40346	647
Oswego	Kosciusko	41.32526	85.78575	562
Otter	Steuben	41.63506	85.16828	1201
Port Mitchell	Noble	41.35955	85.44205	910
Prairie Creek Reservoir	Delaware	40.14536	85.29137	206
Red Pine	Sullivan	38.97544	87.25105	1295
Redbud	Sullivan	38.97683	87.25130	1296
Ridinger	Kosciusko	41.26191	85.66529	570
Round	Whitley	41.24538	85.43335	1463
Royer	LaGrange	41.61099	85.33819	655
Scales	Warrick	38.06382	87.25142	1401
Schlamm	Clark	38.56525	85.78401	1061
Shakamak	Sullivan	39.17606	87.24513	1299
Shipshewana	LaGrange	41.68815	85.60812	656
Shriner	Whitley	41.24397	85.44630	1465
Skunk	Greene	39.05504	87.34841	1479
St. Joseph Reservoir	Allen	41.11687	85.11451	27
Starve Hollow	Jackson	38.80950	86.08033	428
Summit	Henry	40.01881	85.30945	395
Sycamore	Greene	38.95	87.25	360
T Lake	Sullivan	39.02662	87.25375	1308
Todd	Greene	38.96924	87.23930	362
Trimble	Greene	39.00182	87.22481	363
Turtle	Sullivan	39.05	87.35	1311
Waveland	Montgomery	39.88623	87.08295	792
Wawasee	Kosciusko	41.40306	85.70756	596
Webster	Kosciusko	41.32188	85.67553	597
White Oak #2	Knox	38.74250	87.40641	508
Willow	Sullivan	39.02938	87.25882	1317
Yellowwood	Brown	39.17766	86.33977	88

Appendix B

Sample Field Data Sheet

INDIANA CLEAN LAKES PROGRAM
LAKE ASSESSMENT FIELD DATA SHEET

SITE:_____ Picture Taken: ☐

DATE:_____TIME:_____ WEATHER:_____ Glutarald.Added: ☐

Ethanol Added: ☐

DEPTH (m)	TEMP (^{o}C)	D.O. (ppm)
Sur		
1.0		
2.0		
3.0		
4.0		
5.0		
6.0		
7.0		
8.0		
9.0		
10.0		
11.0		
12.0		
13.0		
14.0		
15.0		
16.0		
17.0		
18.0		
19.0		

	E	**H**
COND		
pH		
% TRANSM. @ 1m =		surface
1% level (m) =		
SECCHI (m) =		
ANCHOR DEPTH (m)		
HYPO (m)		
ZOOPLANKTON TOW (m)		
CHL *a* FILTERED (ml)		
Chl-a Dup FILTERED (ml)		

RAMP TYPE: _____

Latitude: _____

Longitude: _____

INITIALS: _____

COMMENTS:

Appendix C

Quality Control Chart

QUALITY CONTROL CHART

+3sd Upper Control Limit (UCL)

+2sd Upper Warning Limit (UWL)

0 Zero Deviation

-2sd Lower Warning Limit (LWL)

-3sd Lower Control Limit (LCL)