

Quality Assurance Project Plan

for

Indiana Clean Lakes Program (2025-2026)

Contract # 91225

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Indiana Department of Environmental Management

Office of Water Quality

Watershed Planning & Restoration Section

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2.5 List of Acronyms

ANC – Acid Neutralizing Capacity

CWA – Clean Water Act

FOM – Field Operations Manual

HDPE – High Density Polyethylene

IDEM – Indiana Department of Environmental Management

INCLP – Indiana Clean Lakes Program

LCS – Lab Control Standard

LOM – Laboratory Operations Manual

MDL – Minimum Detection Limit

PAR – Photosynthetically Available Radiation

QA – Quality Assurance

QA/QC – Quality Assurance/Quality Control

QC – Quality Control

RSD – Relative Standard Difference

SD – Standard Deviation

O'Neill School – O'Neill School of Public and Environmental Affairs

TTL – Training Tracking Log

TSI – Carlson's Trophic State Index

U.S. EPA – United States Environmental Protection Agency

USGS – United States Geological Survey

3 Distribution List

This quality assurance project plan (QAPP), which includes the associated manuals or guidelines, will be distributed to the Indiana Department of Environmental Management (IDEM) and all key project staff in Table 1.

Table 1: QAPP Distribution List

Title	Name	Contact Information
IDEM Project Manager	Timothy Neher	Watershed Management Section Indiana Department of Environmental Management 100 North Senate Avenue MC 65-44 Shadeland Indianapolis, IN 46204
O'Neill School Project Manager	Sarah Powers	sarellis@iu.edu 1315 East 10 th Street Bloomington, IN 47405
O'Neill School Project Lead, Laboratory Manager and Quality Control Officer	Lab Manager	1315 East 10 th Street Bloomington, IN 47405
Project Staff	Indiana University Students	QAPP distributed during training

4 Project Organization and Responsibility

The Indiana University O'Neill School of Public and Environmental Affairs (O'Neill School) in Bloomington, IN, coordinates the Indiana Clean Lakes Program (INCLP) with technical support from IDEM.

Program Director: Sarah Powers is the Project Manager. Works with IDEM Project Manager to select lakes for sampling; hires all personnel; sets employee tasks and work schedules; coordinates employee training; prepares quarterly progress reports

O'Neill School of Public and Environmental Affairs, 1315 East Tenth Street,
Bloomington, IN 47405; 812-856-1274
sarellis@indiana.edu

Laboratory Manager and Quality Control Officer: Megan Gokey is the current Laboratory Manager and Quality Control Officer. Maintains and operates analytical instruments and ensures laboratory quality assurance/quality control (QA/QC); assists in INCLP field and laboratory training; prepares quality control (QC) samples; compiles QA./QC results; responsible for performing corrective action when necessary. Trains the laboratory staff. Also assists in all laboratory analytical procedures.

O'Neill school of Public and Environmental Affairs, 1315 East Tenth Street,
Bloomington, IN 47405; 812-855-1600; mgokey@indiana.edu

Laboratory & Field Staff: Qualified undergraduate and graduate students enrolled in or recently graduating from Indiana University will collect and analyze samples, following extensive training under the direction of the Program Director and Laboratory Manager.

4.1 Project Organization

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 AQ400 discrete analyzer. Also conducts blind analysis of QC samples.

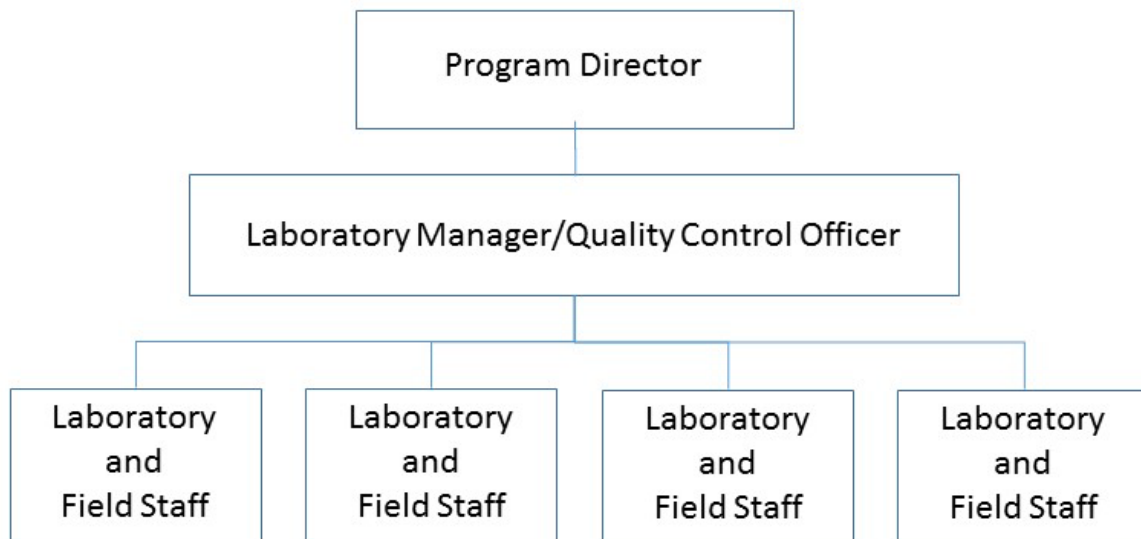


Figure 1: Project Organization Chart

4.2 Special Training Needs/Certification & Qualifications

Qualified students will implement much of this plan, following extensive training under the direction of the Program Director and Laboratory Manager. All laboratory and field

staff undergo 3-4 weeks of training for all laboratory and field techniques in accordance with the Indiana Clean Lakes Program Laboratory Operations Manual (LOM) and Field Operations Manual (FOM).

All staff members are required to demonstrate the ability to calibrate and operate all field equipment for in situ measurements, water sample collection and handling for laboratory analysis. Staff are required to maintain training tracking logs (TTL) to demonstrate competence (Appendix C). Training verification is conducted during the first sampling trip of the season where the Program Director and Laboratory Manager shadow field staff to verify compliance with FOM protocols. Field crews will collect duplicate samples at one site each day of sampling to monitor QA/QC throughout the sampling project.

All staff members are required to demonstrate the ability to process chemical samples for analysis and operate lab instruments. Staff will specialize on specific analysis and instruments to ensure quality results. Staff will maintain TTL for lab analysis including making standard dilution curves and processing of samples including QA/QC results. Staff are required to demonstrate the ability to meet MDLs for each type of analysis before processing seasonal samples.

5 Problem Definition/Background

5.1 Problem Statement

Indiana has many lakes and reservoirs, and many have a variety of problems. Eutrophication, watershed runoff, boating impacts, shoreline erosion, septic system discharge, and bacterial contamination, are all water quality issues but some of the problems are associated with nonpoint source pollution in these waterbodies. In the most recent Indiana Lake Water Quality Assessment Report covering 2023-2024, over 54% of lakes were classified as eutrophic or hypereutrophic using the Carlson Trophic State Index (TSI).

The Indiana Clean Lakes Program (INCLP) helps to meet a nonpoint source monitoring need that IDEM has but which the agency cannot meet with current staff. Having INCLP monitor and assess the state's public freshwater lakes meets that need with the added benefit of the volunteer program, which monitors lakes throughout the state by recruited citizen scientists.

Section 314 (modified by Section 319) of the Clean Water Act charges IDEM with responsibility for monitoring, assessing, and reporting conditions of Indiana's lakes. Continued assessment of lake nutrient levels and effects, as begun by the State in the early 1970s, is needed to do the following:

- a) Report the status of lake eutrophication levels to the United States Environmental Protection Agency (U.S. EPA) in the State's 305(b) Water quality reports and 303(d) reports listing of impaired waterbodies,
- b) Ascertain and track any trends in lake eutrophication levels for IDEM and U.S. EPA use.

INCLP through its probabilistic random design allows for this program to assess and list the lakes and reservoirs of Indiana statewide.

5.2 Historical & Background Information

The INCLP was created in 1989 as a program within the IDEM Office of Water Management. The program is administered through a grant to the O'Neill School in Bloomington. Faculty, staff, and students associated with Indiana University implement the tasks associated with the program. INCLP is a comprehensive, statewide public lake monitoring program having five components:

1. Public information and education
2. Technical assistance
3. Volunteer lake monitoring
4. Lake water quality assessment (LWQA)
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 LWQA component of the INCLP.

INCLP helps to meet the nonpoint source monitoring needs. The educational benefits to students and volunteer lake monitoring program extend the reach of monitoring activities.

6 Quality Objectives & Criteria for Measurement Data

6.1 Study Goals

Approximately 70-80 public lakes each year are selected randomly for detailed analysis designed to provide descriptive statistics for all Indiana public lakes that are accessible to trailered boats, totaling 160 lakes during the two-year period. This assessment work has the following goals:

Goal 1: Collect data to generate the LWQA Report for the condition of Indiana lakes and identifies improving or declining water quality trends.

Goal 2: Collect data that meets IDEM's data quality and other requirements for use in Clean Water Act Section 314 trend and trophic state assessments.

Goal 3: Collect data to meet IDEM's data quality and other requirements for use in Clean Water Act (CWA) 305(b)/303(d) assessment and listing processes.

Goal 4: Produce data of known quality that is publicly available.

6.2 Study Site Description

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.

6.3 Sampling Design

Since 2010, lakes to be sampled are randomly selected each year and integrated with the two-year Integrated Reporting cycle. The capacity of the survey crew is approximately 80 lakes per summer. 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. This will contribute to the Clean Water Act Section 314 trend and trophic state assessments.

The July-August sampling period is used as a reflection of worst-case and stable conditions (warm temperatures, thermal stratification, hypolimnetic anoxia, algal blooms) are expected. Samples collected during this period of relative summer stability can be compared over time.

Field sampling generally occurs on Monday through Thursday of each week. The lakes in a particular summer draw will not be sampled in random order as that would unnecessarily waste energy, money, and time. Instead, lakes will be sampled according to transects originating in Bloomington and spread around the State. For example, on any given Monday-Thursday sampling period, sample scheduling for lakes may start 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 determined using lake bathymetry and depth sonars to locate the deepest location in the lake (index site). GPS coordinates are recorded at the index site using decimal degrees. The deepest depth is selected to standardize the sampling location with the highest probability to capture thermal stratification. Samples of epilimnetic (an integrated sample from the surface to 2-meter depth) and hypolimnetic water (~1 meter above the lake bottom) will be collected.

The parameters chosen for in-field sampling and lab analysis are essential to calculate the Carlson Trophic State Index (TSI) for each lake to meet IDEM's data needs for both CWA Section 314 assessments and CWA Sections 305(b)/303(d) assessments and listing processes, and reporting to the U.S. EPA. Staff will collect, preserve, and store water samples for future lab analysis of: 1) ANC, (2) total phosphorus, (3) soluble reactive phosphorus, (4) total nitrogen, (5) nitrate-nitrogen, (6) ammonia-nitrogen, and (7) chlorophyll a. Staff will collect field measurements for: (8) water temperature, (9) dissolved oxygen (10) Secchi disk transparency, (11) percent light transmission, (12) conductivity, and (13) pH. Staff will collect biological samples for plankton and preserve them for future analysis.

This sampling design allows us to meet our goals to identify and analyze the condition of Indiana lakes. The spatially and temporally scheduled field season ensures the most representative survey of Indiana lakes. Since this design captures a "snap-shot" picture of each lake, sampling along transects from the peripheral edge of the state towards Bloomington, ensures all ecoregions are surveyed throughout the growing season.

6.4 Study Schedule

Table 2: Study Schedule.

Activity	Timeline
Planning	May 2025
Training	June 2025, June 2026
Project Sampling	June - August 2025, June – August 2026
Lab Analysis	June 2025 – December 2026
Data Analysis and Reporting	April 2027

7 Data Quality Indicators (for Measurement Data)

7.1 Precision

There are several procedures used to ensure precision with field sampling and laboratory analysis:

(1) Duplicates – Duplicates are two separate samples collected or measurements made from the same location and depth one right after another. For example, a duplicate nitrate sample at the 1 meter depth would require two separate sample retrievals with the discrete 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 (SD) of values obtained from previous laboratory runs of the same parameter.

(2) Replicate samples – Replicates are two samples drawn off from the same discrete sample or integrated sampler. In this case, field sampling technique is the same and the replicates evaluate bottle preparation and laboratory technique. 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 nitrogen all require preparation of standard curve for analysis. The standard curve must contain no less than 5 points along a range of expected values for analysis. The regression coefficient is evaluated against the laboratory control standard (LCS). The standard curve is accepted if the LCS measured concentrations relative percent difference from the expected concentration is $<10\%$.

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

7.2 Accuracy

Proper maintenance, calibration, and use of field and laboratory meters will ensure their accuracy. The accuracy of each instrument is given by the manufacturer and is included in Table 3. The main procedure used to ensure accuracy in field samples is lab control standards (LCS). LCS are samples that have a known quantity of analyte added to them before they are run through the analytical techniques. LCS solutions are purchased from outside sources with documented reliability. One LCS sample is evaluated for every ten field samples. The LCS analysis must result in $\pm 10\%$ recovery. If outside this range, the analytical run is rejected.

% Recovery = found value/true value x 100%

Field method blanks are analyzed against the method detection limits as a test of accuracy by ensuring 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 method blanks should theoretically be 0, however since some contamination is inevitable, we will compare the field method blank values with the method detection limits for each chemical parameter tested. Field method blank values should not exceed sensitivity values (Table 3).

Table 3: Data quality Objectives

Parameter	Precision	Accuracy	Sensitivity
Temperature	± 2 degrees	±0.1°C	0 °C
Conductivity	± 2 SD	± 5% full scale	0.1 uS
Dissolved oxygen	± 2 SD	± 2% Air Saturation ± 0.3% mg/L	0 mg/L
Secchi disk transparency	RPD	No QA Standards are available	n/a
Percent light transmission	± 2 SD	No QA Standards are available	0% photosynthetically available radiation (PAR)
pH	± 2 SD	± 0.5% full scale	Range 0-14 standard units
Total plankton	± 2 SD	No QA Standards are available	n/a
ANC	± 2 SD	± 10%	n/a
Nitrate-nitrogen	± 2 SD	± 10%	0.007 mg/L
Ammonia-nitrogen	± 2 SD	± 10%	0.005 mg/L
Total nitrogen	± 2 SD	± 10%	0.030 mg/L
Total phosphorus	± 2 SD	± 10%	0.003 mg/L
Soluble reactive phosphorus	± 2 SD	± 10%	0.0015 mg/L
Chlorophyll a	± 2 SD	No QA Standards available	Wavelength range 190 to 1,100 nm

SD = Standard Deviations

7.3 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 for one year falls below the 70-80 lake total because this is an ongoing program allowing us to sample or resample additional lakes in the following year. For the purposes of this section, DQO can be met with 90% completeness.

7.4 Representativeness

Refer to Section 6.3, Sampling Design.

7.5 Comparability

Methods are common and U.S. EPA approved as recommended in Standard Methods, 24th Edition (APHA, 2023).

7.6 Sensitivity

The sensitivity is the reporting limit or the method detection limit. Table 3 lists the reporting limit for each parameter.

8 Non Direct (Secondary Data)

No secondary data will be used to meet the goals of this project.

9 Monitoring Requirements

9.1 Sampling Procedures

In-situ Measurements

In-situ measurements are taken using a multiparameter sonde for dissolved oxygen, conductivity, pH, and temperature. 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 transparency 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. They will lower the disk into the water until it is no longer visible. It is then lowered some more and raised until it again becomes visible. The depth where the disk disappears and reappears is recorded. INCLP will report disappearance, reappearance and the average of these two values.

The percentage of light transmitted is determined using a Li-Cor LI-193 Underwater Spherical Quantum Sensor attached to a LI-189 Quantum Photometer. Measurements will be at one-meter intervals until the light transmission equals zero.

Appendix D 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 discrete sampler for hypolimnetic samples for laboratory analysis of ANC, total nitrogen, ammonia-nitrogen, nitrate-nitrogen and chlorophyll a. Samples are collected in appropriately sized high-density polyethylene (HDPE) bottles (Table 4). Soluble reactive phosphorus and total phosphorus samples will be collected in acid washed glassware. Ammonia-nitrogen, nitrate-nitrogen, total nitrogen and total phosphorus samples will be preserved by acidifying each sample to a pH<2 by addition of a H₂SO₄ solution and stored at 4 degrees C in an ice chest in the field and later in the laboratory refrigerator/freezer until analysis. Soluble reactive phosphorus, and ammonia-nitrogen, nitrate-nitrogen, and chlorophyll a samples are filtered in the field.

A 250-ml sample will be collected for acid neutralizing capacity (ANC) 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 field and then stored at 4 degrees C in the laboratory refrigerator until analysis, which is preferably within 1 day.

A filtered sample 60-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 of collection unless kept in the freezer.

A 60-ml sample in a HDPE bottle will be collected for analysis of total 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 of collection unless kept in the freezer.

A 60-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 of collection unless kept in the freezer.

A filtered sample of 60 ml in an acid-washed glass bottle will be taken for analysis of soluble phosphorus. The sample will be filtered on site (using membrane filter of 0.45µm pore space using a syringe filter apparatus) 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 of collection unless kept in the freezer.

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 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 conical tube covered in foil (to shade) 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.

Algal samples will be collected by lowering a two-meter integrated sample. 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 refrigerator until analysis. The glutaraldehyde preservative provides preservation of plankton cells for at least one year and does not stain the specimens. Algal samples are enumerated by Phycotech Inc. for analysis to identify and classify by HAB taxa.

Zooplankton will be collected by lowering a Wisconsin-style tow net (243 micron mesh) to one meter from the bottom after hypolimnion samples are collected and slowly raising the net to the water surface. The collection bucket will be placed in a pail filled half full with lake water and two CO₂ (alka-seltzer) tablets. The CO₂ narcotizes the zooplankton to relax their external structure prior to preservation in 95% ethanol. The concentrated sample will be poured into a 125 mL bottle and preserved with 95% ethanol (Section 10200 B in Standard Methods for the Examination of Water and Wastewater, 21st edition). Preserved samples will be placed into an ice chest in the field and later stored in the laboratory until analysis.

9.2 Field QC Activities

INCLP collects field method blanks after each sampling sequence at the end of the sampling day in the field. INCLP collects duplicate samples for each chemical parameter at one site for each day of field sampling. Additionally, for in-situ measurements INCLP will record a full profile of temperature and dissolved oxygen and recheck the surface measurements at the end of sampling to ensure that oxygen measurements are within 0.5 mg/L of the original measurement. In-situ measurement recheck at the surface is consistent with National Lakes Assessment (NLA) sampling process and field check (NLA 2022 QAPP).

Table 4: Sampling Methods

Parameter	Sampling Frequency	Sampling Method	Sample Container	Sample Volume	Holding Time
Temperature	1 measurement for each 1-meter depth per lake	YSI Pro DSS or In-Situ Aqua Troll 600 or 500 Oxygen, Conductivity, Salinity, Turbidity and Temperature probe. 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. In-Situ Inc. Method 1002-8-2009 Dissolved Oxygen Measurement by Optical Probe (ATP Case No. N05-0014)	NA	NA	NA
Conductivity	1 epilimnetic & 1 hypolimnetic measurement per lake	YSI Pro DSS or In-Situ Aqua Troll 600 or 500 Oxygen, Conductivity, Salinity, Turbidity and Temperature probe. Standard Method 2510 B-1997 and U.S. 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 Pro DSS or In-Situ Aqua Troll 600 or 500 Oxygen, Conductivity, Salinity, Turbidity and Temperature probe. In-Situ Inc. Method 1002-8-2009 Dissolved Oxygen Measurement by Optical Probe (ATP Case No. N05-0014) 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	2 Measurements 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, the depth the disk disappears is recorded. It is then lowered some more and raised until it again becomes visible, the depth the disk reappears is recorded. The midpoint between these two measurements is recorded as the Secchi disk depth.	NA	NA	NA
% Light transmission	profile until measure drops to zero	Percentage of light transmitted is determined by the use of a Li-Cor LI-193 Underwater Spherical Quantum Sensor. Measurements are collected at one meter intervals until the PAR level reaches zero. We will calculate the 1% light level based on this profile.	NA	NA	NA
pH	1 epilimnetic & 1 hypolimnetic sample per lake	YSI Pro DSS or In-Situ Aqua Troll 600 or 500 Oxygen, Conductivity, Salinity, Turbidity and Temperature probe. Standard Methods 4500-H+ 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
Acid Neutralizing Capacity	1 epilimnetic & 1 hypolimnetic sample per lake	Water samples are collected using integrated and discrete depth samplers	HDPE Nalgene	250 ml	48 hours
Ammonia-nitrogen	1 epilimnetic & 1 hypolimnetic sample per lake	Water samples are collected using integrated and discrete depth samplers The sample will be filtered on site (using membrane filter of 0.45µm pore space using a syringe filter apparatus in the field)	HDPE Nalgene	60 ml	28 days or frozen
Nitrate-nitrogen	1 epilimnetic & 1 hypolimnetic sample per lake	Water samples are collected using integrated and discrete depth samplers The sample will be filtered on site (using membrane filter of 0.45µm pore space using a syringe filter apparatus in the field) and acidified to a pH <2	HDPE Nalgene	60 ml	28 days or frozen
Total nitrogen	1 epilimnetic & 1 hypolimnetic sample per lake	Water samples are collected using integrated and discrete depth samplers and acidified to a pH <2	HDPE Nalgene	60 ml	28 days or frozen
Total phosphorous	1 epilimnetic & 1 hypolimnetic sample per lake	Water samples are collected using integrated and discrete depth samplers and acidified to a pH <2	HDPE Nalgene	60 ml	28 days or frozen
Soluble phosphorus	1 epilimnetic & 1 hypolimnetic sample per lake	Water samples are collected using integrated and discrete depth samplers. The sample will be filtered on site (using membrane filter of 0.45µm pore space using a syringe filter apparatus in the field)	HDPE Nalgene	60 ml	48 hours
Chlorophyll a	1 epilimnetic sample per lake	Water samples are collected using an integrated sampler. A specific amount of lake water is filtered through a Whatman GF/F filter using a Nalgene PSF filter holder with receiver and a hand-operated vacuum pump.	HDPE Nalgene-opaque	30 ml	28 days
Algae	1 sample per lake	Algae are collected using an integrated sampler and preserved with glutaraldehyde	HDPE Nalgene-opaque	250 ml	One year

Table 4: Sampling Methods

Parameter	Sampling Frequency	Sampling Method	Sample Container	Sample Volume	Holding Time
Zooplankton	1 sample per lake	Zooplankton are collected using a Wisconsin-style tow net (50 micron mesh) towed from one full meter from the bottom of the lake and sample is preserved with ethanol	HDPE Nalgene- opaque	125 ml	One year

10 Analytical Requirements

10.1 Sample Analysis Procedures

Each ANC 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 edition). A Fisher Scientific Accumet AE150 selective ion meter and an Accumet 13-620-299B pH probe will be used. The meter and probe will be calibrated using three-point calibration of known standard buffer solutions.

Temperature, dissolved oxygen, pH and conductivity are measured in situ using YSI ProDSS or In-Situ Aqua Troll 500 or 600 (Standard Method 2550 B-2000, In-Situ Inc. Method 1002-8-2009, Standard Method 2510 B-1997, U.S. EPA Method 120.1, respectively, Standard Methods 4500-H+).

Each nitrate-nitrogen sample will be analyzed by cadmium reduction method (Seal Method EPA-126-C Revision 2, U.S. EPA Method 353.2) and each ammonia-nitrogen sample will be analyzed by the salicylate and hypochlorite method (Seal Method EPA-150-C Revision 1, U.S. EPA Method 350.1 Rev. 2.0) using discrete analysis on a Seal Analytical discrete analyzer model AQ400. Calibration will be with at least five serial dilutions of a standard nitrate-nitrogen or ammonia-nitrogen solution and a solution blank.

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

The final dissolved phosphorus measurement will be determined by ascorbic acid methodology of colorimetry listed in Standard Methods for the Examination of Water and Wastewater, 22nd edition (Section 4500-P J.) Analysis will be done on the Seal Analytical discrete analyzer model AQ400 (Seal Method EPA-119-C Revision 3A). This method can and will also be used for analysis of soluble reactive phosphorus samples without digestion. Calibration will be with at least five serial dilutions of a standard phosphate solution and solution blank.

The final total nitrogen values are measured from the nitrogen compounds as they are oxidized by persulfate to nitrate. Nitrate is measured by U.S. EPA 353.2 methodology the same as the nitrate-nitrogen methods listed above for the Seal Analytical discrete analyzer model AQ400 (Seal Method EPA-126-C Revision 2). Calibration will be with at least five serial dilutions of a standard nitrate-nitrogen 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, 22nd edition (method 4500-P-F). Seal Analytical discrete analyzer model

AQ400 will be used for analysis (Seal Method EPA-118-C Rev 1). 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 edition (Section 10200 H). An Evolution 220 spectrophotometer is used for this analysis.

Phytoplankton samples are analyzed using an Imaging FlowCytobot (IFCB) that is an automated submersible imaging flow cytometer, manufactured by McLane Research Laboratories, that generates high-resolution images of suspended particles in water. PhycoTech, Inc. uses the IFCB as a benchtop application to capture algal images for identification. The IFCB can reliably capture particulates between 8 and 250 μm (average phytoplankton $\sim 50 \mu\text{m}$). The general process of analysis includes sample injection, image processing, and classification. Classification of algal images uses a random forest model developed and trained by Dr. Ann St. Amand and can accurately identify algal images to genus and species (70-80% accuracy). However, classification to functional group is more accurate and allows for sufficient assessment of ecological conditions in a waterbody.

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) and Haney et. al (2013).

10.2 Analytical QC Activities

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/ANC, total phosphorus, soluble reactive phosphorus, nitrate-nitrogen, ammonia-nitrogen, total nitrogen, 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 heterogeneity of the lake.

Ten percent of the samples for ANC, total phosphorus, soluble reactive phosphorus, nitrate-nitrogen, ammonia-nitrogen, total nitrogen 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.

Analyses for total phosphorus, soluble phosphorus, nitrate-nitrogen, ammonia-nitrogen and total nitrogen all require preparation of a standard curve. The fit of each standard curve (regression coefficient) is evaluated against the relative standard difference (RSD) the laboratory control sample (LCS). The RSD value of acceptance is <10% variability.

Accuracy

An entire set of field method blanks (all parameters) will be analyzed for 10% of each test's samples to check sampling procedures and bottle preparation. Laboratory method blanks will be analyzed with each sample run to test laboratory procedures. Field method blank results exceeding the sensitivity of the method act as an upper warning limit 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.

LCSs prepared from certified solutions by the QC Officer will be used to test the accuracy of lab and field operations for the following parameters on each sample analysis: soluble reactive phosphorus, total phosphorus, nitrate-nitrogen, ammonia-nitrogen, and total nitrogen. The research assistants will process all LCSs as blind analyses. LCS samples are purchased from outside sources with documented reliability. One LCS sample is evaluated for every ten field samples. The LCS analysis must result in an RSD <10% recovery. If outside this range, the analytical run is rejected.

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

11 Sample Handling and Custody Procedures

Field staff collect all samples and store on ice post sample collection documenting date and time of sample collection. Field staff will document the time and date when samples are delivered to the O'Niell School lab. Lab staff will check in and document all notes from field staff upon return to lab and note the date and time they receive samples. Lab staff will additionally document the date of sample preparation if method indicates specific preparation (digestion of TN/TP). Lab staff will document date of analysis in the sample custody log. The QAQC officer will make note of the date QA was reviewed and samples are acknowledged for disposal. Upon disposal samples will have a date of disposal noted (Appendix E).

12 Testing, Inspection, Maintenance and Calibration

Testing, Inspection, and Maintenance

All equipment used to perform the tests for this study will be inspected according to manufacturer's instructions or more as needed. Each sampling trip includes trip preparation checklists for consumables. Other equipment such as the research boats and trailers receive annual inspections.

Calibration of Field Equipment

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 per manufacture recommendations. Staff will do an annual calibration at the beginning of

season for all water quality sondes. Weekly calibration checks will occur with a quick calibration solution to check working order and will be logged weekly electronically to maintain record. GPS units will be checked annually for updates at the beginning of the season as well and updated per manufacture recommendation. Depth units do not have a calibration verification; however, field staff will take out a handheld sensor to check unit function regularly.

Calibration of Laboratory Analytical Equipment

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: Fisher Scientific Accumet AE150 selective ion meter and an Accumet 13-620-299B pH probe

Calibration: Fisher calibration buffer (10.0 and 4.0) or equivalent prior to each two measurements.

Parameters: pH, ANC

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

Calibration: Sensor is factory calibrated

Parameters: depth of 1% light level

Equipment: Seal Analytical discrete analyzer model AQ400

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, soluble phosphorus

Equipment: Evolution 220 UV-Visible Spec 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: 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

13 Assessment, Oversight, and Data Quality Assessment and Decision Rules

13.1 Data Quality Indicators

Precision

Evaluation of precision, accuracy, control samples, and standard curve correlation coefficients will be kept for each analytical method. If values are suspect or outside of the RSD of <10% samples analysis will be rejected and analyzed again. In the event of field or laboratory control measures that RSD values exceed <10% corrective actions will be assessed to correct any field or laboratory method.

Accuracy

If RSD values of LCS samples is <10% of the true value, the parameter run for which the LCS sample was analyzed is accepted as being accurate. If RSD 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.

13.2 Corrective Action

The Quality Control Officer will be responsible for performing corrective action when a data point is found to be out of control limits. The Quality Control 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 method blanks are taken after each sampling sequence, so if it is determined that the field method blanks are contaminated, 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 within control limits, all analyses done will be repeated. The 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.

The nature of field work offers many opportunities for last minute adjustments and unexpected circumstances. The field staff are prepared for such situations with back up lakes, extra field supplies, and equipment repair kits. Concerns include inaccessible lakes, equipment failure, and not completing the field season due to significant obstacles like a damaged and inoperable boat.

13.3 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.

Field audits include instrument checks and calibration, field method blanks, duplicates, and replicate sampling. Field staff will perform a verification of site location upon arrival at each site. Valid verification tools can include signage at boat launch, lake shape, maps, direction to boat launch, and local contacts or other methods as determined in the field. Lab staff will check all GPS coordinates in google earth or maps prior to data entry to database to ensure accuracy.

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

13.4 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.

14 Data Review, Verification, Validation and Reconciliation

14.1 Data Review and Verification

Review of analysis results are checked after each processing by the Quality Control Officer and/or Laboratory Manager. Particular attention is devoted to reviewing duplicate field measures, laboratory replicate sample analysis, and field and laboratory method 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.

14.2 Validation & Qualifiers

Flags, will be used to identify problems with samples analyzed (Table 5).

Table 5: Flags for Validation of Analytical Results.

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-of-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 1. 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) 2. 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: 3. If the SS RPD is between the established control limits and two times the established control limits then the sample will be estimated. (DJ) 4. 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 laboratory method 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.

14.3 Reconciliation with User Requirements

Data Reduction

Total phosphorus, soluble reactive phosphorus, nitrate-nitrogen, ammonia-nitrogen, and total nitrogen will be reported in milligrams per liter. ANC will be reported in milligrams per liter of calcium carbonate. Chlorophyll a will be reported in micrograms per cubic meter. pH units should be standard units. 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, 22nd edition and in Methodology: U.S. EPA 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 on a scale of 0-14 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 for ease of analysis. Field data sheets and lab analysis files will be maintained in permanent files.

Data Analysis

Results from analyses are used to look at descriptive statistics and trends in lake in Indiana over the sampling period. Descriptors include frequency distributions of water quality parameters including pH, conductivity, ANC, SRP, TP, NO₃-N, NH₃-N, and Org-N, including comparison to lake type and location in the state. Results from the analyses will be used to calculate the Carlson Trophic State Index (TSI) for each lake (Carlson, 1977). TSI scores are calculated for total phosphorus, chlorophyll a, and Secchi depth. TSI will be compared to algal samples for blue-green dominance. The data will be used to identify changes that may have occurred in the lakes since other analyses were carried out. These results may also be incorporated into the Integrated Reports that the State submits to U.S. EPA.

15 Reports, Documentation, Records

15.1 Data Reporting

All final 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, which includes all parameters analyzed. All data will be made available via the INCLP website upon publication.

All raw data and data analysis files will be maintained at the University and available via specific request for additional quality control as needed.

15.2 Data Management

All data analysis results will be maintained in a database, constructed 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.

15.3 Quality Assurance Reports

Quality Assurance (QA) reports will be available to IDEM's Watershed Management Section as requested.

These reports can 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. Since the current IDEM database does not accommodate data flags, this information must be separately documented and submitted.

16 References

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