OWQ- WATERSHED ASSESSMENT & PLANNING BRANCH IDEM/OWQ/WAPB/WM VIRTUAL FILE CABINET INDEX FORM

Program:	Water Monitoring
Document Type:	Report
*Document Date:	3/31/2017
*Security:	Public
*Project Name:	2017 Diel Oxygen Nutrient Pilot Project
*Project Type:	Special Projects
*Report Type:	Work Plan
HUC Code:	No Selection
Site #:	
Route Name:	
Document Control #	B-033-OWQ -WAP-PRB-17-W-R0
Analysis Set #	
County:	No Selection
Cross Reference ID:	
Comments:	Nutrients/Diel Dissolved Oxygen Pilot Study: Sampling Work Plan 2017
Redaction Reference ID:	



Nutrients/Diel Dissolved Oxygen Pilot Study: Sampling Work Plan 2017

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March 31, 2017

B-033-OWQ -WAP-PRB-17-W-R0

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Nutrients/Diel Dissolved Oxygen Pilot Study: Sampling Work Plan 2017

Indiana Department of Environmental Management Office of Water Quality Watershed Assessment and Planning Branch Indianapolis, Indiana B-033-OWQ -WAP-PRB-17-W-R0

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Nutrients/Diel Dissolved Oxygen Pilot Study Work Plan B-033-OWQ -WAP-PRB-17-W-R0 April 28, 2017

WORK PLAN ORGANIZATION

This Sampling and Analysis Work Plan is an extension of the existing Watershed Assessment and Planning Branch, October 2004 "*Quality Assurance Project Plan (QAPP) for Indiana Surface Water Quality Monitoring and Total Maximum Daily Load (TMDL) Program*" and serves as a link to the existing QAPP as well as an independent QAPP of the project. Per the United States Environmental Protection Agency (U.S. EPA) 2006 QAPP guidance (U.S. EPA 2006), this Work Plan establishes criteria and specifications pertaining to a specific water quality monitoring project that are usually described in the following four groups (phases) or sections as QAPP elements:

Section I. Project Management/Planning

- Project Objective
- Project/Task Organization and Schedule
- Background and Project/Task Description
- Data Quality Objectives (DQOs)
- Training and Staffing Requirements

Section II. Measurement/Data Acquisition

- Sampling Procedures
- Analytical Methods
- Sample and Data Acquisition Requirements
- Quality Control (QC) Measures Specific to the Project

Section III. Assessment/Oversight

- External and Internal Checks
- Audits
- Data Quality Assessments (DQAs)
- Quality Assurance/Quality Control (QA/QC) Review Reports

Section IV. Data Validation and Usability

- Data Handling and associated QA/QC activities
- QA/QC Review Reports

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Figure 4. Map of final 28 sites from a randomized list of the 93 candidate sites.	Seven sites from
each of four bins are targeted.	

LIST OF ACRONYMS

AFDM:	Ash Free Dry Mass
AIMS:	Assessment Information Management System
ALUS:	Aquatic Life Use Support
ASTM:	American Society for Testing and Materials
AU:	Assessment Unit
BCG:	Biological Condition Gradient
BOD ₅ :	Biological Oxygen Demand
CAC:	Chronic Aquatic Criterion
CALM:	Consolidated Assessment Listing Methodology
CFR:	Code of Federal Regulations
CFU:	Colony Forming Unit
CHL-a:	Chlorophyll-a
CPR:	Cardio-Pulmonary Resuscitation
CRO:	Community Relations Office
CWA:	Clean Water Act
DELT:	Deformity, Eroded Fin, Lesion, Tumor
DIC:	Differential Interference Contrast (Microscope)
DNR:	Department of Natural Resources
D.O.	Dissolved Oxygen
DQA:	Data Quality Assessment
DQO:	Data Quality Objective
E. coli:	Escherichia coli
EMAP:	Environmental Monitoring Assessment Program (http://www.epa.gov/emap/)
GPP:	Generator Powered Pulsator
GPS:	Global Positioning System
HDPE:	High-density Polyethylene
HUC:	Hydrologic Unit Code
IAC:	Indiana Administrative Code
IBI:	Index of Biotic Integrity
IC:	Indiana Code
IDEM:	Indiana Department of Environmental Management
ISDH:	Indiana State Department of Health
LDB:	Left descending bank
MHAB:	Multi-habitat
MPN:	Most Probable Number
MS/MSD:	Matrix Spike/Matrix Spike Duplicate
N/A: NBI:	Not Applicable Nutrient Biotic Index
NHD:	National Hydrography Database
NPDES:	National Pollutant Discharge Elimination System
NTU:	Nephelometric Turbidity Unit(s)
OEA:	Office of External Affairs
OHEPA:	Ohio Environmental Protection Agency
OWQ:	Office of Water Quality
PFD:	Personal Floatation Device
PPE:	Personal Protective Equipment
QA:	Quality Assurance QC: Quality Control
QAPP:	Quality Assurance Project Plan
QHEI:	Qualitative Habitat Evaluation Index
RPD:	Relative Percent Difference
RDB:	Right descending bank
SM:	Standard Method

SOLAS:	Safety of Life at Sea
SOP:	Standard Operating Procedure
SRP:	Soluble Reactive Phosphorus
S.U.:	Standard Units
TMDL:	Total Maximum Daily Load
U.S. EPA:	United States Environmental Protection Agency
USGS:	Unites States Geological Survey
WAPB:	Watershed Assessment and Planning Branch
WQMS:	Water Quality Monitoring Strategy

DEFINITIONS

Ash Free Dry Mass	A measurement of the organic content weight of a standardized sample material after drying. Drying occurs by baking the sample at a low temperature for a determined period of time and then weighing. After weighing, the sample is combusted at a high temperature to burn off the organic content. The sample is then re- weighed. The difference between the low temperature weight and post high temperature weight is the organic content weight of the sample.
Backwater	A part of the river not reached by the current, where the water is stagnant.
Biological Condition Gradient (BCG)	The Biological Condition Gradient is a conceptual model that describes changes in aquatic communities. It is consistent with ecological theory and has been verified by aquatic biologists throughout the United States.
Biological Oxygen Demand (BOD₅)	The amount of dissolved oxygen needed (i.e., demanded) by aerobic biological organisms to break down organic material present in a given water sample at certain temperature over a specific time period. The BOD value is most commonly expressed in milligrams of oxygen consumed per liter of sample during 5 days of incubation at 20 °C.
Data Logger	A water quality monitoring device for collecting a data point at regular intervals without human intervention. The device is deployed for a pre-determined period of time, allowed to collect data points for the desired parameter, retrieved, and the data off-loaded for management and assessment.
Diel (Diurnal) Dissolved Oxygen Concentration	Of or relating to the maximum swing of dissolved oxygen concentration in water of a stream or lake between the light and dark periods of a 24-hour day.
Elutriate	To purify, separate, or remove lighter or finer particles by washing, decanting, and settling.
Fifteen (15) minute pick	A component of the IDEM multihabitat macroinvertebrate field sampling method used to maximize taxonomic diversity in which the one-minute kick sample and fifty- meter sweep sample collected at a site are first combined

and elutriated. Macroinvertebrates are then manually removed from the resulting sample for 15 minutes.

- Fifty (50) meter sweep
 A component of the IDEM multi-habitat macroinvertebrate sampling method in which approximately 50 meters (50 m) of shoreline habitat in a stream or river is sampled with a standard 500 micrometer (500 µm) mesh width D-frame dipnet by taking 20-25 individual "jab" or "sweep" samples, which are then composited.
 Glide
 Glides are wide habitats with even flow and low to
- Sildes are wide habitats with even now and low to moderated velocities and little or no surface turbulence. Substrates in glide reaches are usually sand, gravel or cobble. Glides often form a transition from a pool to the upper end of a riffle.
- Impoundment A body of water confined within an enclosure, such as a reservoir.
- Lotic A waterbody, such as a stream or river, in which the water is flowing.
- Macroinvertebrate Aquatic animals which lack a backbone, are visible without a microscope, and spend some period of their lives in or around water.
- Marsh A frequently or continually inundated wetland characterized by emergent herbaceous vegetation adapted to saturated soil conditions.
- Nutrient Biotic Index (NBI) A calibration and approach to identifying and assessing biological community response mechanisms to corresponding nutrient concentrations in aquatic systems.
- One (1) minute kick sample A component of the IDEM multi-habitat macroinvertebrate sampling method in which approximately one square meter (1 m²) of riffle or run substrate habitat in a stream or river is sampled with a standard 500 micrometer (500 µm) mesh width D-frame dipnet for approximately one (1) minute.
- Ocular reticle A thin piece of glass marked with a linear or areal scale that is inserted into a microscope ocular, superimposing the scale onto the image viewed through the microscope.
- Perennial A stream that has continuous flow in the stream bed all year during years of normal rainfall. Water must be present in at least 50% of the stream reach during the time of fish community sampling.

Periphyton

Reach

- Algae attached to an aquatic substrate.
 - A segment of a stream used for fish community sampling equal in length to 15 times the average wetted width of the stream, with a minimum length of 50 meters and a maximum length 500 meters.

Seston	Organisms and non-living matter swimming or floating in a water body.
Target	A sampling point which falls on a perennial stream within the basin of interest and the boundaries of Indiana.
Wetland	"Wetland" is a generic term for a habitat characterized by the presence of water, either at the surface or within the root zone (saturated, anoxic soils or standing water for a sustained period of time), undrained, hydric soils, and hydrophytic vegetation. Often described as ecotones or transition zones between uplands and aquatic systems, wetland types vary greatly and, in the United States, are classified or defined by their characteristics.

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I. PROJECT MANAGEMENT/PLANNING

Project Objective

The primary objective of the nutrient/diel dissolved oxygen (D.O.) pilot study is to further trace the steps from nutrients to periphyton (as chlorophyll-a (CHL-a)), from periphyton to D.O., and from D.O. to aquatic macroinvertebrates and fish community responses, with the goal of identifying benchmarks at each step that would help define where a given water body is positioned along a continuum of enrichment. Diel D.O. minimum/maximum and biological oxygen demand (BOD₅), not normally a part of the suite of analyses used by the Office of Water Quality-Watershed Assessment and Planning Branch for biological community assessments, will be evaluated for their potential as secondary response indicators that could help to further refine nutrient thresholds from biological community response.

The use of multiple lines of evidence, including nutrient concentrations, algal biomass, oxygen demand; and diatom, aquatic macroinvertebrate and fish communities' responses, is the most effective procedure for the identification of the nutrient condition of a stream and will provide defensible evidence in support of the methodology for the development of numeric nutrient criteria. Data collected during the nutrient/diel D.O. pilot study will be assessed and used to:

- Support the further development of numeric nutrient criteria for Indiana's water quality standards;
- Further the development of a weighted approach to numeric nutrient criteria development utilizing multiple response variables;
- Develop biological response indicators for use in making water quality assessments on the impacts of excessive nutrient loads in Indiana's rivers and streams;
- Determine the eutrophication status of Indiana's rivers and streams;
- Conduct water quality assessments for eutrophication in Indiana's rivers and streams pursuant to CWA Section 305(b) to support the development of Indiana's *Integrated Report* to U.S. EPA;
- Develop Indiana's CWA Section 303(d) List of Impaired Waters where excessive nutrient inputs are occurring;
- Develop total maximum daily load (TMDL) models (40 CFR, Part 130.7) to address impairments due to excessive nutrients in Indiana's rivers and streams;
- Identify water quality improvements accomplished by watershed restoration efforts to abate excessive nutrient loads to Indiana's rivers and streams'
- Support efforts in abating excessive eutrophication and cyanobacteria growth in Indiana's rivers and streams that pose risks to human, wildlife, domestic pets, and livestock health in Indiana;
- Support the development of national pollutant discharge elimination system (NPDES) permit limits to address excessive nutrient loads from point sources into Indiana's rivers and streams;
- Support U.S. EPA's Strategic Plan to:
 - Protect human health;
 - Protect and restore watersheds and aquatic ecosystems; and
 - Expand applied research.

- Conduct and integrate U.S. EPA nitrogen and co-pollutant research efforts across multiple media and various temporal and spatial scales, including support for developing numeric nutrient criteria, decision-support tools, and cost-effective approaches to nutrient reduction; and
- Promote enhanced compliance through more comprehensive monitoring and surveillance programs.

Project/Task Organization and Schedule

Table 1. 2017 Nutrients/Diel dissolved oxygen Pilot St	udv Tasks. Schedule, and Evaluation.
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Activity	Date(s)	Number of Sites	Frequency of Sampling-related activity	Parameter to be sampled	How evaluated
Site Selection	Sept,, 2016	93 candidate sites	N/A	N/A	Candidate sites previously sampled by IDEM OWQ that met criteria for inclusion to a multivariate cluster analysis on gradients of total phosphorus, total nitrogen, total IBI score, and QHEI > than 53.
Site Reconnaissance	Feb. – Mar., 2017	28 target sites	At least one visit but may require several to obtain final approval	N/A	Land owner approval, stream access, and safety characteristics for first seven sites approved from each of four bins established from multivariate clusters (total 28 sites); "Target" or "Non-target" designations.
			Once each in July, August and Sept with a	CBOD5	Ordinal ranking classification across the spectrum of results for each of the
			minimum 30 days between sampling events		28 sites sampled
				Ortho-phosphate	
				Phosphorous, Total	Once@ >0.3 mg/L (for nutrients)
			Nitrogen (NO3 & NO2)	Once@ >10.0 mg/L (for nutrients)	
				Dissolved O2	<4.0 mg/L; >12 mg/L (for nutrients)
			рН	>9.0 Standard Units (for nutrients)	
				Algal conditions	Excessive (for nutrients, based on a visual inspection)
Water Chamistry	July - Sept.,	20 torget sites		TOC*	
Water Chemistry	2017	28 target sites		DOC*	
				Total Metals *	CAC based on hardness
			Dissolved Metals *(See Table 9)		
			Arsenic (III)*	190 µg/L	
			Nitrogen Ammonia	CAC based on pH and temperature	
			Chloride	CAC based on hardness and sulfate	
				Sulfate	Based on hardness and chloride
				Dissolved Solids	750 mg/L

			Fish Community Macroinvertebrate Community	Fish Community Macroinvertebrate Community	Fish Index of Biotic Integrity (IBI); Nutrient Biotic Index (NBI) Macroinvertebrate IBI
Biological Sampling	Aug., 2017	28 target sites	Qualitative Habitat Evaluation Index (QHEI) Habitat Quality Measurements of canopy cover and density Image: Comparison of Compa		QHEI (evaluated separately for fish and macroinvertebrate communities)
				% Canopy open.	Spherical densiometer
				Angle of canopy to stream	Clinometer - angle from center of stream channel to the top of the riparian
				channel	canopy at three points in the stream reach.
Dissolved oxygen continuous monitoring	Aug., 2017	28 target sites	Deployment of Onset Hobo® U26-001 Dissolved oxygen data logger. Minimum of 7 days at data collection rate of no less than one data point every 15 minutes. (August 2017)	Dissolved oxygen (mg/l) and temperature (degrees Celsius)	The increase in dissolved oxygen from dawn to dusk reflects net primary productivity. Minimum, maximum and average change in dissolved oxygen for the seven day period. Ordinal ranking classification across the spectrum of results for each of the 28 sites sampled
Algal Samples (periphyton)	Aug., 2017	28 target sites	Once - 14 days maximum of other biological community sampling and within 7 days of the retrieval of dissolved oxygen data loggers. (August 2017)	Algal Diatoms Algal Biomass	Diatom identification and enumeration CHL-a and Phaeophytin- <i>a</i> Ash Free Dry Mass (AFDM)

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Background and Project/Task Description

Nutrients are essential constituents to ecosystem functioning in aquatic systems and are essential to the life that inhabits them. Phosphorus and nitrogen are two of the major macronutrients required for energy fixation in plant growth and primary productivity. Excessive amounts of nutrients in aquatic ecosystems can lead to shifts in species composition, reduced D.O. concentrations, fish kills, and toxic algal blooms (Caskey et al. 2012). Although understanding ecological responses to excessive amounts of these nutrients can be complicated, nutrient enrichment from anthropogenic sources has long been recognized to have significant impacts on the health of aquatic ecosystems as land use has been shown to be highly correlative to excessive nutrient loads into aquatic systems (Omernik 1977; Fraterrigo and Downing 2008; Miltner 2010).

The development of nutrient criteria in Indiana and other midwestern Corn Belt Ecoregion states has been difficult as most streams are thought to have reached nutrient saturation (Caskey et al. 2012). Fish and invertebrate communities reflect that saturation by the dominance in streams of organisms that can use algal biomass (i.e., herbivores, omnivores, scrapers) (Caskey et al. 2013). Biological response is a critical component of nutrient criteria development and requires identifying low nutrient sites (Caskey et al. 2012). In a study utilizing 312 Indiana river and stream sites (2005-2009) previously sampled by IDEM across the State, Caskey et al. (2012) assessed nutrients, periphyton CHL-a, aquatic macroinvertebrate and fish community data with the objective of determining which biological community taxa attributes best reflected the conditions of streams in Indiana along a gradient of nutrient concentrations. Although they identified lower and upper threshold nutrient concentrations, they found the biological community response relationship, when measured directly against the nutrient gradient, to be weak. Because nutrient concentrations in Indiana often are so high, response relationships between algal biomass and nutrients can also be weak, and other datasets are needed to develop a meaningful nutrient threshold. Out of 312 sites, guerying for low nutrients and low algal biomass identified a subset of seven nutrient reference sites (IDEM 2015c).

Numerous field studies have demonstrated the links between nutrients and algae, aquatic macroinvertebrates and fish such that a reasonable picture exists of how biological condition changes across a nutrient gradient (Miltner 2010). A dose-response relationship is thought to occur, but it is an indirect path influenced by numerous environmental variables (i.e. land use, light, temperature, flow gradients, in-stream and riparian habitats, and substrate types) that can impact whether a given amount of nutrient enrichment is limiting, sustaining, or detrimental to the aquatic communities (Munn et al. 2010). The impact of eutrophication on higher trophic levels is difficult to quantify because fish and aquatic macroinvertebrate communities are strongly influenced by physical habitat. However, the dose-response relationship can be exploited, because there is a reasonably predictable and consistent response between increasing nutrient concentrations and periphyton (Hillebrand 2002), and between periphyton and D.O. concentrations (Morgan et al. 2006; Huggins and Anderson 2005; Heiskary et al. 2010; Miltner 2010). For example Sabater et al. (2000) observed a 10.0 mg/l difference between daytime and nighttime D.O. at an enriched site where periphytic CHL-a levels exceeded 500 mg/m² and that the short episodes of hypoxia were responsible for fish kills. In a study of large Minnesota rivers the daily ranges in D.O. were positively correlated with total phosphorus and periphytic CHL-a, and in turn, the number of Ephemeroptera, Plecoptera, and Trichoptera taxa were negatively associated with the increasing range of daily D.O. (Heiskary and Markus 2003).

In order to further the development of a weighted approach to nutrient criteria development utilizing multiple response variables, the proposal of this pilot study is to further trace the steps from nutrients utilization to periphyton biomass as CHL-a, from periphyton to D.O., and from D.O. to diatom, macroinvertebrate, and/or fish communities responses, with the goal of identifying benchmarks or change points at each step that would help define where a given water body is positioned along a continuum of enrichment or nutrients utilization. Diel D.O. and BOD₅, not normally a part of the suite of analyses with biological community assessments by the OWQ-WAPB, have been identified as additional secondary response indicators that might help to further refine nutrient thresholds from biological community response.

With that, the objectives of this pilot study are two-fold. The first is to measure whether concentrations of primary nutrients (phosphorus and nitrogen) are positively associated with periphytic CHL-a, and, in turn, increasing daily variation in D.O. concentrations. If those relationships hold, then determine if the increasing expression of nutrient enrichment given by either of these secondary response indicators corresponds to decreasing condition of diatom, macroinvertebrate, and/or fish communities (biological condition indicators). Where clear associations between stressor and response variables are found, the second objective becomes identifying concentrations or levels in the stressors over which the respective response variables change appreciably through further more expanded sampling and modelling in a subsequent study. The change points then form the basis for defensible water quality standards for nutrients in small rivers and streams.

This nutrient/diel D.O. pilot study consists of a single season of sample collection and field observations to assess and characterize overall water quality and biological integrity at the target sites (see Section II on MEASUREMENT/DATA ACQUISITION for random site selection details, QAPP ELEMENT B1). For each target site, the following categories of data will be investigated and utilized for assessment purposes: monthly water chemistry, instantaneous *in situ* water quality parameters collected with each site visit, continuous data acquisition for D.O. diel minimum and maximums, periphyton algal samples (including CHL-a/phaeophytin-a, Ash Free Dry Mass (AFDM), and diatom community enumeration), fish and macroinvertebrate community assemblages, standardized qualitative habitat evaluations, and physical measures of the arboreal canopy cover.

Organizations that help IDEM OWQ with data preparation, collection, and analysis include the United States Geological Survey (USGS), private laboratories under contract with the State of Indiana (e.g., Pace Analytical), the Department of Biological and Environmental Sciences at Georgia College and State University, U.S. EPA Region V, the Indiana Department of Natural Resources (DNR), and the Indiana State Department of Health (ISDH). Landowners and property managers throughout the state also participate by assisting staff to access remote stream locations to collect samples.

The U.S. EPA recommends the use of multiple bio-indicators (i.e. using periphyton, fish and aquatic macroinvertebrate communities and the amount of CHL-a derived from algae), facilitating the "weight-of-evidence" approach to interpretation of biomonitoring results (U.S. EPA, 2004). This approach involves interpreting data from multiple sources to arrive at conclusions about an environmental system or stressors such as nutrients. Multiple lines of evidence utilizing more than one bio-indicator can be valuable in correlating critical levels of nutrients to stream biota. Diatom identification and enumeration will also aid in establishing algal metrics as part of nutrient criteria being developed for Indiana's lotic surface waters.

Data Quality Objectives (DQO)

The DQO process (U.S. EPA 2006) is a planning tool for data collection activities. It provides a basis for balancing decision uncertainty with available resources. The DQO is required for all significant data collection efforts for a project and is a seven-step systematic planning process used to clarify study objectives, define the types of data needed to achieve the objectives, and establish decision criteria for evaluating data quality. The DQO process for the nutrient/diel D.O. pilot study is identified in the following seven steps.

1. State the Problem

Assessments: Indiana is required to assess all waters of the state to determine their designated use attainment status. "Surface waters of the state are designated for full-body contact recreation" and "will be capable of supporting" a "well-balanced, warm water aquatic community"

[327 IAC 2-1-3]. This pilot study will gather biological (algal, fish and macroinvertebrate), chemical, and physical habitat data for the purpose of assessing and tracing the steps from nutrients to periphyton (as CHL-a), from periphyton to D.O., and from D.O. to diatom, aquatic macroinvertebrate and/or fish community responses, with the goal of identifying benchmarks at each step that would help define where a given water body is positioned along a continuum of enrichment.

Nutrient Criteria: The U.S. EPA mandated that states either adopt U.S. EPA's nutrient criteria or develop criteria specific to waters within each state by the year 2004 (U.S. EPA 2000a: 2000b: 2000c). An extension was given to several states (including Indiana) that submitted plans describing data needs, analyses, and protocols that would be used in developing nutrient water guality criteria. Since 2001. IDEM and the USGS have collaborated on several projects which have provided the technical background for developing nutrient criteria for rivers and streams in Indiana. The U.S. EPA has recommended a multiple-lines-of-evidence approach for developing nutrient criteria and has, therefore, approved the implementation of a program that includes the identification and enumeration of diatoms. In order to develop numeric nutrient criteria for rivers and streams in Indiana, IDEM and the USGS have statistically analyzed water chemistry, fish, macroinvertebrate, and CHL-a data from 2005-2009 (Caskey et al. 2013). The addition of taxonomic analysis of periphyton samples to the existing data set will add another line of evidence in this endeavor. However, diel D.O. and BOD_5 , not normally a part of the suite of analyses with biological community assessments by the Office of Water Quality-Watershed Assessment and Planning Branch, have been identified as the additional secondary response indicators that might help to further refine nutrient thresholds from biological community response.

2. Identify the Decision

The primary objective of this project is to gather biological (algal, fish and aquatic macroinvertebrate), chemical, and physical habitat data for the purpose of assessing and tracing the steps from nutrients to periphyton (as CHL-a), from periphyton to D.O., and from D.O. to periphyton, macroinvertebrate and fish communities responses, with the goal of identifying benchmarks or change points at each step that would help define where a given water body is positioned along a continuum of enrichment. Exploratory methodologies and correlative associations of the data will be employed in the assessment for this pilot study to identify those significant change points of enrichment with biological community response factors. Our null hypothesis is that there are no differences in diel D.O. daily ranges, algal biomass as measured by CHL-a and AFDM, and biological community structure response across the gradient of nutrient concentrations or nutrient utilization in streams between each of four bins or clusters (candidate sites previously sampled by IDEM OWQ that met criteria for inclusion to a multivariate cluster analysis on gradients of habitat quality from good to excellent, total phosphorus, total nitrogen, and total IBI score.

Biological Criteria:

Indiana narrative biological criteria [327 IAC 2-1-3; 327 IAC 2-1.5-2] states that "all waters, except as described in subdivision (5)," (i.e., limited use waters) "will be capable of supporting" a "well-balanced, warm water aquatic community". The water quality standard definition of a "well-balanced aquatic community" is "an aquatic community that: (A) is diverse in species composition; (B) contains several different trophic levels; and (C) is not composed mainly of pollution tolerant species" [327 IAC 2-1-9; 327 IAC 2-1.5-5].

To assist in the development of numeric nutrient criteria fish, aquatic macroinvertebrates, and periphyton based diatoms will be collected in conjunction with chemical, CHL-a, phaeophytin-a, and AFDM data from each site, along with field parameters and physical and habitat site descriptions. Fish identifications and enumerations will occur in the field. Any unknown fish will be photographed or an example preserved for further identification in the IDEM laboratory. Once collected, diatom and macroinvertebrate samples will be preserved and transported to the IDEM laboratory where each will be identified and enumerated.

3. Identify the Input to the Decision

Under this pilot study, field monitoring activities are required to collect physical, chemical, biological (algal as periphyton diatoms, fish and macroinvertebrates), and habitat data that will be used to address the necessary assessments and decisions previously described. Monitoring activities will take place at target sites for which permission to access has been granted by the landowners or property managers, or at bridge easements as appropriate. Due to the statistical nature of the survey design, historical data may also be used in the calculation of nutrient response benchmarks as well as aquatic life use support (ALUS) classifications supporting or non-supporting aquatic life uses. Collection procedures for field measurements, algal, chemical, biological, and habitat data will be described in detail under Section II. MEASUREMENT/DATA ACQUISITION.

4. Define the Boundaries for the Study

For the purpose of this pilot study, the Corn Belt Plains Ecoregion (Ecoregion Level III) (Figure 1), which includes Indiana portions of both the Eastern and Central Corn Belt Plains ecoregions, is geographically defined as within the borders of Indiana contained in all or portions of 32 8-digit Hydrologic Unit Codes (HUCs) (Table 2).

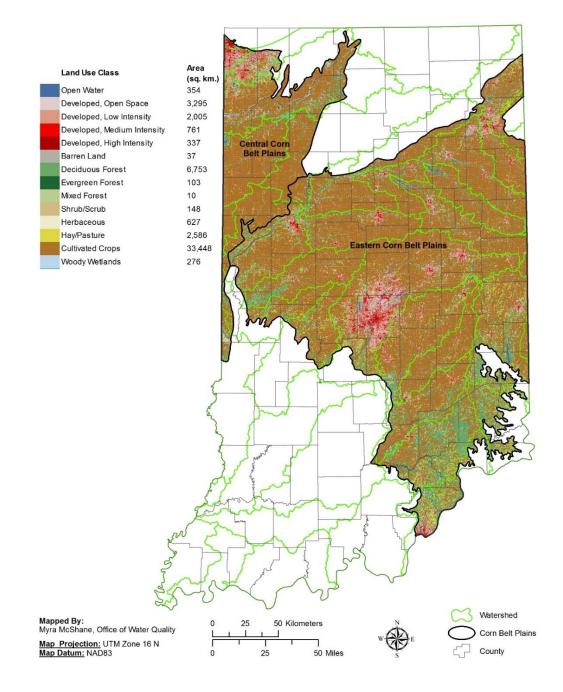


Figure 1. Inclusive 8-digit USGS HUCs of the Eastern and Central Corn Belt Plains ecoregions in Indiana (Homer et al. 2015)

Table 2. All or part of USGS Hydrologic Unit Codes (HUCs) contained within the Eastern and Central Corn Belt Plains ecoregions within Indiana.

HUC_8	HUC_8_NAME
04040001	Little Calumet-Galien
05120203	Eel (WFWR)
05120208	Lower East Fork White
04100007	Auglaize
05120101	Upper Wabash
05120103	Mississinewa
05120206	Upper East Fork White
05120207	Muscatatuck
04050001	St Joseph (MI)
05120104	Eel (WR)
05120110	Sugar
05120111	Middle Wabash-Busseron
05120204	Driftwood
04100004	St Marys
04100005	Maumee
05140101	Silver-Little Kentucky
05080002	Lower Great Miami
05120107	Wildcat
05120201	Upper White
07120002	Iroquois
07120003	Chicago
04100003	St Joseph (OH)
05080001	Upper Great Miami
05120108	Middle Wabash-Little Vermilion
05120205	Flatrock-Haw
07120001	Kankakee
05080003	Whitewater
05090203	Middle Ohio-Laughery
05120102	Salamonie
05120105	Middle Wabash-Deer
05120106	Tippecanoe
05120109	Vermilion

The Corn Belt Plains Ecoregion in Indiana (Level III: Ecoregions 54 and 55 combined)

The total land area for the combined Corn Belt Plains ecoregions in Indiana is 50,821 square kilometers (km²) (19,622 square miles (miles²)). Of this, the top five land uses include: 1) cultivated land - 66%; 2) deciduous forest - 13%; 3) developed open space - 6.5%; 4) low, medium and high density development combined - 6.1%; and hay/pasture -5.1%. These five land uses make up 97% of the land uses in the combined ecoregions in Indiana (Figure 1, Homer et al. 2015).

Central Corn Belt Plains (Ecoregion Level IV 54)

The Central Corn Belt Plains Ecoregion consisted of extensive prairie communities intermixed with oak hickory forests that were native to the glaciated plains of the Central Corn Belt Plains; they were a stark contrast to the hardwood forests that grew on the drift plains of ecoregions to the east. Ecoregions to the west were mostly treeless except along larger streams. Beginning in the nineteenth century, the natural vegetation was gradually replaced by agriculture. Farms are now extensive on the dark, fertile soils of the Central Corn Belt Plains and mainly produce corn and soybeans; cattle, sheep, poultry, and especially hogs are also raised, but they are not as dominant as in the drier Western Corn Belt Plains to the west. Agriculture has affected stream chemistry, turbidity, and habitat (Omernik 1987; USEPA 2000a).

Eastern Corn Belt Plains (Ecoregion Level IV 55)

The Eastern Corn Belt Plains is primarily a rolling plain with local end moraines; it had more natural tree cover and has lighter colored soils than the Central Corn Belt Plains. The region has loamier and better drained soils than the Huron/Erie Lake Plain, and richer soils than the Erie/Ontario Hills and Lake Plain. Glacial deposits of Wisconsin age are extensive. They are not as dissected nor as leached as the pre-Wisconsin till which is restricted to the southern part of the region. Originally, beech forests were common on Wisconsin soils while beech forests and elm-ash swamp forests dominated the wetter pre-Wisconsin soils. Today, extensive corn, soybean, and livestock production occurs and has affected stream chemistry and turbidity (Omernik 1987; USEPA 2000a).

Target Population of Streams

In March 2015, IDEM worked with U.S. EPA and Tetra Tech to develop a framework and criteria for reference site selection (IDEM 2015c). IDEM provided Tetra Tech with a list of 1458 sites that were previously sampled between 2003 and 2013 for possible reference site selection. These Indiana stream sites were utilized for the iterative process to develop an initial list of 93 potential sites based on the site controls listed below.

The target sample population for the basin is defined as all perennial streams within the boundaries of the Corn Belt Plains Ecoregion (Ecoregion Level III) within the geographic boundaries of Indiana. The sample frame for selection of 28 target sites is comprised of all wadeable rivers, streams, canals, and ditches as indexed through the NHD-Plus dataset (U.S. EPA and USGS 2005) of drainage area between 52 and 2590 km² (20 and 1000 miles²), having been previously sampled by IDEM between 2003- 2013, with an in-stream gradient between the 25th and 75th percentile for all Indiana wadeable streams for which we have gradient data in AIMS (≥2.38 to ≤7.25 ft./mile), a historical fish community assessment (IBI), a QHEI score during the fish community assessment of greater than or equal to 53, and with water quality results for total nitrogen and total phosphorus. Marshes, wetlands, backwaters, impoundments, dry sites, and streams with no apparent channel (i.e. submerged or run underground either through natural processes or by anthropogenic channel alterations) are excluded as they are considered non-target populations.

Site selection for the nutrient/diel D.O. pilot study started with the original 1458 sites and then eliminated sites based on the above controls. A principal component multivariate cluster analysis using Statistica 12[®] software (Statsoft Corporation) was performed to group sites by common

characters (Figure 2). Candidate sites were clustered across the gradient of the total QHEI metric, to the Tetra Tech (2015) variates of the phosphorus metric, total nitrogen metric, and fish community IBI metric rankings. Four distinct clusters or bins of sites were identified across the habitat quality gradient (sites clustered between bins 2 and 3 were omitted from the selection process). Bin 1 contains sites with high quality habitat, great fish community structure, and have excess nutrients (e.g., nitrogen and phosphorous) passing through the watershed without being absorbed. Bin 2 consists of sites with the best quality habitat, great fish communities structure, and, similar to Bin 1, have nutrients passing through without absorption occurring. Sites within Bin 3 contain good quality habitat, good fish community structure, and are seeing nutrients being absorbed into the watershed. Bin 4 is comprised of sites which have the lowest quality habitat and fish community structure of the four bins, and, similarly to Bin 3, has nutrient absorption occurring.

A randomized draw was performed to rank all of the sites in each of the four identified bins to a priority list for sampling (Table 3). From this list the first seven sites listed for each bin was selected as a priority site for sampling. If after site reconnaissance a site was no longer considered "target," then the next site down on the list for that bin group was selected as a replacement. This process was to assure that the study would sample seven sites from each of the four bins (total 28 sites for the pilot project). Thirty of the 93 potential sites were also identified as candidate reference sites (Table 3) (Tetra Tech 2015).

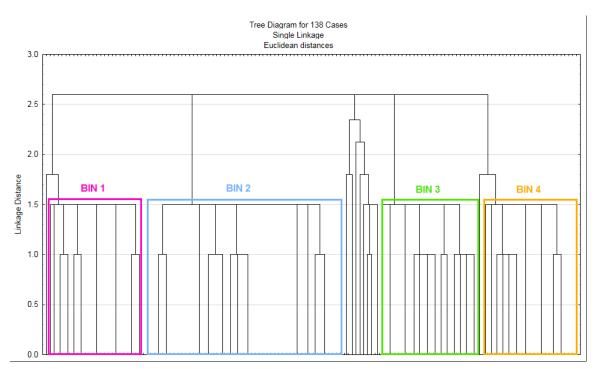


Figure 2. Multivariate cluster classification of candidate Pilot Study sites (n = 93) into four distinct bins across gradients of QHEI total nitrogen, total phosphorus, and fish community IBI metric rankings.

Table 3. Prioritized list of 93 potential candidate sites for the Nutrients/Diel Dissolved Oxygen Pilot Study in the Corn Belt Plains Ecoregion.

<u>Site</u> number	Ref. site	<u>Order</u>	Random Number	BIN	L-Site Code	WATERBODY	COUNTY NAME	LOCATION	LATITUDE MEASURE	LONGITUDE MEASURE	UTM EAST	UTM NORTH	UTM ZONE	HUC14
1	Х	1	10	1	WEF-06-0002	Flatrock River	Bartholomew	CR 900 N	39.33192521	-85.86121339	598148.4312	4354229.979	16N	'05120205050150'
2		1	25	2	WAE060-0012		Miami	CR 190 W	40.8256862	-86.11336948	574763.5281	4519785.061	16N	'05120104060040'
3	X (sub ref.)	1	73	3		Little Salamonie River	Jay	Boundary Pike	40.406944	-84.96137	672985.3421	4474920.927	16N	'05120102010040'
4		1	95	4	WWU100-0078		Madison	Brown St	40.01593861	-85.69206833			16N	'05120201100060'
5		2	16	1	WSU040-0020	0	Montgomery	CR 275 E	40.08778676	-86.85247732	512576.3531	4437511.185	16N	'05120110040010'
6		2	28	2		South Fork Wildcat Creek	Tippecanoe	SR 26	40.41806385	-86.76806189	519676.91	4474185.72	16N	'05120107040130'
7		2	65	3	WED-07-0001		Johnson	River Rd	39.36953801	-85.99488726	586580.8145		16N	'05120204090080'
8		2	96	4	WWU100-0098		Henry	CR 700 N	40.03376611	-85.54764056	623914.1986		16N	'05120201100020'
9		3	7	1		South Fork Wildcat Creek	Clinton	CR 600 W	40.32092985	-86.61818972	532438.1612		16N	'05120107040040'
10	X	3	29	2		South Fork Wildcat Creek	Clinton	W Mulberry-Jefferson Rd	40.329145	-86.647122	529976.4684		16N	'05120107040110'
11	Х	3	67	3		Graham Creek	Jennings	CR 500 S	38.90637908	-85.60614372	620857.8986		16N	'05120207020070'
12		3	85	4	LMG-05-0015		Lake	Clay Street	41.44704867	-87.27761876	476810.298	4588422.82	16N	'04040001030040'
13		4	11	1		Graham Creek	Jennings	CR 230S	38.95664167	-85.49456667	630440.8124		16N	'05120207020030'
14	Х	4	33	2	WDE050-0025		Carroll	Cemetery Rd	40.600487	-86.547286	538302.9685		16N	'05120105050080'
15		4	62	3		Brandywine Creek	Shelby	W 650 N	39.61836716	-85.80054133	602954.5497		16N	'05120204040060'
16		4	91	4	WEU010-0040		Bartholomew	CR 50 N	39.2093675	-85.86791939	597740.5817	4340621.19	16N	'05120206010160'
17		5	20	1		White Lick Creek	Morgan	Carol Ln	39.59170304	-86.36779726	554284.8779		16N	'05120201150130'
18	Х	5	35	2	WDE050-0031		Cass	CR 1100 S	40.60804003	-86.37034006	553267.9937	4495437.78	16N	'05120105050030'
19		5	78	3	WWU100-0040		Madison	C.R. 650W	39.97086972	-85.796175	602803.1303		16N	'05120201100090'
20		5	92	4		Big Raccoon Creek	Montgomery	CR 750 S	39.93023155	-86.76686619	519920.4799		16N	'05120108160020'
21		6	19	1	WWU100-0106		Henry	Mechanicsburg Rd	40.02506028	-85.55712583	623120.5469		16N	'05120201100020'
22	Х	6	37	2		Vernon Fork Muscatatuck River	Jennings	CR 25 W	38.97467929	-85.6141175	620051.2278		16N	'05120207070010'
23		6	61	3		Carpenter Creek	Jasper	CR 1300 S	40.82431705	-87.17677482	485093.5584		16N	'07120002030070'
24		6	97	4	WWU-11-0005	Eagle Creek	Marion	Belmont Ave	39.72427286	-86.19600909	568904.0751	4397463.903	16N	'05120201120140'
25	Х	7	9	1	WEF-06-0001		Bartholomew	CR 400 N	39.260094	-85.92208196	592997.2301	4346193.773	16N	'05120205050190'
26		7	53	2	WWU100-0064	Fall Creek	Madison	CR 750 W	39.96432222	-85.81504806	601201.0415		16N	'05120201100090'
27		7	71	3		Big Raccoon Creek	Boone	@ CR 500S	39.96679722	-86.68392222	526993.3915		16N	'05120108160010'
28	Х	7	89	4	WED-04-0001		Hancock	E CR 1000 N	39.93160872	-85.69904306	611161.705	4420976.626	16N	'05120204060020'
29		8	13	1	WEU010-0039	Clifty Creek	Decatur	CR 420 W	39.38764378	-85.55871433	624122.1776	4360786.03	16N	'05120206010050'
30		8	27	2	WAW040-0018	South Fork Wildcat Creek	Clinton	CR 200 N	40.31501966	-86.54371579	538768.7808	4462822.145	16N	'05120107040040'
31		8	70	3	WLV040-0032	Big Pine Creek	Benton	@ CR 150 S	40.58414722	-87.16546667	485996.9018	4492608.278	16N	'05120108040050'
32		8	86	4	OML060-0019	Laughery Creek	Ripley	N CR 75 E	39.16019008	-85.2524487	650986.5188	4336007.599	16N	'05090203060110'
33		9	21	1	WWU150-0044	East Fork White Lick Creek	Morgan	Windsong Trail	39.59888422	-86.36656327	554385.2211	4383430.458	16N	'05120201150160'
34		9	30	2	WAW040-0119	Kilmore Creek	Clinton	@ CR 180 East	40.34535556	-86.47304444	544753.4467	4466222.685	16N	'05120107040080'
36		9	93	4	WWU010-0039	West Fork White River	Randolph	CR 200 E	40.187928	-84.93141703	676095.0902	4450667.499	16N	'05120201010020'
37		10	5	1	WAW040-0005	South Fork Wildcat Creek	Clinton	CR 130 E	40.31888889	-86.4825	543967.5723	4463280.217	16N	'05120107040020'
38		10	32	2	WAW050-0010	Wildcat Creek	Tippecanoe	SR 25	40.4527	-86.851684	512576.2112	4478015.017	16N	'05120107050010'
39		10	60	3	UMI020-0018	Ryan Ditch	Jasper	CR 400 S	40.957268	-87.058672	495062.4094	4534015.074	16N	'07120002020050'
40		10	84	4	LMG050-0080	Salt Creek	Porter	CR 600 N	41.52118333	-87.12875833	489256.9744	4596623.979	16N	'04040001050030'
42		11	40	2	WMI050-0017	Deer Creek	Grant	CR 650 S	40.460785	-85.693697	610754.0523	4479721.268	16N	'05120103050070'
43		11	76	3	WVE080-0001	Jordan Creek	Warren	CR 800 W	40.380053	-87.48575	458767.2324	4470054.004	16N	'05120109080020'
44		11	81	4	LMG030-0022	Deep River	Lake	Randolph St	41.463922	-87.233299	480517.4096	4590285.145	16N	'04040001030050'
45		12	6	1	WAW040-0006	South Fork Wildcat Creek	Clinton	CR 00 Rd	40.31527778	-86.50611111	541963.7642	4462867.938	16N	'05120107040020'
46		12	43	2	WSU010-0031	Sugar Creek	Clinton	CR 580 S	40.20265833	-86.37410833	553267.8278	4450438.508	16N	'05120110010030'
47		12	74	3	WTI110-0003	Big Monon Creek	White	N Lowes Rd	40.876389	-86.830992	514240.3562	4525048.956	16N	'05120106110130'

Table 3. Continued.

<u>Site</u> number	<u>Ref. site</u>	<u>Order</u>	Random Number	<u>BIN</u>	L-Site Code	WATERBODY	COUNTY NAME	LOCATION	LATITUDE MEASURE	LONGITUDE MEASURE	UTM EAST	UTM NORTH	UTM ZONE	HUC14
48	Х	12	90	4	WED-07-0003	Sugar Creek	Johnson	CR 700 E	39.45254055	-85.96772609	588815.1588	4367505.873	16N	'05120204080100'
49		13	17	1	WWU-01-0006	White River	Delaware	Sciscoe Rd	40.15774577	-85.3317396	642077.4043	4446599.801	16N	'05120201010120'
50		13	44	2	WSU020-0015	Sugar Creek	Montgomery	CR 1100 E	40.14340826	-86.70051246	525510.6268	4443717.31	16N	'05120110020060'
51		13	58	3	LEJ090-0031	Cedar Creek	Allen	St. Rd. 327	41.258176	-85.131049	656570.3812	4569101.994	16N	'04100003090030'
52		13	83	4	LMG050-0053	Salt Creek	Porter	I-80/90	41.58365	-87.13743611	488543.9731	4603560.157	16N	'04040001050050'
53	Х	14	12	1	WEM070-0032	Vernon Fork Muscatatuck River	Jennings	CR 150 W	38.95198889	-85.63975	617868.3388	4312328.448	16N	'05120207070010'
54	Х	14	46	2	WSU050-0033	Sugar Creek	Parke	CR 750 E	39.94124236	-87.11441882	490224.8769	4421241.99	16N	'05120110050090'
55		14	75	3	WUW080-0008	Rock Creek	Huntington	CR 500 E	40.794148	-85.350157	639187.9718	4517215.419	16N	'05120101080060'
56		14	82	4	LMG050-0009	Salt Creek	Porter	W 500 N	41.50722222	-87.13736667	488536.27	4595075.13	16N	'04040001050030'
57	X (sub ref.)	15	14	1	WLV160-0020	Big Raccoon Creek	Montgomery	@ CR 775	39.94097222	-86.75701111	520759.3182	4421234.002	16N	'05120108160020'
58	X	15	26	2	WAW030-0035	Middle Fork Wildcat Creek	Tippecanoe	CR 1025 E	40.406547	-86.710816	524537.6622	4472921.682	16N	'05120107030070'
59	Х	15	63	3	WED030-0028	Little Blue River	Shelby	German RD	39.53976495	-85.72693629	609396.0903	4377451.432	16N	'05120204030060'
60		15	94	4	WWU-09-0002	Fall Creek	Marion	10th St	39.78024	-86.18719427	569603.1838	4403682.37	16N	'05120201110060'
61	Х	16	8	1	WBU020-0023	Brouilletts Creek	Vermillion	SR 163	39.67111	-87.507203	456498.2032	4391377.599	16N	'05120111020020'
63	Х	16	69	3	WEU030-0047	Sand Creek	Jennings	N CR 1000 W	39.06900567	-85.79524371	604222.1494	4325124.909	16N	'05120206030190'
64		16	88	4	WAW040-0081	Kilmore Creek	Clinton	CR 500 N	40.359707	-86.354598	554801.3478	4467882.299	16N	'05120107040070'
65		17	15	1	WSU030-0007		Montgomery	575 E, S of 800 N	40.147205	-86.796641	517321.337	4444115.556	16N	'05120110030050'
66		16	45	2	WSU050-0030		Montgomery	1 mile D/S SR 43	40.049292	-86.905807	508034.5025		16N	'05120110050010'
67		17	77	3	WWU-05-0001		Hamilton	281st St	40.20015046	-85.87730341	595553.6895	4450576.648	16N	'05120201060040'
68		17	87	4	UMK140-0010		Lake	Chestnut Rd	41.22313	-87.501094	457999.5974	4563648.119	16N	'07120001140030'
69		18	18	1	WWU100-0100		Henry	8th St	40.04986028	-85.53712972	624781.659	4434316.357	16N	'05120201100020'
70		17	47	2	WUW170-0015		Miami	SR 218	40.649116	-85.99316	585124.9197	4500294.044	16N	'05120101170090'
71		18	64	3	WED050-0016	•	Shelby	CR 550	39.443892	-85.88859677	595635.3078		16N	'05120204050030'
72		18	80	4	LEJ050-0068	•	Dekalb	CR 18	41.46349867	-84.81128336	682784.45	4592524.164	16N	'04100003050060'
73	Х	19	2	1	LEJ090-0041		Dekalb	CR 9	41.28242036	-85.14240761	655546.218	4571777.248	16N	'04100003090060'
74		18	31	2		South Fork Wildcat Creek	Tippecanoe	@ CR 200 South	40.38853889	-86.76279444	520132.578	4470909.685	16N	'05120107040130'
75		19	59	3	LMG050-0092		Porter	CR 250 W	41.47826111	-87.11411944	490472.0897	4591857.073	16N	'04040001050020'
76	Х	20	1	1		East Fork Whitewater River	Wayne	Lick Creek Farms Rd	39.78864104	-84.92720325	677484.7007		16N	'05080003070060'
77	X	19	41	2		Mississinewa River	Grant	CR 500 E	40.4559775	-85.57776278	620592.3673		16N	'05120103050040'
78	X	20	68	3		Vernon Fork Muscatatuck River	Jennings	CR 400	38.95429083	-85.68486108	613955.5396		16N	'05120207070020'
80	~~~~	20	50	2	WWU-03-0002		Madison	2nd St	40.11573238	-85.70198457	610612.5535		16N	'05120201040080'
81		21	66	3	WEF020-0015		Rush	CR 415	39.55042095	-85.4944404	629355.7994	4378942.607	16N	'05120205020060'
82	Х	21	54	2	WWU100-0075		Madison	CR 1000 S	39.95983056	-85.72611778	608803.4828	4424075.71	16N	'05120201100100'
83		22	72	3		Big Raccoon Creek	Montgomery	CR 1000 S	39.89448888	-86.83678455	513953.4448		16N	'05120108160030'
84	х	22	36	2	WEM020-0042		Ripley	K Road and NE Exit Road	39.00787	-85.3831	639998.5967	4318893.473	16N	'05120207020030'
85	X	23	79	3	WWU100-0074		Madison	CR 400 W	39.95259639	-85.74909333	606852.323	4423244.986	16N	'05120201100110'
86	X	23	39	2		Mississinewa River	Delaware	CR 400 E	40.30628389	-85.30351194	644166.1165		16N	'05120103030020'
87		24	55	2	WWU100-0080		Madison	Falls Park	40.00842	-85.74018	607526.1685		16N	'05120201100060
88		25	51	2	WWU-07-0001		Hamilton	Strawtown Ave	40.13207388	-85.95249793	589242.8088		16N	'05120201070010'
89	X (sub ref.)	26	38	2		Big Raccoon Creek	Putnam	US 231	39.85445546	-86.88695046	509670.332	4411609.517	16N	'05120108160060'
90	X	27	22	2		Whitewater River	Fayette	CR 450 N	39.70765084	-85.11791036	661343.0251	4397003.447	16N	'05080003020070'
91	~~~~	28	52	2	WWU-09-0003		Marion	Fall Cr Pkwy N Dr	39.86382643	-86.05170975	581107.0012		16N	'05120201110050'
92	х	29	23	2		East Fork Whitewater River	Union	Turkey Creek Rd	39.63502677	-84.9987092	671742.1692		16N	'05080003070110'
93	X	30	56	2	WWU100-0083		Madison	CR 200 E	40.01584861	-85.63388722	616585.9493	4430410.048	16N	'05120201100050
94	~	31	42	2		Salamonie River	Huntington	CR 400 W	40.76064472	-85.51411278	625418.4697	4513248.83	16N	05120102040020
95	х	32	24	2	UMK060-0042		Marshall	13 B West	41.27626342	-86.4498675	546073.355	4569571.58	16N	'07120001060060'
96	~	33	49	2	WWU-03-0001		Delaware	Lincolnshire Dr	40.18141219	-85.4884506	628685.8423	4448987.868	16N	'05120201020060'
30	Х	34	49	2		Big Walnut Creek	Putnam	480 E	39.72372837	-86.76765989	519912.1861	4397120.3	16N	'05120203020030'

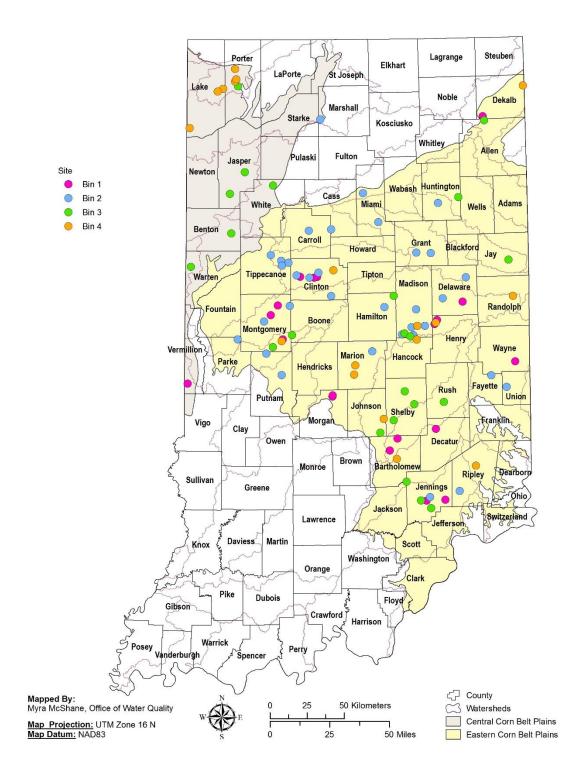


Figure 3. Map of 93 candidate sites for the nutrients/diel dissolved oxygen pilot study color coded by associated cluster bin.

Develop a Decision Rule

Samples will be collected for physical and chemical parameters, algal biomass and biological communities if the flow is not dangerous for staff to enter the stream (e.g., water levels at or below median base flow), and barring any hazardous weather conditions (e.g., thunderstorms or heavy rain in the vicinity) or unexpected physical barriers to accessing the site. The field crew chief makes the final determination as to whether or not a stream is safe to enter. Sample collections for algal and biological communities may be postponed at a particular site for one to four weeks due to scouring of the stream substrate or instream cover following a high water event resulting in non-representative samples.

The primary assessment objective is to determine if there is a statistically defensible relationship between nutrient concentrations, primary productivity, and the resulting diel D.O. patterns, and biological community responses including changes in community composition and diversity. By dividing target sites into bins of commonality based on multivariate associations, statistical comparisons can be made between results from each of the respective bins. Each bin represents a distinct set of sites with common variate features that, across the gradient of conditions, is hypothesized to show statistical differences. If associations can be identified, this will be an impetus for expanding the approach for eventual nutrient criteria development.

Secondarily, this pilot study may provide for, for ALUS assessments in the Indiana Integrated Water Monitoring and Assessment Report by including independent evaluations of chemical, and biological criteria as outlined in Indiana's 2016 CALM (IDEM 2016b; IDEM 2016c, 36 - 39). The fish assemblage will be evaluated at each site using the appropriate IBI (Simon 1991; Simon and Dufour 1998), and BCG (Stamp et al. 2016) classifications. Macroinvertebrate multi-habitat samples will also be evaluated using an IBI in the process of development at this time for lowest practical taxonomic level identifications and BCG.

Given that ecological tolerances for many diatom species are known, changes in diatom community composition can be used to diagnose the environmental stressors affecting ecological health (Stevenson 1998; Stevenson and Pan 1999); thus, periphyton IBI metrics have been developed and tested in many regions (Kentucky Department of Environmental Protection 1993; Hill 1997). The periphyton assemblage may be used to assess biological integrity of a waterbody without any other information; however, periphyton are most effective when used with habitat and macroinvertebrate assessments, particularly because of the close relationship between periphyton and these elements of stream ecosystems (Barbour et al. 1999). For this reason, algal sampling will be conducted at the same sites where macroinvertebrates, fish, habitat, chemical, and physical data will be collected.

5. Specify Tolerable Limits on Decision Errors

Good quality data are essential for minimizing decision error. By identifying errors in the sampling design, measurement, and laboratory for physical, chemical, and biological parameters, more confidence can be placed in the development of nutrient criteria. For this pilot study, our null hypothesis is that there is no significant correlation or response of diel oxygen ranges to increasing nutrient concentrations and thereby no significant measurable biological community responses to diel oxygen extremes.

Actual Status of Sampled Stream Reaches						
Work Plan Findings	Diel oxygen correlative response to nutrient concentrations is significant	No correlative diel oxygen response to increasing nutrient concentrations.				
Diel oxygen correlative response to nutrient concentrations is significant	Stream reach data set is correctly identified as rejecting the null hypothesis	Decision Error (Type 1)				
No correlative diel oxygen response to increasing nutrient concentrations.	Decision Error (Type 2)	Stream reach data set is correctly identified as <u>NOT</u> rejecting the null hypothesis.				

A minimum of 28 target sites will be sampled within the Corn Belt Plains Ecoregion to assess the utility of diel D.O. measures as a linking correlative to biological community response and increasing nutrient loads. Site specific aquatic life use assessments include program specific controls to identify the introduction of errors. These controls include water chemistry blanks and duplicates, biological site revisits or duplicates, and laboratory controls through verification of species identifications as described in field procedure manuals (IDEM 2002) and standard operating procedures (IDEM 1992a, 1992b, 1992c, 1992d, 1992e, 2010a, 2015a, 2016d).

The Quality Assurance (QA)/Quality Control (QC) process detects deficiencies in the data collection as set forth in the IDEM QAPP for the Indiana Surface Water Quality Monitoring Program (IDEM 2004). The QAPP requires all contract laboratories to adhere to rigorous standards during sample analyses and to provide good quality usable data. Chemists within the WAPB review the laboratory analytical results for quality assurance. Any data which is "Rejected" due to analytical problems or errors will not be used for water quality assessment decisions. Any data flagged as "Estimated" may be used on a case-by-case basis and is noted in the QA/QC report. Criteria for acceptance or rejection of results as well as application of data quality flags is presented in the QAPP, Table D3-1: Data Qualifiers and Flags, pages 130-131. Precision and accuracy goals with acceptance limits for applicable analytical methods are provided in the QAPP, Table A7-1: Precision and Accuracy Goals for Data Acceptability by Matrix, pages 45-47 and Table B2-2: Field Parameters, page 81. Further investigation will be conducted in response to consistent "rejected" data in determining the source of error. Field techniques used during sample collection and preparation, along with laboratory procedures will be subject to evaluation by both the WAPB QA Manager and Project Manager in troubleshooting error introduced throughout the entire data collection process. Corrective actions will be implemented once the source of error is determined.

6. Optimize the Design for Obtaining Data

The site selection design facilitates sampling across clusters of conditions for the detection of biological response points to the measures of interest.

Periphyton assemblages are impacted by habitat and aquatic macroinvertebrate community structure; thus, to develop nutrient criteria, periphyton algal samples will also be collected from the same sites.

Training and Staffing Requirements

Role	Required Training/Experience	Responsibilities	Training References		
Project Manager	-Bachelor of Science Degree in biology or other closely related area plus four years of experience in aquatic ecosystems (Master's Degree with two years aquatic ecosystems experience may substitute) - Database experience - Experience in project management and QA/QC procedures	-Establish Project in the Assessment Information Management System (AIMS) II database -Oversee development of Project Work Plan -Oversee entry and QC of field data -Querying data from AIMS II to determine results not meeting Water Quality Criteria -Calculating predicted percentage of perennial stream miles non- supporting for aquatic life uses and recreational uses in the river basin of interest	-AIMS II Database User Guide -U.S. EPA 2006 Quality Assurance (QA) Documents on developing Work Plans(QAPPs)		
Field Crew Chief- Fish or Aquatic Macroinvertebrate Community Sampling	-Bachelor of Science Degree in biology or other closely related area - At least one year of experience in sampling methodology and taxonomy of aquatic communities in the region -Annually review the Principles and Techniques of Electrofishing -Annually review relevant safety procedures -Annually review relevant SOP documents for field operations	-Completion of field data sheets -Taxonomic accuracy -Sampling efficiency and representation -Voucher specimen tracking -Overall operation of the field crew when remote from central office -Adherence to safety and field SOP procedures by crew members -Ensure that multi-probe analyzers are calibrated weekly prior to field sampling activities -Ensure that field sampling	-Barbour et al. 1999 -Hydrolab Corporation 2002 -IDEM 1992a, 1992b, 1992c, 1992d, 1992e,2002, 2010a, 2010b, 2010c, 2015b, 2016d -Klemm et al. 1990 -Plafkin et al. 1989 -Simon 1991 -Simon and Duferent		
Field Crew Chief- Fish or Aquatic Macroinvertebrate Community Sampling (Continued)		equipment is functioning properly and loaded into field vehicles prior to field sampling activities	Dufour 1998, 2005 -U.S. EPA 1995 -YSI 2002		
Field Crew members- Fish or Aquatic Macroinvertebrate Community Sampling	-Complete hands-on training for sampling methodology prior to participation in field sampling activities -Review the Principles and Techniques of Electrofishing -Review relevant safety	-Follow all safety and SOP procedures while engaged in field sampling activities -Follow direction of Field Crew Chief while engaged in field sampling activities	-Barbour et al. 1999 -Hydrolab Corporation 2002 -IDEM 1992a, 1992b, 1992c, 1992d, 1992e, 2002, 2010a,		

Table 5. Project Roles, Experience, and Training.

	B-033-OWQ -WAP-PRB-17-W-R0 April 28, 2017							
Role	Required Training/Experience	Responsibilities	Training References					
	procedures -Review relevant SOP documents for field operations		2010b, 2010c, 2015b, 2016d -Klemm et al. 1990 -Plafkin et al. 1989 -U.S. EPA 1995 -YSI 2002					
Field Crew Chief - Water Chemistry, Algal and/or Bacteriological Sampling	-Bachelor of Science Degree in biology or other closely related area -At least one year of experience in sampling methodology -Annually review relevant safety procedures -Annually review relevant SOP documents for field operations	-Completion of field data sheets -Sampling efficiency and representation -Overall operation of the field crew when remote from central office -Adherence to safety and field SOP procedures by crew members -Ensure that multi-probe analyzers are calibrated weekly prior to field sampling activities -Ensure that D.O. data loggers are appropriately programmed and calibrated before deployment, and data properly off-loaded upon retrieval -Ensure that field sampling equipment is functioning properly and loaded into field vehicles prior to field sampling activities	-Hydrolab Corporation 2002 -IDEM 1997, 2002, 2010b, 2010c, 2015b 2016e -YSI 2002					
Field Crew Members - Water Chemistry, Algal and/or Bacteriological Sampling	-Complete hands-on training for sampling methodology prior to participation in field sampling activities -Review relevant safety procedures -Review relevant SOP documents for field operations	-Follow all safety and SOP procedures while engaged in field sampling activities -Follow direction of Field Crew Chief while engaged in field sampling activities	-Hydrolab Corporation 2002 -IDEM 1997, 2002, 2010b, 2010c, 2015b,2016e - YSI 2002					
Laboratory Supervisor - Fish or Aquatic Macroinvertebrate Community Sample Processing	-Bachelor of Science Degree in biology or other closely related area -At least one year of experience in taxonomy of aquatic communities in the region -Annually review relevant safety procedures -Annually review relevant SOP documents for laboratory operations	-Identification of fish and macroinvertebrate specimens collected during field sampling -Completion of laboratory data sheets -Verify taxonomic accuracy of processed samples -Voucher specimen tracking -Adherence to safety and	-IDEM 1992a, 1992e, 2004, 2010b, 2010c, 2012a -AIMS II Database User Guide					

Dela	Doguirod	Despensibilities	April 28, 2017
Role	Required Training/Experience	Responsibilities	Training References
Laboratory Staff - Fish or Aquatic Macroinvertebrate Community Sample Processing	-Complete hands-on training for laboratory sample processing methodology prior to participation in laboratory sample processing activities -Annually review relevant safety procedures -Annually review relevant SOP documents for laboratory operations	SOP procedures by laboratory staff -Check data for completeness -Perform all necessary calculations on the data -Ensure that data are entered into the AIMS II Database -Ensure that required QA/QC are performed on the data -Querying data from AIMS II to determine results not meeting Water Quality Criteria -Adhere to safety and SOP procedures -Follow Laboratory Supervisor direction while processing samples -Identification of fish and macroinvertebrate specimens collected during field sampling -Completion of laboratory data sheets, perform necessary calculations on data, enter field sheets	-IDEM 1992a, 1992e, 2004, 2010b, 2010c, 2012a -AIMS II Database User Guide
Laboratory Supervisor - Water Chemistry, Algal and/or Bacteriological Sample Processing	-Bachelor of Science Degree in biology or other closely related area -Annually review relevant safety procedures -Annually review relevant SOP documents for field operations	-Completion of laboratory data sheets -Adherence to safety and SOP procedures by laboratory staff -Check data for completeness -Perform all necessary calculations on the data -Ensure that data are entered into the AIMS Data Base -Ensure that required QA/QC are performed on the data -Querying data from AIMS II to determine results not meeting Water Quality Criteria	-IDEM 2010b, 2010c, 2015a -AIMS II Database User Guide
Quality Assurance Officer	-Bachelor of Science in chemistry or a related field of study -Familiarity with QA/QC practices and methodologies -Familiarity with the WAPB	-Ensure adherence to QA/QC requirements of WAPB QAPP -Evaluate data collected by sampling crews for adherence to project work plan	-IDEM 2004, 2012a -U.S. EPA 2006 documentation on QAPP development and data

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_			April 28, 2017
Role	Required	Responsibilities	Training
	Training/Experience		References
	QAPP and data qualification methodologies	 -Review data collected by field sampling crews for completeness and accuracy -Perform a data quality analysis of data generated by the project - Assign data quality levels based on the data quality analysis -Import data into the AIMS data base -Ensure that field sampling methodology audits are completed according to WAPB procedures 	qualification -AIMS II Database User Guide

II. MEASUREMENT/DATA ACQUISITION

Sampling Design and Site Locations

Candidate sites were generated through the use of a compiled data set of previously sampled sites (2003 - 2013) by the WAPB for which biological community data and nutrient data have been collected (IDEM 2015c). The nutrients/diel D.O. study is a pilot study; thus, it has a limited number of sites to test biological response to changes in diel D.O. maximum changes. Sites were selected based on control of environmental factors including:

- Limited to sites only in the Corn Belt Plains Ecoregions.
- Limited to only wadeable sites of drainage area between 52 and 2590 km² (20 and 1,000 miles²).
- Limited to only those sites with gradients between the 25th and 75th percentile of the possibility from all sites in the Assessment Information Management System (AIMS) with gradient determinations.
- Limited to only those sites in which a fish IBI was calculated with an associated QHEI score of 53 and greater.
- Clustering sites into bins based on multivariate cluster analysis of potential sites (2003-2013) based on total phosphorus metric, total nitrogen metric, fish IBI metric and QHEI metric rankings.
- Seven sites randomly selected from each of four distinct clusters or bins of sites across the multivariate clusters. (Total 93 sites in the four bins).

Site reconnaissance activities will be conducted in-house and through physical site visits. Inhouse activities will include preparation and review of site maps and aerial photographs, initial evaluation of target or non-target site status, potential access routes and initial property owner searches. Physical site visits will include property owner consultations, verification of site status (target or non-target), confirmation and documentation of access routes, and determination of equipment needed to properly sample the site (Attachment 1). Precise coordinates for each approved target site will be determined using a Trimble Juno[™] SB or Trimble Juno[™] 3D handheld Series Global Positioning System (GPS) with an accuracy of 2-5 meters (IDEM 2015b). Since all potential sites have been sampled in the past by the WAPB, landowner permission and site access will be determined for only up to the first 28 approved potential sites (7 from each of four bins). Although 8 weeks is the maximum time allotted for site reconnaissance field work (see Section I on PROJECT MANAGEMENT/PLANNING for site reconnaissance activities, QAPP ELEMENT A4), most work for this pilot study should be completed in a four week period (dependent upon weather, driving time to sites, and other unforeseeable constraints such as high water) during the month of August.

Table 6 lists the final sampling sites generated for the nutrient/diel D.O. pilot study. Target sites are sampled for water chemistry, algal community and biomass, diel D.O. 7 day continuous data logs, and diatom, aquatic macroinvertebrate, and fish communities. If a site is considered "non-target" (dry, backwater, marsh/wetland, etc.) or unavailable to sample for some other reason (physical barrier, landowner denial, etc.), the next target site on the list will be taken. Figure 4 depicts the final sampling site locations generated for this pilot study and their approximate locations.

Sampling Methods and Sample Handling

Water Chemistry Sampling

During three discrete monthly sampling events, one team of two staff will collect grab water chemistry samples and record water chemistry field measurements and physical site descriptions on the IDEM Stream Sampling Field Data Sheet (Attachment 2). All water chemistry sampling will adhere to the Water Quality Surveys Section Field Procedure Manual (IDEM 2002). Water chemistry sampling usually takes 30 minutes to complete for each site, depending on accessibility.

Algal Sampling

In addition to standard water chemistry sampling, one team of two staff will collect samples for CHL-a, phaeophytin-a and AFDM from periphyton communities during the second or third round of water chemistry sampling in August (Table 1). In addition a sample for diatom community structure identification and enumeration will be collected at each site. Sampling for an average site that includes all of the above parameters will require approximately 2.5 hours of effort. The Algal Biomass Lab Datasheet (Attachment 3) and Probabilistic Monitoring Section Physical Description of Stream Site Form (Attachment 4) will be used to record information regarding substrates sampled for periphyton and physical parameters of the stream sampling area. See IDEM 2016e for a description of methods used in algal community sampling.

Samples for CHL-a/phaeophytin-a, and AFDM will be delivered to the USGS Indiana Algal Biomass Laboratory in Indianapolis and processed within 24 days of collection. Using U.S. EPA method 445.0, the laboratory will provide measurements for CHL-a, phaeophytin-a and AFDM for the periphyton samples. Samples for diatom community identification and enumeration will be delivered to the IDEM OWQ laboratory located at the Western Select Property at 2525 N. Shadeland Ave, Indianapolis, IN.

Laboratory Procedures for Diatom Identification and Enumeration

See IDEM 2015a for a description of methods used in diatom identification and enumeration.

Site number	Ref. site	Order	Random Number	BIN	L-Site Code	WATERBODY	COUNTY NAME	LOCATION	LATITUDE MEASURE	LONGITUDE MEASURE	UTM EAST	UTM NORTH	UTM ZONE	HUC14	Approved (Y/N)
1	Y	1	10	1	WEF-06-0002	Flatrock River	Bartholomew	CR 900 N	39.33192521	-85.86121339	598148.4312	4354229.979	16N	'05120205050150'	Y
5		2	16	1	WSU040-0020	Sugar Creek	Montgomery	CR 275 E	40.08778676	-86.85247732	512576.3531	4437511.185	16N	'05120110040010'	Y
9		3	7	1	WAW040-0065	South Fork Wildcat Creek	Clinton	CR 600 W	40.32092985	40.32092985 -86.61818972 53		4463448.215	16N	'05120107040040'	Y
13		4	11	1	WEM020-0036	Graham Creek	Jennings	CR 230S	38.95664167	-85.49456667	630440.8124	4313042.641	16N	'05120207020030'	Y
21		6	19	1	WWU100-0106	Fall Creek	Henry	Mechanicsburg Rd	40.02506028	-85.55712583	623120.5469	4431535.792	16N	'05120201100020'	Y
25	Y	7	9	1	WEF-06-0001	Flatrock River	Bartholomew	CR 400 N	39.260094	-85.92208196	592997.2301	4346193.773	16N	'05120205050190'	Y
29		8	13	1	WEU010-0039	Clifty Creek	Decatur	CR 420 W	39.38764378	-85.55871433	624122.1776	4360786.03	16N	'05120206010050'	Y
6		2	28	2	WAW040-0043	South Fork Wildcat Creek	Tippecanoe	SR 26	40.41806385	-86.76806189	519676.91	4474185.72	16N	'05120107040130'	Y
10	Y	3	29	2	WAW040-0080	South Fork Wildcat Creek	Clinton	W Mulberry-Jefferson Rd	40.329145	-86.647122	529976.4684	4464349.872	16N	'05120107040110'	Y
14	Y	4	33	2	WDE050-0025	Deer Creek	Carroll	Cemetery Rd	40.600487	-86.547286	538302.9685	4494507.344	16N	'05120105050080'	Y
18	Y	5	35	2	WDE050-0031	Deer Creek	Cass	CR 1100 S	40.60804003	-86.37034006	553267.9937	4495437.78	16N	'05120105050030'	Y
22	Y	6	37	2	WEM-07-0004	Vernon Fork Muscatatuck River	Jennings	CR 25 W	38.97467929	-85.6141175	620051.2278	4314880.036	16N	'05120207070010'	Y
26		7	53	2	WWU100-0064	Fall Creek	Madison	CR 750 W	39.96432222	-85.81504806	601201.0415	4424469.563	16N	'05120201100090'	Y
30		8	27	2	WAW040-0018	South Fork Wildcat Creek	Clinton	CR 200 N	40.31501966	-86.54371579	538768.7808	4462822.145	16N	'05120107040040'	Y
3	Y (sub ref.)	1	73	3	WSA010-0012	Little Salamonie River	Jay	Boundary Pike	40.406944	-84.96137	672985.3421	4474920.927	16N	'05120102010040'	Y
7		2	65	3	WED-07-0001	Sugar Creek	Johnson	River Rd	39.36953801	-85.99488726	586580.8145	4358267.656	16N	'05120204090080'	Y
11	Y	3	67	3	WEM-02-0002	Graham Creek	Jennings	CR 500 S	38.90637908	-85.60614372	620857.8986	4307310.832	16N	'05120207020070'	Y
15		4	62	3	WED-03-0001	Brandywine Creek	Shelby	W 650 N	39.61836716	-85.80054133	602954.5497	4386088.341	16N	'05120204040060'	Y
19		5	78	3	WWU100-0040	Fall Creek	Madison	C.R. 650W	39.97086972	-85.796175	602803.1303	4425217.881	16N	'05120201100090'	Y
23		6	61	3	UMI030-0044	Carpenter Creek	Jasper	CR 1300 S	40.82431705	-87.17677482	485093.5584	4519269.914	16N	'07120002030070'	Y
27		7	71	3	WLV160-0019	Big Raccoon Creek	Boone	@ CR 500S	39.96679722	-86.68392222	526993.3915	4424119.867	16N	'05120108160010'	Y
4		1	95	4	WWU100-0078	Fall Creek	Madison	Brown St	40.01593861	-85.69206833	611620.3918	4430345.519	16N	'05120201100060'	Y
8		2	96	4	WWU100-0098	Fall Creek	Henry	CR 700 N	40.03376611	-85.54764056	623914.1986	4432515.269	16N	'05120201100020'	Y
12		3	85	4	LMG-05-0015	Deep River	Lake	Clay Street	41.44704867	-87.27761876	476810.298	4588422.82	16N	'04040001030040'	Y
16		4	91	4	WEU010-0040 *	Clifty Creek	Bartholomew	U.S. 31	39.20858111	-85.87337078	597270.997	4340528.058	16N	'05120206010160'	Y
28	Y	7	89	4	WED-04-0001	Sugar Creek	Hancock	E CR 1000 N	39.93160872	-85.69904306	611161.705	4420976.626	16N	'05120204060020'	Y
32		8	86	4	OML060-0019	Laughery Creek	Ripley	N CR 75 E	39.16019008	-85.2524487	650986.5188	4336007.599	16N	'05090203060110'	Y
36		9	93	4	WWU010-0039 *	West Fork White River	Randolph	CR 200 E	39.3869064	-85.55987034	624023.925	4360702.608	16N	'05120201010020'	Y
*= Site m	noved to the	e bridge	on the cro	oss-ro	ad listed.										

Table 6. Final list of 28 sampling sites for the Nutrients/Diel Dissolved Oxygen Pilot Study (seven sites included from each of seven multivariate cluster bins).

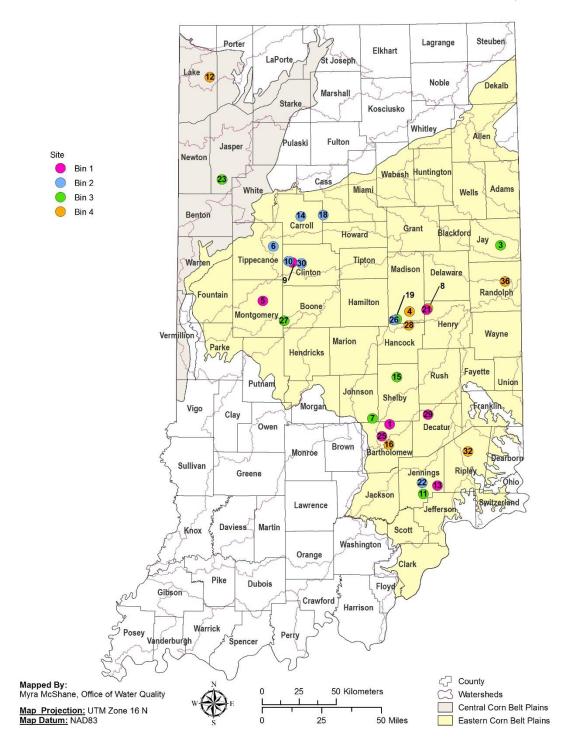


Figure 4. Map of final 28 sites from a randomized list of the 93 candidate sites. Seven sites from each of four bins are targeted.

Fish Community Sampling

Fish community sampling will be performed using various standardized electrofishing methodologies depending on stream size and site accessibility. Fish assemblage assessments will be performed in a sampling reach of 15 times the average wetted width, with a minimum reach of 50 meters and a maximum reach of 500 meters (Simon 1991; Simon and Dufour 1998; U.S. EPA 1995). An attempt will be made to sample all habitat types available within the sample reach to ensure adequate representation of the fish community present at the time of the sampling event. The possible list of electrofishers to be utilized include: the Smith-Root LR-24 or LR-20B Series backpack electrofishers; the Smith-Root model 1.5KVA electrofishing system; the Smith-Root model 2.5 Generator Powered Pulsator (GPP) electrofisher with RCB-6B junction box and rat-tail cathode cable or Midwest Lake Electrofishing Systems (MLES) Infinity Control Box with MLES junction box and rat-tail cathode cable, assembled in a canoe (if parts of the stream are not wadeable, the system may require the use of a dropper boom array outfitted in a canoe or possibly a 12 foot Loweline boat) (IDEM 1992a, 1992b, 1992c, 1992d).

Sample collections during high flow or turbid conditions will be avoided due to 1) low collection rates which result in non-representative samples and 2) safety considerations for the sampling team. Sample collection during late autumn will be avoided due to the cooling of water temperature, which may affect the responsiveness of some species to the electrical field. This lack of responsiveness can result in samples that are not representative of the stream's fish assemblage (Simon 1990; U.S. EPA 1995).

Fish will be collected using dipnets with fiberglass handles and netting of 1/8-inch bag mesh. Fish collected in the sampling reach will be sorted by species into baskets and/or buckets. Young-of-the-year fish less than 20 millimeters (mm) total length will not be retained in the community sample (Simon 1990; U.S. EPA 1995).

For each field taxonomist (generally the crew leader), a complete set of fish vouchers are retained for any different species encountered during the summer sampling season. Vouchers may consist of either preserved specimens or digital images. Prior to processing fish specimens and completion of the fish community datasheet, one to two individuals per new species encountered will be preserved in 3.7% formaldehyde solution to serve as representative fish vouchers if the fish specimens can be positively identified and the individuals for preservation are small enough to fit in a 2000 mL jar. If however, the specimens are too large to preserve, a photo of key characteristics (e.g., fin shape, size, body coloration, etc.) will be taken for later examination (IDEM 2016a). Also, prior to sampling, 10% of the sites will be randomly selected for revisiting and a few representative individuals of all species found at the site will be preserved or photographed to serve as vouchers. Taxonomic characteristics for possible species encountered in the basin of interest will be reviewed prior to field work. Fish specimens should also be preserved if they cannot be positively identified in the field (i.e., those that co-occur like the Striped and Common Shiners or are difficult to identify when immature), individuals that appear to be hybrids or have unusual anomalies, as well as dead specimens that are taxonomically valuable for un-described taxa (e.g., Red Shiner or Jade Darter), life history studies, or research projects.

Data will be recorded for non-preserved fish on the IDEM Fish Collection Data Sheet (Attachment 5) consisting of the following: number of individuals, minimum and maximum total length (mm), mass weight in grams (g), and number of individuals with deformities, eroded fins, lesions, tumors, and other anomalies (DELTs). Once the data have been recorded, specimens will be released within the sampling reach from which they were collected. Data will be recorded for preserved fish specimens following taxonomic identification in the laboratory.

Macroinvertebrate Community Sampling

Aquatic benthic macroinvertebrate samples are collected using a modification of the U.S. EPA Rapid Bioassessment Protocol multi-habitat (MHAB) approach using a D-frame dip net (Plafkin et al. 1989; Barbour et al. 1999; Klemm et al. 1990; IDEM 2010a). The IDEM MHAB approach (IDEM 2010a) is composed of a 1-minute "kick" sample within a riffle or run (collected by disturbing one square meter of stream bottom substrate in a riffle or run habitat and collecting the dislodged macroinvertebrates within the dipnet) and a 50 meter "sweep" sample of shoreline habitats (collected by disturbing habitats such as emergent vegetation, root wads, coarse particulate organic matter, depositional zones, logs and sticks and collecting the dislodged macroinvertebrates within the dipnet). The 50 meter length of riparian corridor that is sampled at each site will be defined using a tape measure or rangefinder. If the stream is too deep to wade, a boat will be used to sample the 50 meter zone along the shoreline that has the best available habitat. The 1-minute "kick" and 50 meter "sweep" samples are combined in a bucket of water which will be elutriated through a U.S. standard number 35 (500 µm) sieve a minimum of five times so that all rocks, gravel, sand and large pieces of organic debris are removed from the sample. The remaining sample is then transferred from the sieve to a white plastic tray where the collector (while still on-site) will conduct a 15-minute pick of macroinvertebrates at a single organism rate with an effort to pick for maximum organism diversity and relative abundance through turning and examination of the entire sample in the tray. The resulting picked sample will be preserved in 70% isopropyl alcohol and returned to the IDEM OWQ laboratory for identification at the lowest practical taxonomic level (usually genus or species level, if possible) and evaluated using the MHAB macroinvertebrate IBI. Before leaving the site, an IDEM OWQ Macroinvertebrate Header Form (Attachment 6) will be completed for the sample.

If time and field conditions permit, three additional macroinvertebrate samples may be collected at target sites that are also part of the WAPB Reference Sites project (IDEM 2015c) and have not been previously collected by the methods listed below (IDEM 2014; IDEM 2015c). These additional samples will include: 1.) the MHAB Remainder – the un-picked remainder of the MHAB sample, 2.) a Transect Kick – a sample in which a bottom kick net is used to take two 0.25 square meter kick samples on each of ten transects (for a sample with a total surface area of 5 square meters) which are equally interspaced along a reach that is equivalent to 15 times the wetted width of the stream and does not overlap the 50 meter reach used for the MHAB sample, 3.) a Transect Jab – a sample in which a dipnet is used to collect three sweep samples (30 sweeps total) on each of the same ten transects used for the Transect Kick samples. None of these additional samples are processed in the field; instead, the elutriated sample is preserved and returned to the laboratory for sub-sampling and analysis. The Macroinvertebrate MHAB Sample Habitat Form (Attachment 7) and Macroinvertebrate Transect Sample Habitat Form (Attachment 8) will be completed for the MHAB Remainder and Transect Kick/Jab samples, respectively. These samples are a part of ongoing methods comparison and development studies and so are not a part of this pilot study. An IBI does not currently exist for these samples collection methods, which will preclude their use for assessment purposes.

Habitat Assessments

Habitat assessments will be completed immediately following macroinvertebrate and fish community sample collections at each site using a slightly modified version of the Ohio Environmental Protection Agency (OHEPA) Qualitative Habitat Evaluation Index (QHEI), 2006 edition (Rankin 1995; OHEPA 2006). A separate QHEI (Attachment 7) must be completed for these two sample types since the sampling reach length may differ (i.e. 50 meters for macroinvertebrates and between 50 and 500 meters for fish). See IDEM 2016d for a description of the method used in completing the QHEI.

The percentage of a typical point in the sampling site that is not covered or shaded by woody bank vegetation, as measured with a spherical densiometer in accordance with the instructions on the lid of the densiometer or in Appendix 9.3 (IDEM 2016d) is the "percent canopy open." A canopy percent open determination will be made at three longitudinal points along the fish sampling transect including the beginning, middle, and end of the sampling reach. For streams less than 10 meters wide to the wetted width, each densiometer reading will be made at center channel. For streams greater than or equal to 10 meters wide to the wetted width, three densiometer readings will be taken in cross-section to the stream including at the right descending bank (RDB), center channel, and left descending bank (LDB). A single densiometer

reading consists of four measurements; 1) looking downstream, 2) looking upstream, 3) facing RDB, and 4) facing LDB. All four of these measures for a densiometer reading are to be recorded on the QHEI form for each point in the stream reach where a densiometer reading occurs. Percent canopy open measures for the macroinvertebrate QHEI will occur as normal for that sampling effort.

A measure of the angle (degrees) from center channel to the top of the canopy along each bank at each transect where densiometer reading is measured will be taken using a Suunto PM-5 Clinometer (Appendix 4). The sum of the two measured angles at each transect point are subtracted from 180 and averaged for the three observation points to yield a "degree of open arc" measure to compliment the densitometer "canopy openness" measure.

Field Parameter Measurements

Dissolved oxygen, pH, water temperature, specific conductance, and D.O. percent saturation will be measured with a data sonde during each sampling event or site visit regardless of the sample type being collected. This includes during each water quality sampling event, the deployment and the retrieval of the D.O. data loggers, fish and macroinvertebrate community assessments, and during the collection of diatom and CHL-a samples. Measurement procedures and operation of the data sonde shall be performed according to the manufacturers' manuals (Hydrolab Corporation 2002; YSI 2002) and Sections 2.10-2.13 of the Water Quality Surveys Section Field Procedure Manual (IDEM 2002, pp 67-79). Turbidity will be measured with a Hach turbidity kit, and the meter number written in the comments under the field parameter measurements. If a Hach turbidity kit is not available, the data sonde measurement for turbidity will be recorded. All field parameter measurements and weather codes will be recorded on the IDEM Stream Sampling Field Data Sheet (Attachment 2) with other sampling observations. A digital photo will also be taken upstream and downstream of the site during each sampling event (IDEM 2016d).

Dissolved Oxygen Continuous Data Logger Measurements

Within 30 days (ideally within 7 to 14 days) prior to biological community and algal biomass collections, an Onset Hobo[®] U26-001 D.O. data logger will be deployed in a representative location within the targeted stream segment to record D.O. measures at no less than 15 minute intervals for no less than 7 consecutive days (Appendices 3 and 4). A programmed and calibrated data logger will be attached to a 16x4x8 cinder block, post, or other securing device dependent on the particular conditions observed at the stream sampling site and placed in a calm glide portion of the stream segment with a water depth of between 0.3 and 1.0 meters. The data logger is not to be placed directly below a riffle, a turbulent run, or in a deep pool. The crosssectional location to the channel for placement should be near the center if possible. GPS coordinates point of the exact placement will be collected using a Trimble Juno™ SB or Trimble Juno[™] 3D handheld Series Global Positioning System (GPS) with an accuracy of 2-5 meters (IDEM 2015b). At least one photograph/digital image will be taken of this placement point in relation to the stream reach to document location and stream flow conditions to the extent possible in a photograph. In-situ water quality measurements will be recorded at the time of each data logger deployment. Upon retrieval of the D.O. data logger all data will be off-loaded to a Hobo U-DTW-1 Waterproof shuttle. Once data are off-loaded the data logger will be returned to the WAPB calibration room at the Western Select Property IDEM OWQ laboratory and prepared (programmed and calibrated) for redeployment at another location. In-situ water quality measurement will also be recorded during the retrieval of each D.O. data logger.

Analytical Methods

Table 7 lists the field parameters with their respective test method and IDEM quantification limits. Table 8 lists the algal parameters with test method and USGS quantification limits. Table 9 lists water chemistry sample container, preservative, and holding time requirements (all samples iced to 4 Degrees Celsius °C). Table 10 lists numerous parameters (priority metals, anions/physical, and nutrients/organic) with their respective test methods, IDEM reporting limits, and contract laboratory reporting limits. The IDEM OWQ Chain of Custody Form (Attachment 8) and the 2017 Diatom samples will be collected in the field according to protocols described in IDEM 2016e. See Appendix 4 in IDEM 2015a for a list of taxonomic references used in diatom identification and enumeration.

Quality Control and Custody Requirements

Quality assurance protocols will follow part B5 of the WAPB QAPP (IDEM 2004, p 119).

Water Chemistry Data

Sample bottles and preservatives certified for purity will be used. Sample collection procedures, including: the container and preservative used for each parameter and holding times will adhere to U.S. EPA requirements for water chemistry testing (see Table 9). Field duplicates and matrix spike/matrix spike duplicates (MS/MSD) shall be collected at the rate of one per sample analysis set or one per every 20 samples, whichever is greater. Additionally, field blank samples using American Society for Testing and Materials (ASTM) D1193-91 Type I water (ASTM 2011) will be taken at a rate of one set per sampling crew for each week of sampling activity. All samples collected for nutrient chemistry will be processed by the Indiana State Department of Health. All sites identified for samples with metals analysis will be processed by Pace Analytical Services, Inc. (Indianapolis, Indiana) following the specifications set forth in Request for Proposals 16-074 (IDEM 2016).

Parameters	Method (SM=Standard Method)	IDEM Quantification Limit
Dissolved Oxygen (data sonde optical)	ASTM D888-12	0.05 mg/L
Dissolved Oxygen (data sonde)	SM 4500-OG	0.03 mg/L
Dissolved Oxygen (Winkler Titration)	SM 4500-OC ²	0.20 mg/L
Dissolved Oxygen % Saturation (data sonde optical)	ASTM D888-09	0.05 %
Dissolved Oxygen % Saturation (data sonde)	SM 4500-OG	0.01 %
pH (data sonde)	U.S. EPA 150.2	0.10 S.U.
pH (field pH meter)	SM 4500H-B ²	0.10 S.U.
Specific Conductance (data sonde)	SM 2510B	1.00 µmhos/cm
Temperature (data sonde)	SM 2550B(2)	0.1 Degrees Celsius (°C)
Temperature (field meter)	SM 2550B(2) ²	0.1 Degrees Celsius (°C)
Turbidity (data sonde)	SM 2130B	0.02 NTU ³
Turbidity (Hach™ turbidity kit)	U.S. EPA 180.1	0.05 NTU ³

Table 7. Field Parameters showing method and IDEM quantification limit.

¹ 1 MPN (Most Probable Number) = 1 CFU (Colony Forming Unit)

² Method used for Field Calibration Check

³ NTU = Nephelometric Turbidity Unit(s)

Algal Parameter	Method	USGS Quantification Limit
Ash Free Dry Mass	SM10200I(5)	36 g/m ²
Periphyton CHL-a	U.S. EPA 445.0	0.30 µg/m²
Periphyton Phaeophytin-a	U.S. EPA 445.0	0.30 µg/m ²

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Table 9. Water Chemistry Sample Container, Preservative, and Holding Time Requirements.¹

Parameter	Container	Preservative	Holding Time
¹ Alkalinity as CaCO ₃ *	1 L, plastic, narrow mouth	None	14 days
² Ammonia-N**	1 L, plastic, narrow mouth	H ₂ SO ₄ < pH 2	28 days
Biological Oxygen Demand	1 L, plastic, narrow mouth	lce	48 hours
Chloride*	1 L, plastic, narrow mouth	None	28 days
Chemical Oxygen Demand**	1 L, plastic, narrow mouth	H ₂ SO ₄ < pH 2	28 days
Hardness (as CaCO ₃ *)	1 L, plastic, narrow mouth	HNO₃ < pH 2	6 months
Calculated			
Metals (Total & Dissolved)	1 L, plastic, narrow mouth	HNO₃ < pH 2	6 months
Nitrate + Nitrite-N**	1 L, plastic, narrow mouth	H ₂ SO ₄ < pH 2	28 days
Total Phosphorus**	1 L, plastic, narrow mouth	H ₂ SO ₄ < pH 2	28 days
Ortho-Phosphate**	1 L, plastic, narrow mouth	H ₂ SO ₄ < pH 2	48 hours
Solids (All Forms)*	1 L, plastic, narrow mouth	None	7 days
Sulfate*	1 L, plastic, narrow mouth	None	28 days
Total Kjeldahl Nitrogen**	1 L, plastic, narrow mouth	H ₂ SO ₄ < pH 2	28 days
Total Organic Carbon**	1 L, plastic, narrow mouth	H ₂ SO ₄ < pH 2	28 days

¹All samples iced to 4°C ²General chemistry includes all parameters noted with an * ³Nutrients include all parameters noted with a **

		Priority	Metals				Anions/Physical					
Parameter	Total	Dissolved	<u>Method</u>	<u>IDEM</u> <u>Reporting</u> Limit (μg/L)	<u>Pace Lab</u> <u>Reporting Limit</u> (μg/L)		Parameter	Method	IDEM Reporting Limit (mg/L)	Pace Lab Reporting Limit (mg/L)		
Aluminum (total and dissolved)	Х	Х	U.S. EPA 200.7	150	20	AI	kalinity (as CaCO ₃)	U.S. EPA 310.2	10	10		
Antimony	Х	Х	U.S. EPA 200.8	1	0.5		Total Solids	SM 2540B	1	10		
Arsenic	Х	Х	U.S. EPA 200.8	5	2.5	Tota	I Suspended Solids	SM 2540D	1	1		
Calcium	Х	Х	U.S. EPA 200.7	40	40		Dissolved Solids	SM 2540C	10	10		
Cadmium	Х	Х	U.S. EPA 200.8	2	1		Sulfate	U.S. EPA 300.0	0.05	0.35		
Chromium	Х	Х	U.S. EPA 200.8	3	1.5		Chloride	U.S. EPA 300.0	1	1		
Copper	Х	Х	U.S. EPA 200.8	2	1	Hai	rdness (as CaCO3)	SM 2340B	0.4	1		
Lead	Х	Х	U.S. EPA 200.8	2	1							
Magnesium	Х		U.S. EPA 200.7	95	100		Nutrients/Organics					
Nickel	х	х	U.S. EPA 200.8	1.5	0.75		Parameter	Method	IDEM Reporting Limit (mg/L)	ISDH Lab Reporting Limit (mg/L)		
Selenium	Х	Х	U.S. EPA 200.8	4	2		COD	SM5220D	3	10		
Silver	Х	Х	U.S. EPA 200.8	0.3	0.3		Nitrogen, Ammonia	EPA 350.1	0.01	0.1		
Zinc	Х	Х	U.S. EPA 200.8	6	6	Nitro	ogen, Nitrate+Nitrite	EPA 353.1	0.01	0.1		
Org=Organic							Phosphorus, Total	EPA 365.1	0.01	0.03		
							ortho-phosphorus	EPA 365.3	0.006	0.006		
SM= Standard Methods for the	Analysis	of Drinking	Water and Wastev	vater		TBO		SM5210B	2	2		
TKN=Total Kjeldahl Nitrogen							TKN	EPA 351.2	0.1	0.3		
U.S. EPA= Unites States Environ	mental F	Protection Ag	gency				TOC	SM5310B	1	1		

Table 10. Water Chemistry Parameters with Test Method and Laboratory Reporting Limits.

Algal Community Data

Excessive algal conditions will be recorded by staff if an algal bloom is observed on the water's surface or in the water column. Staff are not calibrated on this rating (i.e. the decision as to the severity of the bloom is based on best professional judgment), but an algal mat on the surface of the water or a bloom that gives the water the appearance of green paint would be justification for a decision of excessive algal conditions. To decrease the potential for cross contamination and bias of the algal samples, all equipment that has come in contact with the sample will be cleaned with detergent and rinsed with ASTM D1193-91 Type III water after sampling has been completed at a given site. All sample labels must be accurately and thoroughly completed, including AIMS II sample numbers, date, stream name, and sampling location. Chain of Custody forms will be completed in the field to document the collection and transfer of samples to the laboratory. Upon arrival to the laboratory, samples will be checked in by the laboratory manager. For the diatom samples, there will be another Chain of Custody form to document when the sample is removed from storage to be processed and made into a permanent mount.

Methods and quantification limits for CHL-a and phaeophytin-*a* are listed in Table 8. All samples collected for chlorophyll *a* and phaeophytin-*a* determination will be processed by the USGS Indiana Algal Biomass Laboratory (Indianapolis, Indiana) following the specifications set in Joint Funding Agreement (IDEM 2017 - DRAFT contract). Blank filters will be run for periphyton chlorophyll *a*. All CHL-a filters will be processed in quadruplicate for QC purposes (four filters are processed from the same sample). Ten percent of these replicate field samples will be analyzed at the USGS National Water Quality Laboratory in Arvada, Colorado.

Quality control of the diatom sampling, enumeration, and identification project will be documented by QC checks of both field and laboratory data. See IDEM 2015a for description of quality assurance/ quality control protocols used in Diatom Identification and Enumeration. Ten percent of diatom samples will be verified by the Department of Biological and Environmental Sciences of Georgia College and State University (Milledgeville, Georgia), or other entity determined to be recognized as an expert in diatom taxonomy, following the specifications set forth in IDEM 2015a.

Fish Community Data

Replicate fish community sampling will be performed at a rate of 10 percent of the total fish community sites sampled or approximately 3 of the final target sites (IDEM 1992a; U.S. EPA 1995). Replicate sampling will be performed with at least 2 weeks of recovery between the initial and replicate sampling events. The fish community replicate sampling and habitat assessment will be performed with either a partial or complete change in field team members (U.S. EPA 1994; U.S. EPA 1995). The resulting IBI and QHEI total score between the initial visit and the revisit will be used to evaluate precision. The IDEM OWQ Chain of Custody Form is used to track samples from the field to the laboratory (Attachment 8). Fish taxonomic identifications made by IDEM staff in the laboratory may be verified by regionally recognized non-IDEM freshwater fish taxonomists (e.g., Brant Fisher, Nongame Aquatic Biologist, Indiana DNR). All raw data are: 1) checked for completeness; 2) utilized to calculate derived data (i.e. total weight of all specimens of a taxon), which is entered into the AIMS II database; and 3) checked again for data entry errors.

Macroinvertebrate Community Data

Duplicate macroinvertebrate field samples will be collected at a rate of 10 percent of the total macroinvertebrate community sites sampled or approximately 3 of the final target sites. The macroinvertebrate community duplicate sample and habitat assessment will be performed by the same team member who performed the original sample, immediately after the initial sample is collected, from an adjacent reach of the stream that does not overlap the reach used for the original MHAB sample. This will result in a precision evaluation based on a 10% duplication of samples collected. The IDEM OWQ Chain of Custody Form is used to track samples from the field to the laboratory (Attachment 8). Laboratory identifications and QA/QC of taxonomic work is maintained by the laboratory supervisor of the Probabilistic Monitoring Section of IDEM.

Field Parameter Measurements/Instrument Testing/Calibration

The data sonde will be calibrated immediately prior to each week's sampling (IDEM 2002). The D.O. component of the calibration procedure will be conducted using the air calibration method. Calibration results and drift values will be recorded, maintained, stored, and archived in the calibration laboratories at the Shadeland facility. The drift value is the difference between two successive calibrations. Field parameter calibrations will conform to the procedures described in the instrument users manuals (Hydrolab Corporation 2002; YSI 2002). The unit will be field checked for accuracy once during the week by comparison with a Winkler D.O. test (IDEM 2002, page 64), as well as Hach turbidity, pH and temperature meters. Weekly field calibration records will be recorded in the field calibrations portion of Attachment 2 and entered into the AIMS II database. A Winkler D.O. test will also be conducted in the field at sites where the D.O. concentration is 4.0 mg/L or less.

The Onset Hobo[®] U26-001 D.O. data loggers utilize optical D.O. measure technology specified in ASTM D888-12 (ASTM 2012). Calibration and maintenance of these units will follow the manufacturers procedures listed in the "HOBO® Dissolved Oxygen Logger (U26-001) Manual" found in Appendix 2 of this document.

Field Analysis Data

In-situ water chemistry field data are collected in the field using calibrated or standardized equipment. Calculations may be done in the field or later at the office. Analytical results, which have limited QC checks, are included in this category. Detection limits and ranges have been set for each analysis. Quality control checks are performed on information for field or laboratory results to estimate precision, accuracy, and completeness for the project as described in the WAPB QAPP (IDEM 2004) Section C1.1 on page 124.

Algal Community Data

Equipment required for the collection of periphyton include: a toothbrush, cloth measuring tape, petri dish top, spatula, stencil brush, small hobby knife with a chisel blade, a dissection probe, a modified syringe with a rubber o-ring attached, Nalgene HDPE plastic 250 mL sample bottles, plastic bins, and a unitary wash bottle filled with tap water. None of this equipment requires calibration. Equipment has been field tested to ensure its capability of appropriately removing periphyton from different types of substrate (rocks, sticks, and sand/silt).

Laboratory equipment that will be used for the preparation of permanent diatom mounts include: hot plate, fume hood, centrifuge, glass beakers, centrifuge tubes, glass microscope slides, microscope cover glasses, micropipette, and micropipette tips. The micropipette was purchased new and came with a calibration certificate as proof that it was calibrated at the factory. Other than the micropipette, none of the laboratory equipment requires calibration. The micropipette will be checked and recalibrated as necessary according to manufacturer's specifications.

A Nikon Eclipse 80i Normarski Differential Interference Contrast (DIC) microscope equipped with a 100X oil immersion Plan Apochromatic objective and Nikon Elements D camera/imaging system will be used for identification and enumeration of diatoms. Branch staff calibrated the ocular reticle in the microscope. The ocular reticle was calibrated at each magnification with a stage micrometer. The calibration should be checked again if the microscope is moved to a new location.

III.ASSESSMENT/OVERSIGHT

Field and laboratory performance and system audits will be conducted to ensure good quality data. The field and laboratory performance checks include precision measurements by relative percent difference (RPD) of field and laboratory duplicate (IDEM 2004, pp. 41, 45-46), accuracy measurements by percent of recovery of matrix spike and matrix spike duplicate (MS/MSD) samples analyzed in the laboratory (IDEM 2004, pp. 43, 45-46), and completeness measurements by the percent of planned samples that are actually collected, analyzed, reported, and usable for the project (IDEM 2004, p. 43).

Field audits will be conducted to ensure that sampling activities adhere to approved SOPs. Audits are systematically conducted by WAPB Quality Assurance staff to include all WAPB personnel that engage in field sampling activities. WAPB field staff involved with sample collection and preparation will be evaluated by QA staff trained in the associated sampling SOPs, and in the processes related to conducting an audit. QA staff will produce an evaluation report documenting each audit for review by those field staff audited, as well as WAPB management. Corrective actions will be communicated to, and implemented by, field staff as a result of the audit process (IDEM 2004, p. 126).

Data Quality Assessment Levels

The samples and various types of data collected by this program are intended to meet the quality assurance criteria and rated DQA Level 3, as described in the WAPB QAPP (IDEM 2004, pp. 128-129).

IV. DATA VALIDATION AND USABILITY

Quality assurance reports to management and data validation and usability are also important components of the QAPP which ensures good quality data for this project. A quality assurance audit report will be submitted to the QA Manager and Project Manager for review for this project should problems arise and need to be investigated and corrected. Data are reduced (converted from raw analytical data into final results in proper reporting units), validated (qualified based on the performance of field and laboratory QC measures incorporated into the sampling and analysis procedures), and reported (described so as to completely document the calibration, analysis, QC measures, and calculations). These steps allow users to assess the data to ensure it meets the project data quality objectives.

Quality Assurance/Data Qualifiers and Flags

The various data qualifiers and flags that will be used for quality assurance and validation of the data are found on pages 130-131 of the WAPB QAPP (IDEM 2004).

Data Usability

The environmental data collected and its usability are qualified per each lab and/or field result obtained and classified into one or more of the four categories: Acceptable Data, Enforcement Capable Results, Estimated Data, and Rejected Data as described on page 130 of the WAPB QAPP (IDEM 2004).

Information, Data, and Reports

Data collected in 2017 for this pilot study will be recorded in the Office of Water Quality AIMS II database. Data not capable of being stored in the AIMS II database at this time (e.g. D.O. continuous log data, and multiple transect densiometer readings) will be entered and stored in an Excel Spreadsheet environment. All data will be compiled into Excel electronic formats and

submitted to U.S. EPA Region 5 in partial fulfillment of FFY16 Grant #l96555712. A status report will also be submitted to U.S. EPA Region 5 for the partial fulfillment of this Grant. Data summaries will be compiled into three summary packages:

- 1. The first summary will be a general compilation of field and water chemistry data including diel D.O. data logger results (December 2017).
- 2. The second summary will be in database report format containing biological results and habitat evaluations (March 2018).
- 3. The third summary will include diatom species taxa names and enumerations, and CHLa/phaeophytin-a results (October 2018).

All data will be subjected to data reduction, statistical analysis, assessment, and data sharing. An internal IDEM OWQ report that further traces the steps from nutrients to periphyton (as CHL-a), from periphyton to D.O., and from D.O. to macroinvertebrates and fish community response will be produced. Results of this study will be presented to IDEM OWQ management as a further decision tool on developing a more robust sampling effort toward the development of phosphorus and nitrogen threshold criteria, based on multiple response variables, for Indiana's rivers and streams.

All data and reports will be made available to public and private entities which may find the data useful for municipal, industrial, agricultural, and recreational decision making processes (TMDL, NPDES permit modeling, Watershed Restoration Projects, Water Quality Criteria refinement, Water Quality Assessments, etc.).

Laboratory and Estimated Cost

Laboratory analysis and data reporting for this pilot study will comply with the QAPP for Indiana Surface Water Quality Monitoring and TMDL Program (IDEM/100/29/338/073/2004, see IDEM 2004), Request For Proposals 16-074 (see IDEM 2016), and the Office of Water Quality Assessment Branch Quality Management Plan (B-001-OWQ-A-00-08-R00, see IDEM 2012). Analytical tests on the water chemistry parameters outlined in Table 11 will be performed by Pace Analytical Services in Indianapolis, Indiana and or by the ISDH Water Laboratory. Accreditation related to Pace Indy is included as Appendix 1. Algal samples will be collected by IDEM staff. CHL-a, phaeophytin-a, and AFDM will be analyzed by the USGS Indiana Algal Biomass Laboratory in Indianapolis, Indiana. Diatom identification and enumeration will be performed by IDEM staff and/or an outside contractor. The Department of Biological and Environmental Sciences, Georgia College and State University, o other recognized expert in diatom taxonomy, will verify diatom taxa from ten percent of the sites sampled. All fish and macroinvertebrate samples will be collected, identified and enumerated by IDEM staff. The anticipated budget for laboratory cost for the project is outlined in Table 11.

Sample Group	# Events <mark>▼</mark>	Sample Se 🔻	QC Sets -	Parameters -	Lab ▼	Cost	t/Event <mark></mark> ▼	Co	st 🔻	C	ost + 10%
Reference Sites	27	12	0	BOD, Ophos	ISDH	\$	39.05	\$	958.50	\$	1,054.3
	27	12	0	Nx	ISDH	\$	112.75	· ·	2,767.50	\$	3,044.2
	27	12	12	Gc, Mt, D	Pace	\$	447.09		0,974.00	\$	12,071.4
										\$	-
19 Other Sites	57	12	12	GC, Nx	ISDH	\$	266.75	\$1	3,822.50	\$	15,204.7
	57	12	12	BOD, Ophos	ISDH	\$	50.40	\$	2,611.50	\$	2,872.6
JSGS parameters	28	12		Chl-A /Pheophyton, AFDM	USGS	\$	367.46	\$1	0,288.88	\$	10,288.8
Totals								\$4	1,422.88	\$	44,536.2
ISDH - Lab Account					ISDH						18,249.0
ISDH - Supplemental 106					ISDH					\$	3,927.0
Pace - Lab Account					Pace					\$	12,071.4
USGS - Supplemental 106					USGS					\$	10,288.8
Totals		1		1						\$	44,536.2
Total Lab Account					Pace					\$	12,071.4
ISDH Services at no additional	costs				ISDH					\$	-
Total Supplemental 106					ISDH/L	JSGS	6			\$	14,215.8
Total laboratory Expenditures											26,287.2

Table 11. Total Estimated Laboratory Cost for the Project.

No additional QA/QC for reference site samples sent to ISDH since they are included with the other samples collected.

Pace Bot	ttle Orde	:				
Bottles:	Blank	MS/MSD	Normal	DUP	Totals	Preserv CS
Ice or None	12	36	30	12	90	0
H ₂ SO ₄	0	0	0	0	0	0
HNO ₃	24	24	30	36	114	4.75
NaOH	0	0	0	0	0	0
Totals:	36	60	60	48	204	17
# Samples:	12	12	15	12	51	
ISDH Bottles	s - All Sites (i	34 Events)				
Bottles:	Blank	MS/MSD	Normal	DUP	Totals	Preserv CS
Ice or None	24	60	144	24	252	0
H ₂ SO ₄	24	36	144	24	228	9.5
HNO3	12	12	72	12	108	4.5
NaOH	0	0	0	0	0	0
Totals:	60	108	360	60	588	49
# Samples:	12	12	72	12	108	
Bottle Co	sts	49*40	\$1,960.00			
Bottle Co HNO3	sts	49*40 5*\$69	\$1,960.00 345			

Table 12. Total Estimated Water Sample Bottles Needed.

Role	Required Training/Experience	Training References	Training Notes
All Staff that Participate in Field Activities	-Basic First Aid and Cardio-Pulmonary Resuscitation (CPR)	-A minimum of 4 hours of in-service training provided by WAPB (IDEM 2010b)	-Staff lacking 4 hours of in-service training or appropriate certification will be accompanied in the field at all times by WAPB staff that meet Health and Safety Training requirements
	-Personal Protective Equipment (PPE) Policy	-IDEM 2008	
	-Personal Flotation Devices (PFD)	-February 29, 2000 WAPB internal memorandum regarding use of approved PFDs	-When working on boundary waters as defined by Indiana Code (IC) 14-8-2-27 or between sunset and sunrise on any waters of the state, all personnel in the watercraft must wear a high intensity whistle and Safety of Life at Sea (SOLAS) certified strobe light.

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Attachment 1. IDEM OWQ-WAPB Site Reconnaissance Form.

					Recon #: Trip #:						
tte Number:	si [Stream:		County:						
ocation Des	cription:										
	and a second	ance Data Collect	and the second se		wner/Contact In						
8	Recon Date	Crew	Members	First Name Last Name							
vg. Width (m)	Avg. Depth (m)	Max. Depzh (m) Nearesz Town		Street A ddress	- <u>1</u> 2						
Water	Site Wadeable?	Riffle/Run	Road/Public	City		State	Zíp				
Present?		Present?	Access Possible?	,			-6				
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Acces	ss Route	Pre-Recon		1 555			2				
	ž.	Recon In proce Approved Site	166			Backpack Boat					
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	ę.	No, Impounder No, Marsh/We				Seine					
Sampli	ing Effort	No, Bridge gor	e or not accessible e to traffic or location			Weighted Waders	Handline				
		No, Site Impac No, Other	ted by backwater			Gill Net					
					8.00						
omments											

Attachment 2. IDEM OWQ-WAPB Stream Sampling Field Data Sheet.

	ЧY] <u>St</u>	ream	Samp	ling F	ield	l Da	ata S	heet	Analysis	Set #	EPA Site ID	Rank	
Sample #	t i	Site #			Sample N	lediun	n		S	ample Type	mple Type		ple #	
Stream Nam							R	iver Mile:	¢ .		Cour	ny:		
Site Descript				8t-	O all a da d	_			Vater	_			_	
Survey Crew Chief		le Collect			Collected	Ну	drolat #		h/Gage H	t Water Flo (cf/sec)		Flow Algae	Aquatic	
CION CITIO	1 4	2 3	4	Date	Time	_		_	(ft)	(0000)	Loc			
Samo	ie Taken?		Alle	uots	Wa	ter Flo			v	ater Appeara	nce	Canopy C		
Ves Ves					Riffie	Dry		_	Clear	Green	Sheer	0-20%	60-80%	
No; Stream No; Owner r				12 24 A8-Flow] Run] Eddy		Flood Other	G Murky Brown		Other		80-100%	
Special	enuced Add			LI AS-FIOW		1 Eddy		Uther		Li Gray (sep	uuraewa	age) 🔲 40-80%		
Notes:														
Field Data	a:													
Date (m/d/yy)	24-hr Tin (hh:mm		pH .	Water Temp (°C)	Spec Cond (µohms/om)	Turbi (NT		% Sat.	Chiorine (mg/l)	Chloride (mg/l)	Chioro (m)		WS AT	
Comments														
Comments														
Comments														
Comments														
Comments														
Comments														
					leter Measurer					Weather Cod	e Defin	itions		
			ags	 > Max. Meter Measurement E Estimated (See Comments) R Rejected (See Comments) 				SC Sky Conditions		WD Ons Wind Direction		WS Wind Strength	AT Air Temp	
Field Cali	bration	s:						Clear Scattered	8 Rain 9 Snow	00 North (0 deg 09 East (90 deg		rees) 0 Calm 1<32		
Date	Time	Calibrat	or	Calib	rations		3	Partly	10 Sleet	18 South (180	degrees)	2 Mod./Light	346-60	
(m/d/yy)	(hh:mm)	Initials	Туре	Meter	# Value	Uni	5	Cloudy Mist		27 West (270 d	egrees)	3 Moderate 4 Mod./Strong	461-75	
					_			Fog Shower				5 Strong 6 Gale	6>86	
				<u> </u>		-								
		Calibratio	DO											
Preservat	tives/Bo	Type offle I o	Turbidity fs:					Groups	: Presen	vatives		Bottle Types		
Group: Pres				Bottle Typ	e Bottle L	.ot#	GC	General C		ce		2000mL Plastic, Na	mow Mouth	
								Nutrients: Metals: Hi	NO3		500P	1000mL Plastic, Na 500mL Plastic, Nan	row Mouth	
								Cyanide: I Oli & Grea	ise: H280	4	1000G	250mL Plastic, Nan 1000mL Glass, Nan	rrow Mouth	
							Ecoli	Toxics: Ice Bacteriolo	gy: ice		250G	500mL Glass, Wide 250mL Glass, Wide	Mouth	
							Pest	Pesticides	cice	CI & Thiosulfate	40GV	125mL Glass, Wide 40mL Glass Vial		
							Phen Sed	Phenols: H Sediment:			1000PF	120ml Plastic (Bact 1000mL Plastic, Co	ming Filter	
							Gly Hg	Glyphosat Mercury(1	e: Thiosulf 631): HCl	ate	500PF 60P	500mL Plastic, Con 50mL Plastic	ning Filter	
							Cr6 MeHg	Chromium	VI(1636):		250T 500T	250mL Teflon 500mL Teflon		
										-	125T	125mL Tefion		

Data Entered By: _____ QC1: _____ QC2: _____

Stream Sampling Field Data Sheet

Attachment 3. IDEM OWQ-WAPB Algal Biomass Lab Data Sheet.

								-1					
ample #		Site						Stream					
		L					_						
upporting) Site Inform	ation											
aditional f	Forestry % CI	losed Cano	py: □ <	-10m C				nly if width <=10		to nearest	whole per	rcent)	
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iytoplank	ton Informa	tion											
Sampling	Method: 🗆 (Grab Sampi			e Verticle				Number o	f Verticles	-		
	Chiorphyli A		E	Blank		Filter 1		Filter 2		Filter 3		Filte	r 4
		mple Time											
	Sample Vo	Nume (mL)											
	Chlorphyll A		E	Blank	_	Filter 1		Filter 2		Filter 3		Filte	r 4
		mple Time											
	Sample Vo	lume (mL)											
ariphyton	Area Calcul												
eriphyton Cylinder								Area Scrape (Using SG	;-92)			
	Scrape	ation	umference	e		Area		Area Scrape (Rock#	Using SG	-92) 2	3	4	
Cylinder Snag #		ation	umferenci U ₂	e Us	U	Area (L°U)		Rock# Area (cm ²)			7.38	4 7.38	
Cylinder Snag # 1	Scrape Length	lation Circ			U			Rock#	1	2			
Cylinder Snag # 1 2	Scrape Length	lation Circ			U			Rock# Area (cm ²)	1	2	7.38		
Cylinder Snag # 1	Scrape Length	lation Circ			U			Rock# Area (cm ²) Total (cm ²)	1 7.38	2 7.38	7.38		
Cylinder Snag # 1 2 3	Scrape Length	lation Circ			U			Rock# Area (cm ²) Total (cm ²) Petri Dish	1 7.38 crete San	2 7.38 nples (n):	7.38	7.38	
Cylinder Snag # 1 2 3 4	Scrape Length	lation Circ		Us	U ea (cm²)			Rock# Area (cm ²) Total (cm ²) Petri Dish Number of Dis	1 7.38 crete San One Samp	2 7.38 nples (n): ler (a):	7.38 36.9	7.38	
Cylinder Snag # 1 2 3 4 5	Scrape Length (cm)(L)		U2	Us				Rock# Area (cm ²) Total (cm ²) Petri Dish Number of Dis Total Area of C	1 7.38 crete San One Samp	2 7.38 nples (n): ler (a):	7.38 36.9	7.38	
Cylinder Snag # 1 2 3 4 5	Scrape Length		U2	Us				Rock# Area (cm ²) Total (cm ²) Petri Dish Number of Dis Total Area of C	1 7.38 crete San One Samp	2 7.38 nples (n): ler (a):	7.38 36.9	7.38	
Cylinder Snag # 1 2 3 4 5 5	Scrape Length (cm)(L)	ation Circ	U ₂	U ₃ Total Ar	ea (cm²)		-	Rock# Area (cm²) Total (cm²) Petri Dish Number of Dis Total Area of C Total Sample /	1 7.38 crete San One Samp Area (n * a	2 7.38 nples (n): ler (a): a):	7.38 36.9	7.38	
Cylinder Snag # 1 2 3 4 5 5 ream Dis Nearest U River mile	Scrape Length (cm)(L) charge / Rate SGS Gage S s from site:	ation Circ	U ₂	U ₃ Total Ar	ea (cm²)	(L * U)	Dis	Rock# Area (cm²) Total (cm²) Petri Dish Number of Dis Total Area of (Total Sample / Charge CFS at a	1 7.38 crete San Dre Samp Area (n * a	2 7.38 nples (n): ier (a): a):	7.38 36.9	7.38 2m²	
Cylinder Snag # 1 2 3 4 5 tream Dis Nearest U River mile Gage loca	Scrape Length (cm)(L) charge / Rate SGS Gage S s from site: tton:	ation Circ U ₁	U2	U ₃ Total Ar Downstr	ea (cm²) eam 🗆	(L*U)	Dis Dis	Rock# Area (cm²) Total (cm²) Petri Dish Number of Dis Total Area of C Total Sample /	1 7.38 crete San Dre Samp Area (n * a sampling: ce 50% fit	2 7.38 nples (n): ler (a): a): CFS ow exceed	7.38 36.9 19.01 c	7.38 2m²	

Review 2 Completed

Review 1 Completed

Attachment 4. IDEM OWQ-WAPB Physical Description of Stream Site Form (front).

Revised 4/20/12		
Probabilistic Monito	oring Section Physical	Description of Stream Site
Stream :	AIMS #	Program #:
		Crew
General Stream Description	:	
Characteristics at the site a	and immediately upstrean	n (check All that apply).
Outer Riparian Zone L R □ Agricultural Row crop □ Agricultural Pasture □ Devoid of Vegetation □ Fallow □ Forested □ Residential □ Commercial/Industrial □ Weeds and Scrub □ Other	Agricultural Devoid of Ve Devoid for the second sec	Rowcrop
Flow above site Riffle Pool Eddy Run Glide Other	Flow at site Riffle Pool Eddy Run Glide Other	Substrate (if visable) Cobble Boulder Sand Muck Silt Gravel Bedrock Other
Characteristics at site and	immediately upstream (cl	heck ONE).
Water Description Clear Grey (Septic) Murky Black Brown	Sinuosity of Channel High Moderate Low Channelized	Discharge Pipe Present ☐ No ☐ Yes If yes, Effluent Flowing? ☐ No ☐ Yes

Continued on back

□ Green □ Other Description of Effluent

Attachment 4. IDEM OWQ-WAPB Physical Description of Stream Site Form (back).

Revised 4/20/12			
Stream Bank			
<u>Functional Slope</u> : <u>L</u> <u>R</u> 0-30° 31-50° 51-70° 1 71-90°	Bank Erosion: <u>L R</u> D Low Moderate High	Percent Canopy Clo Stream Stage 1-5 (J Velocity of Stream	
Visible Stream Degr	adation? □ Yes □ No	D	
Description:			
Aquatic Life Observe			
Description:			
Algae Observed?	Yes 🗆 No		
Description:			
Rooted Macrophytes	Observed? □ Yes □	No	
Description:			
Additional Comment	ts:		
Follow Up Date:	Time:	_Crew Chief:	Crew:
Follow Up Date:	Time:	_Crew Chief:	Crew:
			; ; ; nown measurement, etc.)

Attachment 5. IDEM OWQ-WAPB Fish Collection Data Sheet (front).

	OWQ-WA	IDEM TERSHED ASSESSMENT	AND PLANNING BRANCH	
Voltage	Time fished (sec)	Distance fished (m)	Equipment Max. depth (m) If no, why	
Elapsed time at si	te (hh:mm): Comm	nents		· · · · · · · · · · · · · · · · · · ·

Museum data: Initials_____ ID date_____ Jar count_____ Fish Total_____

 $\begin{array}{l} \mbox{Coding for Anomalies: } D-deformities \ E-eroded fins \ L-lesions \ T-tumor \ M-multiple \ DELT anomalies \ O-other (A-anchor worm \ C-leeches \ W-swirled scales \ Y-popeye \ S-emaciated \ F-fungus \ P-parasites) \ H-heavy \ L-light (these codes may be combined with above codes) \end{array}$

TOTAL # OF FISH	L # OF FISH (mass g) (length						ANOMALIES							
	(11055 g)		(length mm) Min length	D	E	L	т	м	0					
			May log oth											
V P			Max length											
			Min length	D	E	L	т	м	0					
V P			Max length											
			Min length	D	E	L	т	м	0					
			Mary Israeth											
V P			Max length											
			Min length	D	E	L	т	м	0					
	l e l		Max length											
V P			Maxiengen											
			Min length	D	E	L	т	м	0					
			Max length											
V P														
			Min length	D	E	L	т	м	0					
			Max length											
V P														

MKM: Rev/February 19, 2014

Attachment 5. IDEM OWQ-WAPB Fish Collection Data Sheet (back).

Event ID		 			Page		_of	_
		Min length	D	E	L	Т	м	0
		Max length						
V P								
		Min length	D	E	L	т	м	0
V P		Max length						
		Min length	D	E	L	т	м	0
	┦────┦────							
V P		Max length						-
•		Min length	D	E	L	т	м	0
	┦────┦───		-	-	-			
		Max length						
V P		Min length						
			D	E	L	Т	M	0
		Max length						
VP	 	 Min los oth					-	
		 Min length	D	E	L	Т	м	0
		Max length						
V P								
		Min length	D	E	L	т	м	0
		Max length						
V P								
		Min length	D	E	L	т	м	0
V P		 Max length						\vdash

	Office of	Water Quality:	Macroinve	ertebrate	e Header	
L-Site #	Event ID	Stream Name	Locatio	m .	County	Surveyor
Sample Date Si	_	Quality Rejected	Macro Sample Black Light CPOM Hester-Dendy	□ Kick □ MHAB	□ Normal □ Duplicate _ □ Replicate _	
<u>Riparian Zo</u>	one/Instrea	am Features				
Watershed Eros Heavy Moderate None		/atershed NPS Pollution: No Evidence Obvious Sources Some Potential Sources				
Stream Depth Riffle (m):	Stream Depth Run (m):	Stream Depth Pool (m):	Distances Riffle-Riffle (m):	Distances Bend-Bend (
Stream Width (m): High W	/ater Mark (m): Velocity	(ft/s):			
Stream Type: Cold Warm	Turbidity Clear Opaque Dam Pr	Slightly Turbid	ty (mg/L):	ORP (mV):]	
Predominant Su Other	irrounding Lan	d Use: □ Forest □ Field/Pastu	re 🗆 Agricultural	□ Residential □	Commercial	Industrial

Attachment 6. IDEM OWQ-WAPB Macroinvertebrate Header Form.

Sediment

Sediment Odors: Normal Sewage Petroleum Chemical Anaerobic None O	ther
Sediment Deposits: Sludge Sawdust Paper Fiber Sand Relic Shells Other	
Sediment Oils: Absent OModerate Profuse OSight	

Are the undersides of stones, which are not deeply embedded, black?

Substrate Components

(Note: Select from 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% for each inorganic/ organic substrate component)

	Inorgan	ic Substrate C	omponents (%	Diameter)	Org	anic Substr	ate Components (% 1	Type)		
Bedrock	Boulder	Cobble	Gravel	Sand	Cit	Clay	Detritus	Detritus	Muck/Mud	Marl(gray w/
Bedrock	(>10 in)	(2.5-10 in)	(0.1-2.5 in)	(gritty)	SIIC	(slick)	(sticks, wood)	(CPOM)	(black, fine FPOM)	shell fragments)

Water Quality

Water Odors:
Normal
Sewage
Petroleum
Chemical
None Other
Water Surface Oils:
Slick
Sheen
Glob
Flocks
None

IDEM 03/14/13

Sample AB	Macro			Strea	m				Loc				Cnty	CntyDate				
	Flow Type	BR	BL	CB	GC	GF	SA	FN	HP	AR	AL	MA	WD	DT	ov	UB	01	
Sweep 1	RF RN PL GL																	
Sweep 2	RF RN PL GL																	
Sweep 3	RF RN PL GL							Î.										
Sweep 4	RF RN PL GL																	
Sweep 5	RF RN PL GL																	
Sweep 6	RF RN PL GL																	
Sweep 7	RF RN PL GL																	
Sweep 8	RF RN PL GL																	
Sweep 9	RF RN PL GL																	
Sweep 10	RF RN PL GL							-										
Sweep 11	RF RN PL GL			-	· · · · ·													
Sweep 12	RF RN PL GL													-				
Sweep 13	RF RN PL GL																	
Sweep 14	RF RN PL GL																	
Sweep 15	RF RN PL GL																	
Sweep 16	RF RN PL GL													-				
Sweep 17	RF RN PL GL																	
Sweep 18	RF RN PL GL																	
	RF RN PL GL				-		-	-		-				-				
Sweep 20	RF RN PL GL									-				×				
Sweep 21	RF RN PL GL																	
	RF RN PL GL			· · · · ·	·													
Sweep 23	RF RN PL GL			-				-			-			-				
•	RF RN PL GL							-						-				
•	RF RN PL GL																	
	RF RN PL GL				2 A													
Sweep 27	RF RN PL GL							-		-				-				
	RF RN PL GL																-	
	RF RN PL GL			-													-	
Sweep 30	RF RN PL GL							-	_					-				
Sweep 31	RF RN PL GL																-	
	RF RN PL GL			-													-	
Sweep 33	RF RN PL GL			-				-		-							-	
Sweep 34	RF RN PL GL			-														
<u> </u>	RF RN PL GL							-						-				
•	kick (in 10%)				-												-	
Flow Type: 250 mm; G Hardpan (fi	RF Riffle, RN R C Gravel, Coars irm, consolidat Petritus (wood	se (16 - ed fine	- 64 mr e subst	m); GF (rate); /	Gravel, AR Arti	Fine fical su	(2 - 16 Ibstrate	mm); es (rip	SA San rap); A	d (0.06 L Filam	6 - 2 mi entou	m); FN s Algae	Silt/Cl ; MA N	aγ/Mu /lacrop	ck (no hytes;	gritty WD W); HI ood	
Sweeps: Cir	cle the flow ty	pe the	n rank	by do	minand	e (4, 3	, 2, 1) t	he fou	r most	preval	ent sul	ostrate	s in th	e swee	p sam	ple.		
	Enter the perc	-				199				-								

Attachment 7. Macroinvertebrate MHAB Sample Habitat Form.

Sa	mple AB	М	acro	Str	eam		_	_Lo	_		_	_				Cnt		_		te	_	_
Co	ISW	at X	_SW*15=_	SW*0.0)9=	SW*0.1=	BR	BL	СВ	GC	GF	SA	FN	HP	AR	AL	MA	WD	DT	٥V	UB	C
-	ERL L M H	RWL >	50 10-50 5	5-10 V N		K1 RF RN PL GL																L
ans	ERR L M H	RWR	>50 10-50 5	5-10 V N	L L	K2 RF RN PL GL																
ĥ	SWLD_	_MD	RD_U_R	_D_L_	T VI	F M S IS IM ED																
2	ERL L M H	RWL>	50 10-50 5	5-10 V N		K1 RF RN PL GL																
ans.	ERR L M H	RWR	>50 10-50 5	5-10 V N	L Z	K2 RF RN PL GL																Г
Ē	SWLD_	_MD	RD_U_R	_D_L_	T VI	F M S IS IM ED																Γ
ŝ	ERL L M H	RWL>	50 10-50 5	i-10 V N		K1 RF RN PL GL																Г
Trans.	ERR L M H	RWR	>50 10-50 5	5-10 V N	Ξ	K2 RF RN PL GL																Γ
Ē	SWLD_	MD_	RD_U_R	_D_L_	ΤV	F M S IS IM ED																T
4	ERL L M H	RWL>	50 10-50 5	5-10 V N	~	K1 RF RN PL GL																t
us.	ERR L M H	RWR	>50 10-50 5	5-10 V N	Σ	K2 RF RN PL GL																t
Tra	SWLD_	MD_	RD_U_R	_D_L_	τv	F M S IS IM ED																t
ŝ	ERLLMH	-			œ	K1 RF RN PL GL					_										_	t
us.	ERR L M H	RWR	>50 10-50 5	5-10 V N	Σ	K2 RF RN PL GL																t
Tra	SW LD	MD	RD_U_R	DL		F M S IS IM ED		-													-	t
9	ERL L M H				æ	K1 RF RN PL GL			-		_			_							_	t
	ERR L M H	-				K2 RF RN PL GL		-			_										-	t
Tra	SW LD		RD_U_R		_	F M S IS IM ED								_								t
2	ERLLMH	-			_	K1 RF RN PL GL										_					_	t
ŝ	ERRLMH	-			Σ	K2 RF RN PL GL															-	t
Tra	SW LD	-	RD_U_R	1025 20		F M S IS IM ED			-		-				-	_						t
8	ERL L M H					K1 RF RN PL GL					_					-			_			t
ns.	ERRLMH	_				K2 RF RN PL GL			-		8											t
Tra	SW LD		RD_U_R		-	F M S IS IM ED					<u> </u>				2							t
6	ERLLMH	-				K1 RF RN PL GL			_		_				_				_		_	┢
us.	ERRLMH					K2 RF RN PL GL		-				-				-		-				t
Tra	SWLD_	_	RD U R	5.03 10	_	F M S IS IM ED			-								_					t
10	ERLLMH		50 10-50 5		_	K1 RF RN PL GL			_		_			-	_			_	_		_	┢
•	ERRLMH					K2 RF RN PL GL		-	2		2				8 - 11 9 - 12		-	3			i i	┢
Trans		-	RD_U_R	1000 00		F M S IS IM ED	\vdash		-		_	-			-	_					-	┢
-	Trans 1.	<u></u>				1 11 5 15 11 12 5																_
	Trans 2.	-																				-
	Trans 3.	+																				
es	Trans 4.	+																				
Not	Trans 5.	1																				
neral	Trans 6.	1																				
ene	Trans 7.																					-
9	Trans 8.	1																				
	Trans 9.	+																				
	Trans 10.	+																				
																						_

Attachment 8. Macroinvertebrate Transect Sample Habitat Form.

ERosion, Left, Right - Low, Med, High; Riparian Width, Left, Right - Very narrow, Narrow; Stream Width; Left Depth, Middle Depth, Right Depth; Densiomter - Upstream, Right, Downstream, Left; Kick Location - Left, Middle, Right; Kick, 1, 2 - RiFfle, RuN, PooL, GLide; Current velocity - Torrential, Very Fast, Fast, Moderate, Slow, InterStitial, InterMittent, EDdies

Substrate: BR Bedrock (>4000mm); BL Boulder (250 - 4000 mm); CB Cobble (64 - 250 mm; GC Gravel, Coarse (16 - 64 mm); GF Gravel, Fine (2 - 16 mm); SA Sand (0.06 - 2 mm); FN Silt/Clay/Muck (not gritty); HP Hardpan (firm, consolidated fine substrate); AR Artifical substrates (rip rap); AL Filamentous Algae; MA Macrophytes; WD Wood (logs); DT Detritus (woody debris, CPOM); OV Overhanging Vegetation; UB Undercut Banks (incl. rootwads/rootmats); OT Other (list in notes)

Attachment 9. IDEM OWQ-WAPB Biological Qualitative Habitat Evaluation Index (front).

IDEM		OWQ Bio	ogical QHEI	(Qualitati	ve Habitat I	Evaluation	Index)	
	Sample #		bioSample #	Strea	m Name		Location	
	Surveyor	Sample Date	County	Macro Sa	nple Type	🗆 Habitat		
			,			Complete	QHEI So	ore:
1] <i>SU</i>	BSTRATE	Check ONLY Two pr				Charle ONE (2.2.8	
PREDOMIN	BEST TYPE			IER TYPES PRESENT TOT		IGIN	•	LITY
	LDR/SLABS[1			4]		TONE[1] [1]	s□ HEAVY I□ MODE	[-2] VATE[-1]
	OULDER[9] OBBLE[8]	· == ==	DE DETRITUS DE MUCK[2]	3 == =	- Wetla Hardi	NDS[0]	L□ NORM/ □ FREE[1	
	RAVEL 7		□□ SILT[2]		SANDS	TONE [0]	5	<u> </u>
	and[6] Edrock[5]	88	(Score natura	l substrates; igr	CIP/R/ Nore □ LACUS	AP[0] TRINE[0]	D EXTEN	
NUMB	ER OF BEST	TYPES: 4 or 3 or		rom point-source	≝) □ Shale □ Coale	[-1] -INES [-2]		
Comm							8	-
quality; 1	2-Moderate an	OVER Indicate pre nounts, but not of hi	sence 0 to 3 and e ghest quality or in :	timate percent: small amounts o	0–Absent; 1–Ve f highest quality;	ry small amount 3-Highest		non of marginal MOUNT
		greater amounts (e. sloped root wad in d						: (Or 2 & average) IVE > 75% [11]
% Amour			% Amount		Amount	ackwaters[_ □ MODER/	ATE 25 - 75% [7] 5 - < 25% [3]
	OVERHANGI	IG VEGETATION [1]	ROOTV	IADS[1]	AQUATIC M	ACROPHYTES	[Î]□ NEARLY	ABSENT < 5%[1]
	ROOTMATS[1	NSLOW WATER)[: L]	L] BOULD	HO[1]		1000 V DEBRIS	[1]	Cover Maximum
Comm	nents							20
		RPHOLOGY				CTAD		
□ HIG	OSITY H[4]		BNT[7] [CHANNELIZ		STAB:	H[3]	a 1999
		□ GOOD [□ FAIR [3	l' (RECOMEREI	Ġ[3]		XERATE[2] /[1]	Channel Maximum
Comm	Æ[1] vents	POOR[ij i	□ RECENTOR	NORECOVERY[1]		20
4] BAI	NK EROSIO	ON AND RIPAR	IAN ZONE Che	ck ONE in each	category for EAC	H BANK (Or 2 p	er bank & average	±)
River	right looking down	stream L R RIPA	RIAN WIDTH		D PLAIN QU	ALITY	L R	TION TILLAGE [1]
	ONE/LITTLE	3] 🗆 MODE	RATE 10-50m[3]	D SHRUB	OROLDFIELD[2]	URBANOR	INDUSTRIAL [0]
	ioderate[2] Eavy/severe	E[1] 🗆 🗆 VERVI	DW 5-10m[2] WARROW [1]		NTIAL, PARK, NE) PASTURE [1]	Indica	te predominant la	nd use(s)
			[0]	D D OPEN P	ASTURE, ROWC	ROP[0] past 1	00m riparian.	Riparian Maximum
Comm 51 PO		AND RIFFLE/	RUN OUALITY	,				10
MAX	IMUM DEP	TH CHAN	NEL WIDTH		CURRENT VE			reation Potential
	ONE (ONLY!) 1m[6]		(Or 2 & average) OTH > RIFFLE WID		Check ALL the RRENTIAL [-1]	□ SLOW[1]	` □	e and comment on back) Primary Contact
	7-<1m[4] 4-<0.7m[2]		OTH=RIFFLEWID OTH <rifflewid< td=""><td></td><td>RYFAST[1] ST[1]</td><td> INTERŠTĪ INTERMUT </td><td></td><td>Secondary Contact Pool/ N</td></rifflewid<>		RYFAST[1] ST[1]	 INTERŠTĪ INTERMUT 		Secondary Contact Pool/ N
	2-<04m[1] :02m[0] [me				ODERATE[1] licate for reach -	EDDIES[1	-	Current Maximum
Comm	ents	-				poors and mile		12
	ate for function fle-obligate spe	nal riffles; Best area ecles:	must be large end	ugn to support		Or 2 & average)		FLE [metric = 0]
	E DEPTH	RUND m[2] □ MAXD			N SUBSTRAT	E RIFFL	e/run embe None[2]	DDEDNESS
BES	TAREAS5-10	lamī[1] □ MAXO	4UM < 50cm[1] [MOD.STABL	E(eg,LargeGra	avel)[1] □	LOW[1]	Riffle/
		m nic=0]			eg, Fine Gravel,		EXTENSIVE	
6] GR	ADIENT (ft/mi)	U VERY LOW -		%POOL:] %GL	(DE:	Gradient
DR	AINAGEA	REA (mi ²)	MODERATE[%RUN: [Maximum 10
								IDEM 11/15/12

Attachment 9 (continued). IDEM OWQ-WAPB Biological QHEI (back).

		owo	2 Biological	QHEI (Qualit	ative Hal	bitat Evaluation Index)	
A-CANOPY	B-AESTHETIC	s		C-RECRE	ATION	D-MAINTENANCE	E-ISSUES
□ >85%-Open	Nuisance algae		heen	Area	Depth	Public Private	WWTP COO NPDES
□ 55%-<85%	Invasivemaar	ophytes 🗆 Tras	h/Litter	Pook □ > 100 ft ²	□ >3ft	Active Ellistoric	□ Industry □ Urban
□ 30%-<55%	🗆 Excess turbidit	y ⊡ Nuis	anceodor			Succession: 🗆 Young 🗆 Old	□ Hardened □ Dirt & Grime
□ 10%-<30%	Discoloration	□ Slud	gedeposits			□ Spray □ Islands □ Scoured	Contaminated Landii
10%-Cosed	Foam/Soum	CS0	s/SSOs/Outfails			Snag: □ Removed □ Modified	BMPs: Construction Sediment
						Leveed: 🗆 One sided 🗆 Both banks	Logging Imigation Cooling
Looking upstream (> 10m, 3 rea	adings, ≤ 10m, 1 reading"	in middle); Round	to the nearest w	hole percent		Relocated Outoffs	Erosion: Bank Surface
Right	Middle	Left	Total Averag	e		Bedload: 🗆 Moving 🗆 Stable	🗆 Falsebank 🗆 Manure 🗆 Lagoon
% open %	%	%	9/0			Amound Sumps	□Wash H₂O □ Tile □ H₂O Table
						Impounded Desiccated	Mine: 🗆 Acid 🗆 Quany
	~ /	· ·				Flood control Drainage	Flow: 🗆 Natural 🗆 Stagnant
\sim	\sim	\sim					🗆 Wetland 🗆 Park 🗆 Golf
	\sim	\sim					🗆 Lawn 🗆 Home
	/ \	<pre>/</pre>					Atmospheric deposition
							□ Agriculture □ Livestock

Stream Drawing:

IDEM 11/15/12

Attachment 10. IDEM OWQ-WAPB Chain of Custody Form.



Indiana Department of Environmental Management OWQ Chain of Custody Form Project:

OWQ Sample Set or Trip #:

I Certify that the sample(s) listed below was/were collected by me, or in my presence. Date:_

Signature:									Se	ction:									
Sample Media (□	Water, 🗆 Alga	ie,⊡ Fisl	h, 🗆 Ma	acro, 🗆 (Cyanob	acteria/I	Microcy	stin, ⊡	Sedime	nt)									
Lab Assigned	IDEM	Sample Type	ID	ш.	ш.	40 ml Vial	120 ml P (Bact)	2000 ml Nalgene	250 ml Nalgene	125 ml Glass	a s	ul ss	ml ss	a s	ene ml	Date and Tir	me Collected		check bottle
Number / Event ID	Control Number	San T ₃		1000 ml P.N.M.	1000 ml G.N.M.	40 Vi	120 P (E	200(Nalg	250 Nalg	125 Gla	Date	Time	per	sent					
													<u> </u>						
													<u> </u>						
													+						
													+						
													<u> </u>						
													<u> </u>						
													+						
													+						
													+						
P = Plastic	G = Glass	N.	M. = Na	rrow Mo	outh	Bact =	Bacter	iologica	l Only	L	Should samples	be iced?	Y	N					
M = MS/MSD	MS/MSD B = Blank D = Duplicate R = Revisit																		

Carriers

I certify that I have received the above sample(s).							
Signature	Date	Time	Seals Intact		Comments		
Relinquished By:			v	N			
Received By:				, N			
Relinquished By:			v	N			
Received By:			T	IN			
Relinquished By:			v	N			
Received By:			ſ	N			
IDEM Storage Room #							

Lab Custodian

I certify that I have received the above sample(s), which has/have been recorded in the official record book. The same sample(s) will be in the custody of competent laboratory personnel at all times, or locked in a secured area.

Signature:

Date:_____ Time:____

Lab:____

Address:

Revision Date: 4/27/2016

Attachment 11. IDEM OWQ-WAPB Water Sample Analysis Request Form.



Indiana Department of Environmental Management Office of Water Quality Watershed Planning and Assessment Branch www.idem.IN.gov

Water Sample Analysis Request

Project Name:

Composite 🗌 Grab 🖂

IDEM Sample Nos.	
Lab Sample Nos.	
Lab Delivery Date	
	IDEM Sample Nos. Lab Sample Nos.

Parameter	Test Method	Total	Dissolved
Alkalinity (as CaCO ₃)	EPA 310.2	**	
Total Solids	SM 2540B	**	
Suspended Solids	SM 2540D	⊠ **	
Dissolved Solids	SM 2540C		× 12
Sulfate	EPA 375.2	⊠ **	
Chloride	SM 4500CI-E	**	
Hardness (as CaCO ₃)	EPA 130.1	⊠ **	
Fluoride	380-75WE	*	
Silica (Reactive)	SM 4500-SiD		
Parameter Antimony	Test Method 200.8	Total	Dissolved
Priority Pollutant M			
	17.1 V 1 V 1 V 1		
Arsenic	200.8		
Beryllium	200.8		
Cadmium	200.8		
Chromium (Hex)	SM 3500Cr-D		
Chromium (Total)	200.8		
Copper	200.8		
Lead	200.8		
Mercury,	EPA 245.1		
Nickel	200.8		
Selenium	200.8		
Silver	200.8		
Thallium	200.8		
Zinc	200.7		
Cations and Seco	ndary Metals I	Paramete	rs
Parameter	Test Method	Total	Dissolved
Aluminum	200.7, 200.8		

200.8

200.8

200.7

200.8

200.7

200.7

200.7, 200.8

SM 3500Ca-D

200.7, 200.8

SM 3500-K D

Parameter	Test Method	Total
Priority Pollutants: Oranochlorine Pesticides and PCBs	EPA 608	
Polynuclear Aromatic Hydrocarbons	EPA 610	
Priority Pollutants: VOCs - Purgeable Organics	EPA 624	
Priority Pollutants: Base/Neutral Extractables	EPA 625	
Priority Pollutants: Acid Extractables	EPA 625	
Phenolics, 4AAP	EPA 420.4	
Oil and Grease, Total	EPA 1664A	
Semi-volatile Organics & Pesticides	EPA 525.2	

Parameter	Test Method	Total	Dissolved
Ammonia Nitrogen	EPA 350.1		
CBOD ₆	SM 5210B		
CBODu	SM 5210B		
Total Kjeldahl Nitrogen (TKN)	EPA 351.2		
Nitrate + Nitrite	EPA 353.1		
Dissolved Reactive Phosphorus	SM4500-P		
Total Phosphorus	EPA 365.1		
TOC	SM 5310B		
COD (Low Level)	SM 5220D		
Cyanide (Total)	EPA 335.4		
Cyanide (Free)	SM 4500CN-I	*	
Cyanide (Amenable)	SM 4500CN-G		

Bacteriological V	Vater Parameter	s	\$2
Parameter	Test Method	Total	Dissolved
E. coli (Colilert Method)	SM9223B		

30 day reporting time required.

Notes:

** = DO NOT RUN PARAMETER IF SAMPLE IDENTIFIED AS A BLANK ON THE CHAIN OF CUSTODY

* = RUN ONLY IF TOTAL CYANIDE IS DETECTED

*** = Report Calcium, Magnesium as Total Hardness components if Hardness is calculated

Testing Laboratory: Indiana State Department of Health (ISDH) Environmental Laboratory Division 550 W. 16th Street Indianapolis, IN 46202 Phone: 317-921-5815 (Ray Beebe) (Rev. 6/2013)

Send reports (Fed. Ex. or UPS) to: Deliver reports to: David Jordan - IDEM Mail Code 65-40-2 (Shadeland) 100 N. Senate Ave. Indianapolis, IN 46204-2251

Boron

Calcium

Cobalt

Magnesium

Manganese

Potassium

Sodium

Strontium

Iron

Calcium (as CaCO₃)

David Jordan - IDEM **STE 100** 2525 North Shadeland Ave. Indianapolis, IN 46219 DJordan@idem.in.gov

×**

Nutrients/Diel Dissolved Oxygen Pilot Study Work Plan B-033-OWQ -WAP-PRB-17-W-R0 April 28, 2017

Nutrients/Diel Dissolved Oxygen Pilot Study Work Plan B-033-OWQ -WAP-PRB-17-W-R0 April 28, 2017

Appendix 1. Pace Laboratory Inc., Indianapolis: Accreditation Documents



Michael R. Pence Governor

Jerome M. Adams, MD, MPH State Health Commissioner

CERTIFIED MAIL NO. 7000 0520 0012 9325 6837 RETURN RECEIPT REQUESTED

May 6, 2015

Beth Schrage Pace Analytical Services, Inc. 7726 Moller Road Indianapolis, Indiana 46268

Dear Ms. Schrage:

On April 22, 2015, Philip Zillinger, Chemistry Laboratory Certification Officer, Chemistry Laboratory, ISDH Laboratories, Indiana State Department of Health (ISDH), visited the laboratory of the Pace Analytical Services, Inc., 7726 Moller Road, Indianapolis, to conduct an on-site evaluation. The laboratory was evaluated for purposes of determining the laboratory's capabilities for analyzing samples for metals, cyanide, fluoride, nitrate, nitrite, volatile organic compound (VOC) and trihalomethane (THM) content pursuant to the National Primary Drinking Water Regulations (NPDWR) as implemented by 40 CFR Part 141 and the Indiana Primary Drinking Water Regulations (IPDWR) as implemented by 327 IAC 8-1 and 8-2.

Based on the information contained in the attached evaluation report, the recommendation of the survey officer, and the performance evaluation sample results, the ISDH hereby issues the following determination, pursuant to IC 4-21.5-3-5:

- The laboratory is hereby granted full certification for: *antimony, arsenic, barium, beryllium, cadmium, chromium, cyanide, fluoride, mercury, nickel, selenium, thallium, copper, lead, nitrate, nitrite, the regulated volatile organic compounds (VOC), vinyl chloride and trihalomethanes (THM).*
- This certification is valid for three (3) years from the date of this letter, with continuing successful performance on performance evaluation samples.
- The laboratory has been assigned laboratory number C-49-06. This number is to be used on all reports used for compliance monitoring of public water supplies.



2 North Meridian Street ● Indianapolis, IN 46204 317.233.1325 tdd 317.233.5577 www.statehealth.in.gov

To promote and provide essential public health services.

Beth Schrage

2

May 6, 2015

If you wish to seek review or stay of the effectiveness of this determination, pursuant to IC 4-21.5-3-7, you are required to submit, in writing, a petition, on or before May 25, 2015, to:

Office of the Secretary Indiana State Department of Health 2 North Meridian Street Indianapolis, Indiana 46204-3006

The petition for review or stay must include facts demonstrating that:

- The petitioner is a person to whom the determination is specifically directed;
- The petitioner is aggrieved or adversely affected by the agency determination; or,
- The petitioner is entitled to review under any law.

Dated at Indianapolis, Indiana, this 6th day of May, 2015.

Sincerely,

Julith C Loople

Jugath C. Lovchik, PhD, D(ABMM) Assistant Commissioner, Public Health Protection and Laboratory Services Indiana State Department of Health 550 West 16th Street Indianapolis, Indiana 46202 317 921-5808

A copy of this letter was sent on the above date, postage prepaid first class mail, to:

Matthew Prater Indiana Department of Environmental Management Drinking Water Branch 100 North Senate Avenue Indianapolis, IN 46204



Indiana State Department of Health

SCOPE OF CERTIFICATION PACE ANALYTICAL SERVICES, INC. INDIANAPOLIS, INDIANA

ANALYTE	METHOD	ANALYTE	METHOD
METALS		PCB	-
Antimony	EPA 200.8R5.4	as decachlorobiphenyl	Not certified
Arsenic	EPA 200.8R5.4		
Barium	EPA 200.7R4.4;	VOC	
-	EPA 200.8R5.4		
Beryllium	EPA 200.7R4.4;	20 regulated VOC	EPA 524.2R4.1
	EPA 200.8R5.4		
Cadmium	EPA 200.7R4.4;	Vinyl chloride	EPA 524.2R4.1
	EPA 200.8R5.4	1	
Chromium	EPA 200.7R4.4;	DBCP	Not certified
	EPA 200.8R5.4		
Copper	EPA 200.7R4.4;	EDB	Not certified
	EPA 200.8R5.4		
Lead	EPA 200.8R5.4		
Mercury	EPA 245.1R3.0	TTHM	
Nickel	EPA 200.7R4.4;	4 THM	EPA 524.2R4.1
	EPA 200.8R5.4		
Selenium	EPA 200.8R5.4		
Thallium	EPA 200.8R5.4	PAH	
		Benzo(a)pyrene	Not certified
NONMETALS			
Cyanide	EPA 335.4R1.0	ADIPATE/PHTHALATE	
Fluoride	EPA 300.0R2.1	Di(2-ethylhexyl)adipate	Not certified
Nitrate	EPA 300.0R2.1	Di(2-ethylhexyl)phthalate	Not certified
Nitrite	EPA 300.0R2.1		
	1	CARBAMATES	
PESTICIDES		Carbofuran	Not certified
Alachlor	Not certified	Oxamyl (vydate)	Not certified
Atrazine	Not certified		
Chlordane	Not certified	HERBICIDES	
Endrin	Not certified	2,4-D	Not certified
Heptachlor	Not certified	2,4,5-TP (silvex)	Not certified
Heptachlor epoxide	Not certified	Dalapon	Not certified
Hexachlorobenzene	Not certified	Dinoseb	Not certified
Hexachlorocyclopentadiene	Not certified	Diquat	Not certified
Lindane	Not certified	Endothall	Not certified
Methoxychlor	Not certified	Glyphosate	Not certified
Simazine	Not certified	Pentachlorophenol	Not certified
Toxaphene	Not certified	Picloram	Not certified



SCOPE OF CERTIFICATION PACE ANALYTICAL SERVICES, INC. INDIANAPOLIS, INDIANA

ANALYTE	METHOD	ANALYTE	METHOD
DISINFECTION		MISCELLANEOUS	
BYPRODUCTS		ANALYTES	
HAA5	Not certified	2,3,7,8-TCDD (dioxin)	Not certified
Bromate	Not certified	Asbestos	Not certified
Chlorite	Not certified		



KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT ENVIRONMENTAL LABORATORY ACCREDITATION CLEAN WATER ACT PROGRAM - NON-POTABLE WATER MATRIX

PAGE: 1

Pace Analytical Services, Inc.	Certificate Number: E-10177	
7726 Moller Road	Effective Date: 05/01/2016	
Indianapolis, IN 46268-4163	Expiration Date: 04/30/20	
	Reciprocity:	

The laboratory listed above is hereby approved for environmental laboratory accreditation in accordance with K.S.A. 65-1, 109a for the following:

**DEMANDS BOD {SM 5210 B} cBOD {SM 5210 B} COD {EPA 410.4} COD {HACH 8000} Total Organic Carbon {SM 5310 B, C, or D}

****METALS** Aluminum {EPA 200.7} Aluminum {EPA 200.8} Antimony {EPA 200.7} Antimony {EPA 200.8} Arsenic {EPA 200.7} Arsenic {EPA 200.8} Barium {EPA 200.7} Barium {EPA 200.8} Beryllium {EPA 200.7} Beryllium {EPA 200.8} Boron {EPA 200.7} Boron {EPA 200.8} Cadmium {EPA 200.7} Cadmium {EPA 200.8} Calcium {EPA 200.7} Chromium {EPA 200.7} Chromium {EPA 200.8} Chromium, VI {SM 3500-Cr B} Cobalt {EPA 200.7} Cobalt {EPA 200.8} Copper {EPA 200.7} Copper {EPA 200.8} Iron {EPA 200.7} Lead {EPA 200.7} Lead {EPA 200.8} Magnesium {EPA 200.7} Manganese {EPA 200.7} Manganese {EPA 200.8} Mercury {EPA 1631 E} Mercury {EPA 245.1} Molybdenum {EPA 200.7} Molybdenum {EPA 200.8} Nickel {EPA 200.7} Nickel {EPA 200.8} Potassium {EPA 200.7} Selenium {EPA 200.7} Selenium {EPA 200.8} Silver {EPA 200.7} Silver {EPA 200.8} Sodium {EPA 200.7} Thallium {EPA 200.7} Thallium {EPA 200.8} Tin {EPA 200.7} Tin {EPA 200.8} Titanium {EPA 200.7} Titanium {EPA 200.8} Vanadium {EPA 200.7}

 KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT ENVIRONMENTAL LABORATORY ACCREDITATION RCRA PROGRAM - NON-POTABLE WATER AND SOLID/CHEMICAL WASTE MATRICES
 PAGE: 1

 This certificate supersedes all previous certificates
 Pace Analytical Services, Inc.
 Certificate Number: E-10177

 7726 Moller Road
 Effective Date: 05/01/2016
 Effective Date: 05/01/2016

 Indianapolis, IN 46268-4163
 Expiration Date: 04/30/2017

 Reciprocity:
 The laboratory listed above is hereby approved for environmental laboratory accreditation in accordance with K.S.A. 65-1, 109a for the following:

**CHARACTERISTICS Ignitability {EPA 1010} Synthetic-Precipitation Leaching Procedure {EPA 1312} Toxic-Characteristic-Leaching Procedure {EPA 1311}

**METALS

Aluminum {EPA 6010} Aluminum {EPA 6020} Antimony {EPA 6010} Antimony {EPA 6020} Arsenic {EPA 6010} Arsenic {EPA 6020} Barium {EPA 6010} Barium {EPA 6020} Beryllium {EPA 6010} Beryllium {EPA 6020} Boron {EPA 6010} Cadmium {EPA 6010} Cadmium {EPA 6020} Calcium {EPA 6010} Chromium {EPA 6010} Chromium {EPA 6020} Chromium, VI {EPA 7196} Cobalt {EPA 6010} Cobalt {EPA 6020} Copper {EPA 6010} Copper {EPA 6020} Iron {EPA 6010} Lead {EPA 6010} Lead {EPA 6020} Magnesium {EPA 6010} Manganese {EPA 6010} Manganese {EPA 6020} Mercury {EPA 7470} Mercury {EPA 7471} Molybdenum {EPA 6010} Nickel {EPA 6010} Nickel {EPA 6020} Potassium {EPA 6010} Selenium {EPA 6010} Selenium {EPA 6020} Silver {EPA 6010} Silver {EPA 6020} Sodium {EPA 6010} Thallium {EPA 6010} Thallium {EPA 6020} Tin {EPA 6010} Titanium {EPA 6010} Vanadium {EPA 6010} Vanadium {EPA 6020} Zinc {EPA 6010} Zinc {EPA 6020}

****MINERALS**

KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT ENVIRONMENTAL LABORATORY ACCREDITATION CLEAN WATER ACT PROGRAM - NON-POTABLE WATER MATRIX

PAGE: 2

Vanadium {EPA 200.8} Zinc {EPA 200.7} Zinc {EPA 200.8}

**METALS - 503 Regs Arsenic {EPA 6010} Arsenic {EPA 6020} Cadmium {EPA 6010} Cadmium {EPA 6020} Chromium, Total {EPA 6010} Chromium, Total {EPA 6020} Copper {EPA 6010} Copper {EPA 6020} Lead {EPA 6010} Lead {EPA 6020} Mercury {EPA 7470} Mercury {EPA 7471} Molybdenum {EPA 6010} Molybdenum {EPA 6020} Nickel {EPA 6010} Nickel {EPA 6020} Selenium {EPA 6010} Selenium {EPA 6020} Zinc {EPA 6010} Zinc {EPA 6020}

**MINERALS Acidity {SM 2310 B}

Alkalinity {SM 2320 B} Chloride {EPA 300.0} Chloride {SM 4500-Cl E} Fluoride {EPA 300.0} Fluoride {SM 4500-F C} Hardness {SM 2340 B or C} Sulfate {EPA 300.0} Sulfate {SM 4500-S-2 D}

**MISCELLANEOUS Bromide {EPA 300.0} Chlorine - Total {SM 4500-Cl G} Conductivity {EPA 120.1} Conductivity {SM 2510 B} Cyanide {EPA 335.4} Cyanide {SM 4500-CN C} Cyanide {SM 4500-CN C} Hydrogen Ion (pH) {SM 4500-H B} Oil & Grease {EPA 1664} Phenolics {EPA 420.4} Surfactants {SM 5540 C} Turbidity {EPA 180.1}

**NUTRIENTS Ammonia {EPA 350.1} Ammonia {SM 4500-NH3 G} K Nitrogen {EPA 351.2} Nitrate-Nitrite {EPA 300.0} Nitrate-Ritrite {EPA 353.2} Nitrate {EPA 300.0} Nitrate {EPA 353.2} Nitrite {EPA 353.2} Organic-Nitrogen {TKN-NH3-CAL} Ortho-phosphate {SM 4500-P E} Phosphorus {EPA 365.1}

KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT ENVIRONMENTAL LABORATORY ACCREDITATION CLEAN WATER ACT PROGRAM - NON-POTABLE WATER MATRIX

PAGE: 3

Phosphorus {SM 4500-P E} **ORGANIC CHEMISTRY VOLATILES (MEASUREMENT BY GC/MS) {EPA 624} 1,1-Dichloroethane {EPA 624} 1,1-Dichloroethene {EPA 624} 1,1,1-Trichloroethane {EPA 624} 1,1,2-Trichloroethane {EPA 624} 1,1,2,2-Tetrachloroethane {EPA 624} 1,2-Dichlorobenzene {EPA 624} 1,2-Dichloroethane {EPA 624} 1,2-Dichloropropane {EPA 624} 1,3-Dichlorobenzene {EPA 624} 1,4-Dichlorobenzene {EPA 624} 2-Chloroethyl vinyl ether {EPA 624} Acrolein {EPA 624} Acrylonitrile {EPA 624} Benzene {EPA 624} Bromodichloromethane {EPA 624} Bromoform {EPA 624} Bromomethane (Methyl bromide) {EPA 624} Carbon tetrachloride {EPA 624} Chlorobenzene {EPA 624} Chloroethane {EPA 624} Chloroform {EPA 624} Chloromethane (Methyl chloride) {EPA 624} cis-1,3-Dichloropropene {EPA 624} Dibromochloromethane {EPA 624} Ethylbenzene {EPA 624} Methylene chloride (Dichloromethane) {EPA 624} Tetrachloroethene (Perchloroethylene) {EPA 624} Toluene {EPA 624} trans-1,2-Dichloroethene {EPA 624} trans-1,3-Dichloropropene {EPA 624} Trichloroethene (Trichloroethylene) {EPA 624} Trichlorofluoromethane {EPA 624} Vinyl chloride **ORGANIC CHEMISTRY (MEASUREMENT BY GC) {EPA 608} 4,4'-DDD {EPA 608} 4,4'-DDE {EPA 608} 4,4'-DDT {EPA 608} Aldrin {EPA 608} alpha-BHC (alpha-Hexachlorocyclohexane) {EPA 608} beta-BHC (beta-Hexachlorocyclohexane) {EPA 608} Chlordane {EPA 608} delta-BHC {EPA 608} Dieldrin {EPA 608} Endosulfan I {EPA 608} Endosulfan II {EPA 608} Endosulfan Sulfate {EPA 608} Endrin {EPA 608} Endrin aldehyde {EPA 608} gamma-BHC (Lindane, gamma-Hexachlorocyclohexane) {EPA 608} Heptachlor {EPA 608} Heptachlor epoxide {EPA 608} PCB-1016 {EPA 608} PCB-1221 {EPA 608} PCB-1232 {EPA 608} PCB-1242 {EPA 608} PCB-1248 {EPA 608} PCB-1254 {EPA 608} PCB-1260 {EPA 608} Toxaphene

KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT PAGE: 4 **ENVIRONMENTAL LABORATORY ACCREDITATION CLEAN WATER ACT PROGRAM - NON-POTABLE WATER MATRIX **ORGANIC CHEMISTRY (MEASUREMENT BY GC/MS)** {EPA 625} 1,2,4-Trichlorobenzene {EPA 625} 2-Chloronaphthalene {EPA 625} 2-Chlorophenol {EPA 625} 2-Methyl-4,6-dinitrophenol {EPA 625} 2-Nitrophenol {EPA 625} 2,2'-Oxybis(1-chloropropane) {EPA 625} 2,4-Dichlorophenol {EPA 625} 2,4-Dimethylphenol {EPA 625} 2,4-Dinitrophenol {EPA 625} 2,4-Dinitrotoluene (2,4-DNT) {EPA 625} 2,4,6-Trichlorophenol {EPA 625} 2,6-Dinitrotoluene {EPA 625} 3,3'-Dichlorobenzidine {EPA 625} 4-Bromophenyl phenyl ether {EPA 625} 4-Chloro-3-methylphenol {EPA 625} 4-Chlorophenyl phenyl ether {EPA 625} 4-Nitrophenol {EPA 625} Acenaphthene {EPA 625} Acenaphthylene {EPA 625} Anthracene {EPA 625} Benzidine {EPA 625} Benzo(a)anthracene {EPA 625} Benzo(a)pyrene {EPA 625} Benzo(b)fluoranthene {EPA 625} Benzo(g,h,i)perylene {EPA 625} Benzo(k)fluoranthene {EPA 625} Benzyl butyl phthalate {EPA 625} Bis(2-chloroethoxy)methane {EPA 625} Bis(2-chloroethyl)ether {EPA 625} Bis(2-ethylhexyl)phthalate {EPA 625} Chrysene {EPA 625} Di-n-butyl phthalate {EPA 625} Di-n-octyl phthalate {EPA 625} Dibenzo(a,h)anthracene {EPA 625} Diethyl phthalate {EPA 625} Dimethyl phthalate {EPA 625} Fluoranthene {EPA 625} Fluorene {EPA 625} Hexachlorobenzene {EPA 625} Hexachlorobutadiene {EPA 625} Hexachloroethane {EPA 625} Indeno(1,2,3-cd) pyrene {EPA 625} Isophorone {EPA 625} N-nitroso-di-n-propylamine (NDPA) {EPA 625} N-nitrosodimethylamine (NDMA) {EPA 625} N-Nitrosodiphenylamine {EPA 625} Naphthalene {EPA 625} Nitrobenzene {EPA 625} Pentachlorophenol {EPA 625} Phenanthrene {EPA 625} Phenol {EPA 625} Pyrene ****RESIDUES** Residue, Filterable (TDS) {SM 2540 C} Residue, Non Filterable (TSS) {SM 2540 D} Residue, Settleable {SM 2540 F} Residue, Total {SM 2540 B} **SUPPLEMENTAL {ASTM D516-02} Sulfate {EPA 608} Methoxychlor

{EPA 624} Naphthalene

KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT ENVIRONMENTAL LABORATORY ACCREDITATION CLEAN WATER ACT PROGRAM - NON-POTABLE WATER MATRIX

PAGE: 5

{EPA 624} Xylene (total)
{EPA 625} 1,2-Dichlorobenzene
{EPA 625} 1,3-Dichlorobenzene
{EPA 625} 1,4-Dichlorobenzene
{EPA 8205} Propylene Glycol
{EPA 8260B} Xylene (total)
{EPA 8260B} 1,1,2-Trichlorobenzene
{EPA 8260B} 1,3,5-Trichlorobenzene
{EPA 8270C} Carbazole
{EPA 8270C} Carbazole
{EPA 8270C} Carbazole
{EPA 8270C} CGC/FID}} Ethane
{EPA RSK-175 (GC/FID)} Ethene
{EPA RSK-175 (GC/FID)} Methane

End of Parameter List

KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT PAGE: 1 ENVIRONMENTAL LABORATORY ACCREDITATION RCRA PROGRAM - NON-POTABLE WATER AND SOLID/CHEMICAL WASTE MATRICES

This certificate supersedes all previous certificates

This certificate supersearce an previous certificates	
Pace Analytical Services, Inc.	Certificate Number: E-10177
7726 Moller Road	Effective Date: 05/01/2016
Indianapolis, IN 46268-4163	Expiration Date: 04/30/2017
	Reciprocity:

The laboratory listed above is hereby approved for environmental laboratory accreditation in accordance with K.S.A. 65-1, 109a for the following:

**CHARACTERISTICS Ignitability {EPA 1010} Synthetic-Precipitation Leaching Procedure {EPA 1312} Toxic-Characteristic-Leaching Procedure {EPA 1311}

**METALS

Aluminum {EPA 6010} Aluminum {EPA 6020} Antimony {EPA 6010} Antimony {EPA 6020} Arsenic {EPA 6010} Arsenic {EPA 6020} Barium {EPA 6010} Barium {EPA 6020} Beryllium {EPA 6010} Beryllium {EPA 6020} Boron {EPA 6010} Cadmium {EPA 6010} Cadmium {EPA 6020} Calcium {EPA 6010} Chromium {EPA 6010} Chromium {EPA 6020} Chromium, VI {EPA 7196} Cobalt {EPA 6010} Cobalt {EPA 6020} Copper {EPA 6010} Copper {EPA 6020} Iron {EPA 6010} Lead {EPA 6010} Lead {EPA 6020} Magnesium {EPA 6010} Manganese {EPA 6010} Manganese {EPA 6020} Mercury {EPA 7470} Mercury {EPA 7471} Molybdenum {EPA 6010} Nickel {EPA 6010} Nickel {EPA 6020} Potassium {EPA 6010} Selenium {EPA 6010} Selenium {EPA 6020} Silver {EPA 6010} Silver {EPA 6020} Sodium {EPA 6010} Thallium {EPA 6010} Thallium {EPA 6020} Tin {EPA 6010} Titanium {EPA 6010} Vanadium {EPA 6010} Vanadium {EPA 6020} Zinc {EPA 6010} Zinc {EPA 6020}

**MINERALS

KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT PA ENVIRONMENTAL LABORATORY ACCREDITATION RCRA PROGRAM - NON-POTABLE WATER AND SOLID/CHEMICAL WASTE MATRICES

PAGE: 2

Bromide {EPA 9056} Chloride {EPA 9056} Fluoride {EPA 9056} Sulfate {EPA 9038} Sulfate {EPA 9056}

**MISCELLANEOUS Cyanide {EPA 9012} Cyanide, Amenable to CL {EPA 9012} Hydrogen Ion (pH) {EPA 9040} Hydrogen Ion (pH) {EPA 9045} Paint Filter Liquids Test {EPA 9095} Phenolics {EPA 9066}

**NUTRIENTS Nitrate {EPA 9056} Nitrite {EPA 9056}

**ORGANIC CHEMISTRY VOLATILES (MEASUREMENT BY GC) {EPA 8015} 1-Propanol {EPA 8015} Ethanol {EPA 8015} Ethylene glycol {EPA 8015} Isobutyl Alcohol {EPA 8015} Isobutyl Alcohol {EPA 8015} Methanol {EPA 8015} n-Butyl alcohol {EPA 8015] n-Butyl alcohol {EPA 8021} Benzene {EPA 8021] Benzene {EPA 8021] Ethylbenzene {EPA 8021] ortho-Xylene {EPA 8021] para-Xylene {EPA 8021] Toluene

**ORGANIC CHEMISTRY VOLATILES (MEASUREMENT BY GC/MS) {EPA 8260} 1,1-Dichloroethane {EPA 8260} 1,1-Dichloroethene {EPA 8260} 1,1-Dichloropropene {EPA 8260} 1,1,1-Trichloroethane {EPA 8260} 1,1,1,2-Tetrachloroethane {EPA 8260} 1,1,2-Trichloroethane {EPA 8260} 1,1,2,2-Tetrachloroethane {EPA 8260} 1,2-Dichlorobenzene {EPA 8260} 1,2-Dichloroethane {EPA 8260} 1,2-Dichloropropane {EPA 8260} 1,2,3-Trichlorobenzene {EPA 8260} 1,2,3-Trichloropropane {EPA 8260} 1,2,4-Trichlorobenzene {EPA 8260} 1,2,4-Trimethylbenzene {EPA 8260} 1,3-Dichlorobenzene {EPA 8260} 1,3-Dichloropropane {EPA 8260} 1,3,5-Trimethylbenzene {EPA 8260} 1,4-Dichlorobenzene {EPA 8260} 1,4-Dioxane {EPA 8260} 2-Chloro-1,3-butadiene {EPA 8260} 2-Chloroethyl vinyl ether {EPA 8260} 2-Chlorotoluene {EPA 8260} 2-Hexanone {EPA 8260} 2,2-Dichloropropane {EPA 8260} 4-Chlorotoluene {EPA 8260} 4-Isopropyltoluene {EPA 8260} 4-Methyl-2-Pentanone (MIBK) {EPA 8260} Acetone {EPA 8260} Acetonitrile {EPA 8260} Acrolein

KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT PAGE: 3 **ENVIRONMENTAL LABORATORY ACCREDITATION** RCRA PROGRAM - NON-POTABLE WATER AND SOLID/CHEMICAL WASTE MATRICES {EPA 8260} Acrylonitrile {EPA 8260} Allyl Chloride {EPA 8260} Benzene {EPA 8260} Bromobenzene {EPA 8260} Bromochloromethane {EPA 8260} Bromodichloromethane {EPA 8260} Bromoform {EPA 8260} Bromomethane (Methyl bromide) {EPA 8260} Carbon disulfide {EPA 8260} Carbon tetrachloride {EPA 8260} Chlorobenzene {EPA 8260} Chloroethane {EPA 8260} Chloroform {EPA 8260} Chloromethane (Methyl chloride) {EPA 8260} cis-1,2-Dichloroethylene {EPA 8260} cis-1,3-Dichloropropene {EPA 8260} Dibromochloromethane {EPA 8260} Dibromomethane {EPA 8260} Dichlorodifluoromethane {EPA 8260} Dichloromethane (Methylene chloride) {EPA 8260} Diethyl ether {EPA 8260} Ethyl acetate {EPA 8260} Ethyl methacrylate EPA 8260} Ethylbenzene EPA 8260} Ethylbenzene EPA 8260} Ethylene dibromide (EDB, 1,2-Dibromoethane) {EPA 8260} Hexachlorobutadiene {EPA 8260} Iodomethane {EPA 8260} Isobutyl Alcohol {EPA 8260} Isopropylbenzene {EPA 8260} meta-Xylene {EPA 8260} Methacrylonitrile {EPA 8260} Methyl ethyl ketone {EPA 8260} Methyl tert-butyl ether (MTBE) {EPA 8260} Methylmethacrylate {EPA 8260} n-Butyl alcohol {EPA 8260} n-Butylbenzene {EPA 8260} n-Propylbenzene {EPA 8260} Naphthalene {EPA 8260} ortho-Xylene {EPA 8260} para-Xylene {EPA 8260} Propionitrile {EPA 8260} sec-Butylbenzene {EPA 8260} Styrene {EPA 8260} t-Butyl alcohol {EPA 8260} tert-Butylbenzene {EPA 8260} Tetrachloroethene (Perchloroethylene) {EPA 8260} Toluene {EPA 8260} trans-1,2-Dichloroethylene {EPA 8260} trans-1,3-Dichloropropene {EPA 8260} trans-1,4-Dichloro-2-butene {EPA 8260} Trichloroethene (Trichloroethylene) {EPA 8260} Trichlorofluoromethane {EPA 8260} Vinyl Acetate {EPA 8260} Vinyl chloride **ORGANIC CHEMISTRY (MEASUREMENT BY GC) {EPA 8011} Dibromochloropropane (DBCP) {EPA 8011} Ethylene dibromide (EDB, 1,2-Dibromoethane) {EPA 8081} 4,4'-DDD {EPA 8081} 4,4'-DDE {EPA 8081} 4,4'-DDT {EPA 8081} Aldrin

- {EPA 8081} alpha-BHC (alpha-Hexachlorocyclohexane)
- {EPA 8081} alpha-Chlordane

KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT PAGE: 4 **ENVIRONMENTAL LABORATORY ACCREDITATION** RCRA PROGRAM - NON-POTABLE WATER AND SOLID/CHEMICAL WASTE MATRICES {EPA 8081} beta-BHC (beta-Hexachlorocyclohexane) {EPA 8081} Chlordane (Tech) {EPA 8081} delta-BHC {EPA 8081} Dieldrin {EPA 8081} Endosulfan I {EPA 8081} Endosulfan II {EPA 8081} Endosulfan Sulfate {EPA 8081} Endrin {EPA 8081} Endrin aldehyde {EPA 8081} Endrin ketone {EPA 8081} g-Chlordane {EPA 8081} gamma-BHC (Lindane, gamma-Hexachlorocyclohexane) {EPA 8081} Heptachlor {EPA 8081} Heptachlor epoxide {EPA 8081} Methoxychlor {EPA 8081} Toxaphene {EPA 8082} PCB-1016 {EPA 8082} PCB-1221 {EPA 8082} PCB-1232 {EPA 8082} PCB-1242 {EPA 8082} PCB-1248 {EPA 8082} PCB-1254 {EPA 8082} PCB-1260 {EPA 8141} Atrazine EPA 8141 Azinphos-methyl (Guthion) {EPA 8141} Chlorpyrifos {EPA 8141} Chlorpyrifos methyl {EPA 8141} Demeton-o {EPA 8141} Demeton-s {EPA 8141} Diazinon {EPA 8141} Dichlorovos (DDVP, Dichlorvos) {EPA 8141} Dimethoate {EPA 8141} Disulfoton {EPA 8141} Famphur {EPA 8141} Malathion {EPA 8141} Merphos {EPA 8141} Naled {EPA 8141} Parathion ethyl {EPA 8141} Parathion methyl {EPA 8141} Phorate {EPA 8141} Ronnel {EPA 8141} Simazine {EPA 8141} Stirophos {EPA 8141} Terbufos {EPA 8151} 2,4-D {EPA 8151} 2,4-DB {EPA 8151} 2,4,5-T {EPA 8151} 2,4,5-TP (Silvex) {EPA 8151} 3,5-Dichlorobenzoic acid {EPA 8151} 4-Nitrophenol {EPA 8151} Aciflurofen {EPA 8151} Bentazon {EPA 8151} Chloramben {EPA 8151} Dalapon {EPA 8151} DCPA Di-Acid {EPA 8151} Dicamba {EPA 8151} Dichlorprop {EPA 8151} Dinoseb (2-sec-butyl-4,6-dinitrophenol, DNBP) {EPA 8151} MCPA {EPA 8151} MCPP {EPA 8151} Pentachlorophenol {EPA 8151} Picloram **ORGANIC CHEMISTRY (MEASUREMENT BY GC/MS)

KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT PAGE: 5 ENVIRONMENTAL LABORATORY ACCREDITATION RCRA PROGRAM - NON-POTABLE WATER AND SOLID/CHEMICAL WASTE MATRICES {EPA 8270} 1-Naphthylamine {EPA 8270} 1,2-Dichlorobenzene {EPA 8270} 1,2-Diphenylhydrazine {EPA 8270} 1,2,4-Trichlorobenzene {EPA 8270} 1,2,4,5-Tetrachlorobenzene {EPA 8270} 1,3-Dichlorobenzene {EPA 8270} 1,3-Dinitrobenzene {EPA 8270} 1,4-Dichlorobenzene {EPA 8270} 1,4-Naphthoquinone {EPA 8270} 1,4-Phenylenediamine {EPA 8270} 2-Acetylaminofluorene {EPA 8270} 2-Chloronaphthalene {EPA 8270} 2-Chlorophenol {EPA 8270} 2-Methyl-4,6-Dinitrophenol {EPA 8270} 2-Methylnaphthalene {EPA 8270} 2-Methylphenol (o-Cresol) {EPA 8270} 2-Naphthylamine {EPA 8270} 2-Nitroaniline {EPA 8270} 2-Nitrophenol {EPA 8270} 2-Picoline {EPA 8270} 2,3,4,6-Tetrachlorophenol {EPA 8270} 2,4-Dichlorophenol: {EPA 8270} 2,4-Dimethylphenol {EPA 8270} 2,4-Dinitrophenol {EPA 8270} 2,4-Dinitrotoluene (2,4-DNT) {EPA 8270} 2,4,5-Trichlorophenol {EPA 8270} 2,4,6-Trichlorophenol {EPA 8270} 2,6-Dichlorophenol {EPA 8270} 2,6-Dinitrotoluene {EPA 8270} 3-Methylcholanthrene {EPA 8270} 3-Methylphenol (m-Cresol) {EPA 8270} 3-Nitroaniline {EPA 8270} 3,3'-Dichlorobenzidine {EPA 8270} 3,3'-Dimethylbenzidine {EPA 8270} 4-Aminobiphenvl {EPA 8270} 4-Bromophenyl phenyl ether {EPA 8270} 4-Chloro-3-methylphenol {EPA 8270} 4-Chloroaniline {EPA 8270} 4-Chlorophenyl phenyl ether {EPA 8270} 4-Methylphenol (p-Cresol) {EPA 8270} 4-Nitroaniline {EPA 8270} 4-Nitrophenol {EPA 8270} 5-Nitro-o-toluidine {EPA 8270} 7,12-Dimethylbenz(a)anthracene {EPA 8270} Acenaphthene {EPA 8270} Acenaphthylene {EPA 8270} Acetophenone {EPA 8270} alpha-alpha-Dimethylphenethylamine {EPA 8270} Aniline {EPA 8270} Anthracene {EPA 8270} Aramite {EPA 8270} Benzidine {EPA 8270} Benzoic acid {EPA 8270} Benzo(a)anthracene {EPA 8270} Benzo(a)pyrene {EPA 8270} Benzo(b)fluoranthene {EPA 8270} Benzo(g,h,i)perylene {EPA 8270} Benzo(k)fluoranthene {EPA 8270} Benzyl alcohol {EPA 8270} Bis(2-chloroethoxy)methane {EPA 8270} Bis(2-chloroethyl)ether {EPA 8270} Bis(2-chloroisopropyl)ether {EPA 8270} Bis(2-ethylhexyl)phthalate {EPA 8270} Butyl benzyl phthalate

KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT PAGE: 6 ENVIRONMENTAL LABORATORY ACCREDITATION RCRA PROGRAM - NON-POTABLE WATER AND SOLID/CHEMICAL WASTE MATRICES {EPA 8270} Chlorobenzilate {EPA 8270} Chrysene {EPA 8270} Di-n-butyl phthalate {EPA 8270} Di-n-octyl phthalate {EPA 8270} Diallate {EPA 8270} Dibenzofuran {EPA 8270} Dibenzo(a,h)anthracene {EPA 8270} Diethyl phthalate {EPA 8270} Dimethoate {EPA 8270} Dimethyl phthalate {EPA 8270} Dimethylaminoazobenzene {EPA 8270} Diphenylamine {EPA 8270} Disulfoton {EPA 8270} Ethyl methanesulfonate {EPA 8270} Famphur {EPA 8270} Fluoranthene {EPA 8270} Fluorene {EPA 8270} Hexachlorobenzene {EPA 8270} Hexachlorobutadiene (EPA 8270) Hexachlorocyclopentadiene {EPA 8270} Hexachloroethane {EPA 8270} Hexachlorophene {EPA 8270} Hexachloropropene {EPA 8270} Indeno(1,2,3-cd) pyrene {EPA 8270} Isodrin {EPA 8270} Isophorone {EPA 8270} Isosafrole {EPA 8270} Kepone {EPA 8270} Methapyrilene {EPA 8270} Methylmethanesulfonate {EPA 8270} N-nitroso-di-n-butylamine (NDBA) {EPA 8270} N-nitroso-di-n-propylamine (NDPA) {EPA 8270} N-nitrosodiethylamine (NDEA) {EPA 8270} N-nitrosodimethylamine (NDMA) {EPA 8270} N-Nitrosodiphenylamine {EPA 8270} N-Nitrosomethylethylamine {EPA 8270} N-Nitrosomorpholine {EPA 8270} N-Nitrosopiperidine {EPA 8270} N-Nitrosopyrrolidine {EPA 8270} Naphthalene {EPA 8270} Nitrobenzene {EPA 8270} Nitroquinoline-1-oxide {EPA 8270} o-Toluidine {EPA 8270} O,O,O-Triethylphosphorothioate {EPA 8270} Parathion ethyl {EPA 8270} Parathion methyl {EPA 8270} Pentachlorobenzene {EPA 8270} Pentachloronitrobenzene (PCNB) {EPA 8270} Pentachlorophenol {EPA 8270} Phenacetin {EPA 8270} Phenanthrene {EPA 8270} Phenol {EPA 8270} Phorate {EPA 8270} Pronamide (Kerb) {EPA 8270} Pyrene {EPA 8270} Pyridine {EPA 8270} Safrole {EPA 8270} Tetraethyl dithiopyrophosphate (Sulfotepp) {EPA 8270} Thionazine ****SUPPLEMENTAL** {EPA 8015} Propylene Glycol {EPA 8260B} Xylene (total) {EPA 8260} 1,1,2-Trichloro-1,2,2-trifluoroethane

KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT PA ENVIRONMENTAL LABORATORY ACCREDITATION RCRA PROGRAM - NON-POTABLE WATER AND SOLID/CHEMICAL WASTE MATRICES

PAGE: 7

{EPA 8260} 1,3,5-Trichlorobenzene {EPA 8270C} Carbazole {EPA 8270} 1-Methylnaphthalene

**TOTAL PETROLEUM HYDROCARBONS {EPA 8015} DRO {EPA 8015} GRO

End of Parameter List

Nutrients/Diel Dissolved Oxygen Pilot Study Work Plan B-033-OWQ -WAP-PRB-17-W-R0 April 28, 2017

Appendix 2. Onset HOBO® Dissolved Oxygen Logger (U26-001) Manual







HOBO Dissolved Oxygen Logger with Included Calibration Boot and Sponge (Shown Wet in Photo)

HOBO Dissolved Oxygen Logger

U26-001

Included Items:

Dissolved Oxygen

Sensor Cap

- Protective Guard
- Calibration Boot and Sponge

Required Items:

- Coupler (COUPLER-2-C) with USB Optic Base Station (BASE-U-4) or HOBO Waterproof Shuttle (U-DTW-1)
- HOBOware Pro 3.3.1 or later

Accessories:

- Replacement Dissolved **Oxygen Sensor Cap** (U26-RDOB-1)
- Anti-Fouling Guard (U26-GUARD-2) • Sodium Sulfite
- (U26-CAL-SOL)

You May Also Need:

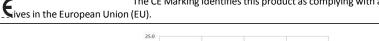
- For saltwater, salinity or conductivity measurements are required; HOBO Conductivity/SalinityLogger (U24-002-C) recommended
- For percent saturation, barometric pressure is required; HOBO Water Level Logger (U20-001-0x or U20L-0x) recommended

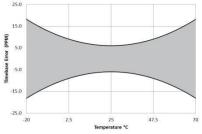
The HOBO Dissolved Oxygen logger is a standalone logger that uses RDO® Basic Technology to measure dissolved oxygen (DO). The logger has an optical sensor that provides 0.2 mg/L accuracy. The logger also features an easily replaceable sensor cap and an integrated temperature sensor. Using HOBOware® software for logger setup and a HOBO Waterproof Shuttle for quick data offload, this logger is easy to deploy in both freshwater and saltwater environments making it an ideal tool for environmental impact studies as well as ecological and oceanographic research. Using the data offloaded from the logger, the HOBOware Dissolved Oxygen Assistant can calculate percent saturation and salinity-adjusted DO concentration as well as correct for measurement drift from fouling (additional meter or logger measurements required).

Specifications

Dissolved Oxygen

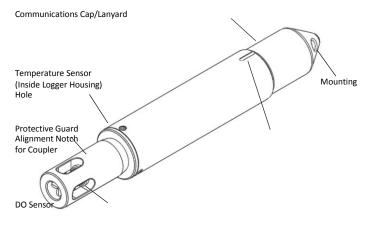
Dissolved Oxygen	
Sensor Type	Optical (dynamic luminescence quenching)
Measurement Range	0 to 30 mg/L
Calibrated Range	0 to 20 mg/L; 0 to 35°C (32 to 95°F)
Accuracy	± 0.2 mg/L up to 8 mg/L; ± 0.5 mg/L from 8 to 20 mg/L
Resolution	0.02 mg/L
Response Time	To 90% in less than 2 minutes
DO Sensor Cap Life	6 months (cap expires 7 months after initialization)
Temperature Temperature Measurement/ -5 Operating Range	5 to 40°C (23 to 104°F), non-freezing
Temperature Accuracy	0.2°C (0.36°F)
Temperature Resolution	0.02°C (0.04°F)
Response Time	To 90% in less than 30 minutes
Logger	
Memory memory); logging stops when memory fills	21,700 sets of DO and temperature measurements (64 KB tota
ogging Rate	1 minute to 18 hours
Fime Accuracy	±1 minute per month at 0 to 50°C (32 to 122°F) (see Plot A)
Battery	3.6 V lithium battery; factory replaceable
Battery Life	3 years (at 5 minute logging)
Download Type	Optical
Depth Rating	100 m (328 ft)
Wetted Materials	Black Delrin®, PVC, EPDM o-rings, silicon bronze screws; rated for saltwater use
Size	39.6 mm diameter x 266.7 mm length (1.56 x 10.5 inches); mounting hole 7.88 mm (0.31 inches)





Plot A: Time Accuracy

Logger Components and Operation



Communications Cap/Lanyard. This removable cap protects the optical communications window. An LED in the communications window of the logger confirms logger operation. When the logger is logging, the LED blinks once every four seconds. The LED also blinks when the logger is recording a sample. When the logger is awaiting a start because it is configured to start "At Interval," "On Date/Time," or "Using Coupler," the LED blinks once every eight seconds until logging begins. See *Connecting the Logger to a Computer or Waterproof Shuttle* for details on using the communications window.

Mounting Hole. Use the hole on the communications cap to mount the logger. See *Deploying the Logger* for more information.

Alignment Notch for Coupler. Use this notch to align the coupler when communicating with the logger. See *Connecting the Logger to a Computer or Waterproof Shuttle* for more information.

DO Sensor. This optical sensor measures dissolved oxygen using RDO[®] Basic Technology. It is shipped with a red dust cap that must be replaced with a green sensor cap that lasts for six months plus a one-month grace period. See *Installing the Sensor Cap* for more details.

Protective Guard. This removable guard protects the DO sensor. Unscrew it to install or replace the sensor cap as needed. See *Installing the Sensor Cap* for more details.

Temperature Sensor. This built-in sensor (not visible in diagram) measures temperature.

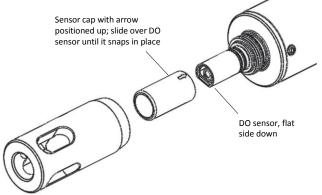
WANING: This logger can be damaged by mechanical shock. Always handle the logger with care. The logger may be damaged if it is dropped. Use proper packaging when transporting or shipping the logger.

Do not attempt to open the logger case or sensor housing. Disassembling of the logger case or sensor housing will cause serious damage to the sensor and logger electronics. There are no user-serviceable parts inside the case. Contact Onset Technical Support at 1-800-LOGGERS (1-800-564-4377) or an authorized Onset dealer if your logger requires servicing.

Installing the Sensor Cap

The logger ships with a replaceable sensor cap that provides six months of continuous use. Once the cap is initialized, an internal clock within the logger will count down until the sensor cap expiration date. When the sensor cap expires, you will need to replace it with a new cap (U26-RDOB-1). The sensor cap is intended for six months of actual deployment, but the expiration date is seven months from the date the cap was initialized. This allows for any time needed between launching the logger and physically deploying as well as extra time in case you are not able to get the logger after exactly six months of deployment. To install the sensor cap:

- 1. Unscrew the protective guard covering the DO sensor (see diagram at left).
- 2. Remove the red dust cap that protects the sensor during shipping.
- 3. Take the green sensor cap out of the canister.
- 4. With the flat part of the DO sensor pointing down and the the green sensor cap oriented with the arrow up, slide the sensor cap over the sensor until it snaps in place. The cap should be snug against the logger housing without any gaps.



5. Screw the external protective guard back on until tight.

IMPORTANT: The sensor cap expires 7 months (to the day) after it has been initialized. The logger will record a value of - 888 mg/L at each logging interval after the cap has expired. Initialization occurs automatically when the cap is installed while the logger is logging. You can also initialize it from the Status window in HOBOware or when using the Lab Calibration tool. To see when the sensor cap expires after being initialized, check the Status in HOBOware for the expiration date. The cap also has a shelf life; check the "Install By" date printed on the canister.

Connecting the Logger to a Computer or Waterproof Shuttle

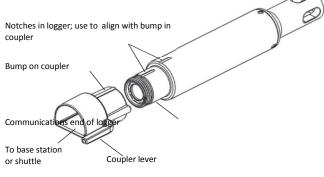
To connect the logger to a computer, use either the Optic USB Base Station (BASE-U-4) or HOBO Waterproof Shuttle (U-DTW-1) with a coupler (COUPLER2-C). To launch and read out the logger in the field, use one of these three methods:

- Laptop computer with Optic USB Base Station (BASE-U-4) and coupler (COUPLER2-C)
- HOBO Waterproof Shuttle (U-DTW-1, Firmware Version 3.2.0 or later) and coupler (COUPLER2-C)

• HOBO U-Shuttle (U-DT-1, Firmware Version 1.16 or later) with Optic USB Base Station and coupler (COUPLER2-C)

IMPORTANT: USB 2.0 specifications do not guarantee operation outside the range of 0°C (32°F) to 50°C (122°F).

- Follow the instructions that came with your base station or Waterproof Shuttle to attach it to a USB port on the computer.
- 2. Unscrew the pointed cap on the communications end of the logger.
- 3. Attach the coupler to the base station or shuttle.
- 4. Insert the logger into the coupler, aligning the bump/arrow on the coupler with the notches on the logger. Be sure that it is properly seated in the coupler. If the logger has never been connected to the computer before, it may take a few seconds for the new hardware to be detected by the computer. Note: If you are using the HOBO Waterproof Shuttle as a base station with a computer, briefly press the coupler lever to put the shuttle into base station mode. A green LED on the shuttle or base station indicates good communication.



5. After logger communications are complete, remove the logger from the coupler. Make sure the o-ring is still in the groove inside the cap and then reinstall the communications cap.

IMPORTANT: When connected to a coupler, the logger is "awake" and consumes significantly more power than when it is disconnected and considered "asleep." The logger will automatically "go to sleep" after being left in the coupler for 30 minutes. It will no longer appear as a USB device connected to the computer. If this occurs, remove it from the coupler and start the instructions to connect the logger to a computer or waterproof shuttle over again.

Calibrating the Logger with the Lab Calibration Tool

Use the Lab Calibration tool in HOBOware when you need to calibrate the logger before deploying it or after replacing an expired sensor cap. The tool sets the gain and offset adjustment values for the logger by:

- Restoring logger calibration values to the factory defaults,
- Using your own gain and offset adjustment values, or
- Calculating the values with a 3-step calibration procedure.

In the three-step procedure, the logger is first calibrated to 100% saturation by placing it in water-saturated air. Then, you can calibrate the logger to 0% saturation by placing it in sodium sulfite or another 0% oxygen environment (recommended if the logger will be deployed in water with DO levels of 4 mg/L or less).

IMPORTANT: Lab calibration only affects future launches; any data saved in the logger will be based on the previous calibration values. If the sensor cap is installed and it has not yet been initialized, you will be prompted to do so. Follow the instructions on the screen.

To complete these steps, you will need fresh water, the calibration boot and sponge supplied with the logger, and a source for current barometric pressure at your current location. You will also need sodium sulfite solution and a 7.6 cm (3 inch) beaker if you will be calibrating to 0% saturation.

The fresh water, logger, and sodium sulfite (if applicable) should be left out in the lab where the calibration is being done long enough so that they are at room temperature. If the logger was deployed previously, make sure the sensor is clean and dry (see *Maintenance* for more details). To use the Lab Calibration tool:

- Connect the logger to the computer as described in the previous section. Stop the logger if it is currently logging or awaiting a coupler or delayed start.
- 2. From the Device menu, click Lab Calibration.
- 3. The current gain and offset adjustments are displayed in the top pane of the Lab Calibration window along with the date and time the last lab calibration was completed (if applicable). Completing Steps 1 through 3 in the Lab Calibration tool will result in new gain and offset adjustment values based on the current logger conditions. Continue to the next section for details on how to complete these steps.

If you already know what the gain and offset values should be (for example, the values from a previous calibration that you want to use again) or want to return to the default factory values, click the "I know my values, skip to Finish" button. This will automatically move you to "Step 3: Finish" in the Lab Calibration window. Either click the "Reset to Factory Defaults" button or type in the desired gain adjustment and offset adjustment values and click the "Send Calibration to the Logger" button. **Note:** If you decide you do not need to change the calibration, click Close to cancel the calibration and revert back to the last saved logger values.

Step 1: 100% Saturation

- In "Step 1: 100% Saturation" in the Lab Calibration window, enter the barometric pressure for your current location. If the barometric pressure reading has been adjusted for sea level (such as a reading taken from the National Weather Service weather station), select the "If using sea level barometric pressure, enter elevation" checkbox and enter your elevation in either meters or feet.
- 2. Make sure the logger either has the protective guard or the anti-fouling guard installed (whichever guard you plan to use in the deployment) so that the sensor is covered.

3. Wet the small sponge with fresh water. Squeeze out any excess water.

4. Place the sponge in the end of the calibration boot.

5. Insert the logger in the calibration boot so that there is approximately a 1 cm (0.5 inch) overlap between the end of the boot and the body of the logger. This will ensure there is enough space between the end of the logger and the sponge (the logger should not be pressed up tightly against the sponge).

- Wait for approximately 15 minutes until the logger reaches temperature equilibrium (and less than 30 minutes so the logger does not go to sleep).
- Click the "Get DO value from the logger" button to display the 100% saturation results. You can click this button as often as needed. The results are updated each time you click the button. To check for equilibrium, click the "Get DO value from the logger" button several times in a row to check the current "DO Conc from logger at 100% Saturation" value. If the value remains the same or varies very little with each button click, then temperature equilibrium has likely been reached.
- When you are satisfied with the results displaying in the "Step 1: 100% Saturation" tab, click the Next button to proceed to "Step 2: 0% Saturation."

Step 2: 0% Saturation (optional)

If the logger will be deployed in water with DO levels greater than 4 mg/L, click the "Skip this Step" button. Otherwise, continue with the following procedure.

- 1. Make sure the logger either has the protective guard or the anti-fouling guard installed (whichever guard you plan to use in the deployment) so that the sensor is covered.
- 2. Pour the sodium sulfite into the beaker so that it is about two-thirds full.
- 3. Place the sensor end of the logger into the solution so that the entire protective guard or anti-fouling guard and at least 2.5 cm (1 inch) of the logger body are submerged in the beaker. Allow it to rest on the bottom of the beaker.
- 4. Wait for approximately 15 minutes until the logger reaches temperature equilibrium (and less than 30 minutes so the logger does not go to sleep).
- 5. Click the "Get DO value from the logger" button to display the 0% saturation results. As with the 100% calibration, you can click this button as often as needed. The results are automatically updated each time you click the button. If the value remains the same or varies very little with each button click, then temperature equilibrium has likely been reached.
- 6. When you are satisfied with the results displaying in the "Step 2: 0% Saturation" tab, click the Next button to proceed to "Step 3: Finish."

Step 3: Finish

The results from the first two steps are displayed as well as the overall calibration results and the new gain and offset adjustment values. If you are satisfied with the results, click the "Send Calibration to Logger" button. The logger will then be calibrated based on the new values. These values will not take effect until the logger is launched. If you do not want to save these values, click Close to cancel the calibration and revert back to the last saved logger values. Or, click "Reset to Factory Defaults" to return to the original values. If you performed Step 2, then remove the logger from the solution and thoroughly rinse it with fresh water to remove any excess sodium sulfite. See *Maintenance* for additional details on cleaning the logger.

Launching the Logger

After calibrating the logger, it needs to be launched to configure it before taking it to the field for deployment. Once launched, the logger will record two types of data: samples and events. Samples are the sensor measurements recorded at each logging interval. Events are independent occurrences triggered by a logger activity, such as Bad Battery or Host Connected. Events help you determine what was happening while the logger was logging. To launch the logger:

- 1. With the logger connected to the computer, open HOBOware. From the Device menu, select Launch.
- 2. Select both the DO and Temperature channels to log. Note: HOBOware provides the option of recording the current battery voltage at each logging interval, which is disabled by default. Recording battery life at each logging interval takes up memory and therefore reduces logging duration. It is recommended that you only record battery voltage for diagnostic purposes. Even with the channel disabled, a bad battery event will still be recorded.
- 3. Select a logging interval.
- 4. Choose when to start logging and click the Start button.
- 5. Remove the logger from the coupler and screw the communications cap back on the logger.

IMPORTANT: If this is the first launch with a new sensor cap, the sensor cap will expire six months (plus a one-month grace period) from the time of the first sensor reading. Two caps per year are required for year-round deployment.

Deploying the Logger

The logger is designed to be easy to deploy in many environments. Follow these guidelines when deploying it:

- Remove the calibration boot before deploying the logger.
- Make sure the logger is located where it will receive an unrestricted flow of the water being monitored to the sensor.
- Make sure the logger is fully submerged and not in direct sunlight to minimize temperature changes that are unrelated to water temperature.
- When deploying the logger in rivers, streams, and ponds, insert the logger in a PVC or ABS pipe for protection from debris (if possible). The pipe should have enough holes to ensure good circulation of water to the sensor.
- If possible, position the logger so the sensor face is oriented vertically. After deploying in the water, move the logger around slightly to eliminate any bubbles that may have formed.

- Do not deploy the logger in freezing water with moving ice where the logger could be crushed.
- Use the optional anti-fouling guard to protect against fouling. Unscrew the protective guard and replace it with the anti-fouling guard.
- If fouling is expected during deployment, use field calibration readings from both the beginning and end of the deployment as described in the next section. These readings can then be entered into the HOBOware Dissolved Oxygen Assistant to compensate for any measurement drift due to fouling. Scrub fouling off the logger with a plastic bristle brush.
- When deploying the logger in saltwater with small changes in salinity, you will need a conductivity or salinity value from either a conductivity meter or salinometer to enter in the Dissolved Oxygen Assistant to adjust the data from the logger for salinity. A single meter reading will add less than 1.1% DO error (assuming the conductivity changes are within ±3,000 µS/cm from the calibration point).

If the conductivity changes, then you will need a data file with salinity or specific conductivity readings for the entire deployment. Consider deploying a HOBO Conductivity logger (U24-002-C) next to this DO logger to use the resulting data file for salinity data. For U24-002-C conductivity readings within a $\pm 30,000 \ \mu$ S/cm range, there will be less than 4% error added to the DO measurements, and for readings over a narrower range, the accuracy will be even better. Refer to the *HOBO Conductivity Logger (U24-002-C) Manual* for more details. For applications that require higher accuracy conductivity logger.

 To generate a percent saturation series, you will need to deploy a barometric pressure logger (such as a HOBO Water Level Logger, U20-001-0x or U20L-0x) or have access to a nearby weather station to gather barometric pressure data. This data is necessary for the Dissolved Oxygen Assistant to calculate percent saturation.

Taking Field Calibration Readings

If fouling is expected during the deployment, you can take calibration readings at the beginning and end of the deployment to enter in the Dissolved Oxygen Assistant. This will adjust the data from the logger to compensate for any measurement drift due to fouling. There are two methods for taking field calibration readings: the first method involves taking readings using a dissolved oxygen meter or titration while the second method involves calibrating the logger in 100% water-saturated air. The first method is recommended because it is quicker to get the necessary calibration readings; the second method can take 40 minutes or more to achieve equilibrium with temperature extremes.

To Take Calibration Readings Using a DO Meter or Titration:

 The logger must be logging. Take a DO measurement of the water where the logger is being deployed using either a DO meter or by titration. If using a meter, make sure it is calibrated and allow time for the meter probe to stabilize (this will occur when three meter measurements taken in a row are within your accuracy tolerance).

If the logger is being deployed in saltwater, adjust the meter measurements for salinity using a meter with both conductivity and DO probes. If the saltwater has a constant salinity, you can use a DO meter where you can enter that salinity value to adjust the readings. If the salinity and/or DO are changing rapidly, then you will need to get a sample of the water in a container large enough for both the logger and meter probe to be completely submerged. Place both devices in the water long enough for them to stabilize and then for the DO logger to log at least two values, and take a concurrent meter reading.

- 2. Record the reading, date, and time of the measurement in a field notebook.
- 3. At the end of the deployment, repeat steps 1 and 2.

To Take Calibration Readings Using 100% Water-Saturated Air:

- 1. The logger must be logging. You will need fresh water, the included calibration boot and sponge, and the current barometric pressure from a HOBO U20 or U20L Water Level logger, a barometer, or a nearby weather station.
- If the logger has been in salt water, clean the logger body and sensor cap as described in the *Maintenance* section. Make sure the sensor cap is dry before continuing.
- 3. Make sure the protective guard or anti-fouling guard is installed on the logger.
- 4. Wet the small sponge with fresh water. Squeeze out any excess water.
- 5. Place the sponge in the end of the calibration boot.
- 6. Insert the logger in the calibration boot so that there is approximately a 1 cm (0.5 inch) overlap between the end of the boot and the body of the logger. This will ensure there is enough space between the end of the logger and the sponge (the logger should not be pressed up tightly against the sponge).
- Allow at least 40 minutes for the logger to reach temperature equilibrium, and then write down the date and time in a field notebook.
- 8. Write down the barometric pressure at that time (note the elevation if the barometric reading has been adjusted for sea level).
- 9. Repeat these steps at the end of the deployment.

Reading Out the Logger and Redeploying

Your readout and maintenance schedule will be determined by the amount of fouling at the site. To read out the logger in the field:

1. Take a field calibration reading as described in the *Taking Field Calibration Readings* section.

2. If the logger was in saltwater and you did not deploy a HOBO Conductivity Logger, then use a conductivity meter or salinometer to take a conductivity reading. Write down the reading and the date and time.

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3. Remove the logger from the water and read out the data from the logger using a shuttle or computer with a base station.

4. If you are deploying it again, clean the sensor (see *Maintenance* for details).

- 5. Check the expiration date for your cap and make sure it will not expire before the end of your deployment. Replace it if needed.
 - 6. Relaunch the logger if it is not already logging.
- 7. Take another field calibration reading after the logger is cleaned.
 - 8. Redeploy the logger.

Using the HOBOware Dissolved Oxygen Assistant

Use the Dissolved Oxygen Assistant to obtain accurate Dissolved Oxygen readings if the logger was deployed in a saltwater environment or if percent saturation is required. Also use this assistant if you took field calibration readings. The Dissolved Oxygen Assistant is only available in HOBOware from the Plot Setup window when you open a file from this logger. To use the assistant:

- 1. Offload the most recent data files from the shuttle or logger to your computer.
- 2. Open a data file in HOBOware.
- 3. In the Plot Setup window, select the Dissolved Oxygen Assistant and click Process.
- 4. In the Dissolved Oxygen Assistant window, enter the salinity, barometric pressure, and field calibration information as needed. Click the Help button in the Dissolved Oxygen Assistant for more details and to learn about the ranges of input data allowed.
- 5. Plot the data and save it as a project file.

Maintenance

To clean the sensor cap:

- 1. Remove the protective guard or anti-fouling guard, but leave the sensor cap on the sensor.
- 2. Rinse the logger with clean water from a squirt bottle or spray bottle.
- Gently wipe the cap with a soft-bristled brush (such as a toothbrush) or soft cloth if biofouling is present. Use Alconox[®] to remove grease.
- If extensive debris or mineral build-up is present, soak the cap end in vinegar for 15 minutes, then soak it in deionized (DI) water for another 15 minutes.
- 5. If the logger is being immediately redeployed with the same sensor cap, a field calibration is adequate. If a new sensor cap is being installed, a lab calibration with HOBOware is recommended. When storing the logger between deployments, keep it in the calibration boot (wet the small

sponge with fresh water, place the sponge in the end of the calibration boot, and then insert the logger in the boot.)

WARNING: Do not use organic solvents; they will damage the sensor. Do not remove the sensor cap from the sensor prior to cleaning with a brush. Only clean the sensor when you replace the sensor cap. See the full instructions that ship with the replacement sensor cap. Do not wet the sensor optical lens area with water or any solution. Remove the cap and gently wipe the window with a soft cloth.

To clean the logger body:

- 1. Make sure the sensor cap is installed on the logger.
- 2. Gently scrub the logger body with a plastic bristle brush or nylon dish scrubber.
- 3. Use Alconox[®] to remove grease.
- 4. Soak in vinegar to remove mineral deposits.
- 5. Rinse the logger with deionized (DI) water.

Battery Guidelines

The battery life of the logger should be three years or more. Actual battery life is a function of the number of deployments, logging interval, and operation/storage temperature of the logger. Frequent deployments with fast logging intervals, continuous storage/operation at temperatures above 35°C (95°), and keeping the logger connected to the coupler will result in significantly lower battery life. For example, the battery may last less than a year with a 1-minute logging interval. To obtain a three-year battery life, a logging interval of five minutes or greater should be used and the logger should be operated and stored at temperatures between 0° and 25°C (32° and 77°F).

The logger can report and log its battery voltage. If the battery falls below 3.2 V, the logger will record a "bad battery" event in the datafile. The logger will record a second "bad battery" event and stop logging when the battery falls below 3.1 V. If the datafile contains "bad battery" events, the logger should be returned to Onset for battery replacement. Note the logger does not have to be recording the battery channel for it to detect bad battery events. The logger will record these events regardless of what channels are logged. To have your logger's battery replaced, contact Onset or your place of purchase for return arrangements. Do not attempt to replace the battery yourself. Severe damage to the logger will result if the case is opened without special tools, and the warranty will be voided.

WARNING: Do not cut open, incinerate, heat above 100°C (212°F), or recharge the lithium battery. The battery may explode if the logger is exposed to extreme heat or conditions that could damage or destroy the battery case. Do not dispose of the logger or battery in fire. Do not expose the contents of the battery to water. Dispose of the battery according to local regulations for lithium batteries.



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Appendix 3. Onset HOBO®® Waterproof Shuttle (U-DTW-1) Manual

App

HOBO® Waterproof Shuttle (U-DTW-1) Manual



veral major functions:

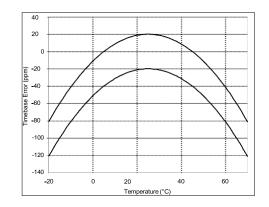
- Reads out all logger information (serial number, deployment number, data, etc.) from loggers in the field for transfer to host computer, and stores each logger's data in a "bank"
- Nonvolatile memory preserves data, even if batteries are depleted
- Relaunches the logger, resetting the logger's time to the shuttle's time and synchronizing the logging interval on relaunch
- Can be used as an optic-to-USB base station
- Can be used to read out and relaunch loggers underwater

Although the HOBO Waterproof Shuttle is easy to use, Onset strongly recommends that you spend a few minutes reading this manual and trying out the procedures described here before taking the shuttle into the field.

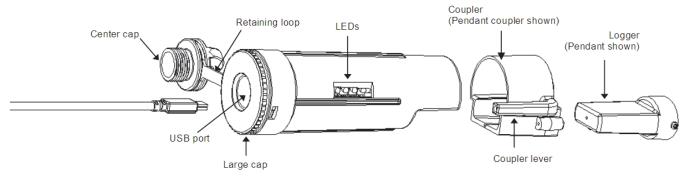
Specifications HOBO Waterproof Shuttle Compatibility All HOBO U-Series loggers with optic USB. Not compatible with the HOBO U-Shuttle (U-DT-1) U-DTW-1 **Data Capacity** 63 logger readouts of up to 64K each Included Items: 0° to 50°C (32° to 122°F) **Operating Temperature** USB cable **Storage Temperature** -20° to 50°C (-4° to 122°F) Set of couplers: For UA Pendant Wetted Materials Polycarbonate case, EPDM o-rings and retaining loop (COUPLER2-A) Waterproof To 20 m (66 feet) For U20 Water Level (COUPLER2-B) **Time Accuracy** ±1 minute per month at 25°C (77°F); see Plot A For U20L Water Level, U22 Logger-to-Shuttle Transfer Reads out one full 64K logger in about 30 seconds Water Temp Pro v2, U24 Conductivity, Speed and U26 DO (COUPLER2-C) For UTBI TidbiT v2 Shuttle-to-Host Transfer Speed Full shuttle offload (4 MB) to host computer in 10 to 20 minutes, depending on computer 2 AA alkaline batteries required for remote operation (COUPLER2-D) **Batteries** For U23 HOBO Pro v2 (COUPLER2-E) **Battery Life** One year or at least 50 complete memory fills, typical use Weight 150 g (4 oz) **Required Items:** 15.2 x 4.8 cm (6.0 x 1.9 inches) Dimensions • HOBOware Pro 2.2 or later The CE Marking identifies this product as complying with all relevant • Compatible logger and matching coupler

 directives in the European Union (EU). To maintain CE compliance, this product must be used with the supplied USB cable or equivalent (less than

3 m long).



HOBO Waterproof Shuttle Features



Preparing to Go on Location

Before using the shuttle for the first time, you must launch it with HOBOware 2.2 or greater. You must also launch any compatible loggers that were last launched with an earlier version of HOBOware, or have never been launched at all.

- Use HOBOware 2.2 or greater to launch each logger you wish to read out and relaunch with the shuttle later. (Read "Using the shuttle as a base station" for instructions if you do not have another base station for the loggers.) The shuttle cannot relaunch loggers that were last launched with an earlier version of HOBOware. (You only have to do this once for each logger.)
- 2. Plug the large end of a USB interface cable into a USB port on the computer. (Avoid using a USB hub, if possible.)
- 3. Unscrew the center cap on the shuttle. If the cap is too tight to loosen by hand, insert a screwdriver through the lanyard hole and rotate counterclockwise until the cap is loosened.
- 4. Plug the small end of the USB interface cable into the USB port in the shuttle. (If the shuttle has never been connected to the computer before, it may take a few seconds for the new hardware to be detected.)
- Follow the instructions in the HOBOware User's Guide to access the Manage Shuttle dialog. Make sure the battery level is good, and change the batteries now if they are weak.

Important: If you change the batteries in the field, the shuttle's clock will stop, and the shuttle will not read out loggers again until you relaunch it in HOBOware.

 If you are using the shuttle for the first time, launch the shuttle as described in the HOBOware User's Guide. Launching synchronizes the shuttle's clock to the host computer and initializes the shuttle's header.

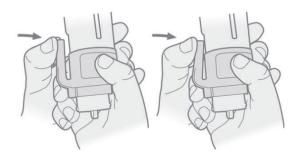
Important: The shuttle's clock is used to set the logger's clock at relaunch. For most accurate results, make sure the host computer's clock is correct before launching the shuttle. If you need to adjust the computer's clock, quit HOBOware, set the computer's clock, then reopen HOBOware and launch the shuttle.

- If you have used the shuttle before, make sure there are enough banks available to accommodate the loggers you plan to read out.
- 8. Disconnect the USB cable from the shuttle and replace the center cap securely.

Reading Out and Relaunching Loggers in the Field

After you have ensured that the shuttle's batteries are good, there is sufficient memory available, and the shuttle's clock is synchronized, follow these steps to read out and relaunch a logger in the field:

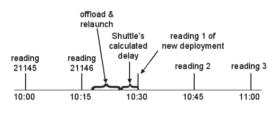
- 1. Make sure the shuttle's large cap and center cap are closed securely. Tighten the center cap until it is just flush with the large cap, or until the O-ring is no longer visible.
- 2. Make sure the communication end of the shuttle is clean. Attach the correct coupler for the logger, and ensure that it is seated properly.
- 3. Insert the logger into the coupler, following the instructions that came with the coupler.
- 4. Momentarily press the coupler lever (pressing hard enough so the lever bends).



Readout should begin immediately. The amber LED blinks continuously while readout and relaunch are in progress. Do not remove the logger when the amber LED is blinking.

5. After reading out the logger, the shuttle synchronizes the logger's clock to the shuttle's internal clock and relaunches the logger, using the description, channels to log, logging interval, and other settings that are already in the logger. (If the logger was launched with multiple logging intervals, the final defined logging interval will be used.) The logger is

launched with a slight delay that causes its readings to be synchronized with those of the previous deployment, as shown in the following diagram.



Important: If the logger was launched with multiple logging intervals, there will be no synchronizing delay. The logger will start immediately with the last defined logging interval.

- 6. When the relaunch has completed, the green LED blinks for 15 minutes, or until you momentarily press the coupler lever to stop it (press hard enough so the lever bends). If the red LED blinks instead, there was an error, and the logger may have stopped. Refer to "Troubleshooting" in this manual for details.
- 7. Remove the logger from the coupler.

Checking Shuttle Status in the Field

The shuttle's memory has 63 "banks." One logger readout can be stored in each bank. To check the shuttle's memory and batteries in the field, remove the logger and press the coupler's lever for at least three seconds (pressing hard enough so the lever bends). When you release the lever, the green LED blinks once for each unoccupied bank in the shuttle's memory. (Press the lever momentarily to stop the blinking, pressing hard enough so the lever bends.)

If the shuttle's batteries are running low, all of the shuttle banks are full, or the clock has not been set, the red LED blinks. (Press the lever momentarily to stop the blinking, pressing hard enough so the lever bends) Use HOBOware to check the shuttle's battery level, available memory, and clock. You may need to change the batteries, or offload the datafiles to the host computer and delete them from the shuttle to free up memory before you can continue reading out loggers.

Offloading Data to the Host Computer

You can offload the data stored in the shuttle even when the batteries are depleted. Take the following steps:

- 1. Connect the shuttle to a host computer running HOBOware.
- Follow the instructions in the HOBOware User's Guide to offload the new datafiles or access the Manage Shuttle dialog. The Manage Shuttle dialog shows you how many banks are occupied, and whether they have already been offloaded and saved to the host computer.
- 3. Offload and save data from the banks of your choice. Refer to the *HOBOware User's Guide* for details on saving datafiles offloaded from the shuttle.
- 4. Review the list of banks and delete any that are no longer needed. Make sure the battery level is good, and change the batteries now if they are weak. (If you change the batteries in the field, the shuttle's clock will stop, and the

shuttle will not read out loggers.) Update the shuttle's clock, if necessary.

5. When finished, disconnect the shuttle from the computer and close the center cap securely.

Using the Shuttle as a Base Station

You can use the shuttle as a base station for any U-Series logger with an optic USB interface. (This function is available even when the batteries are depleted.) To use the shuttle as a base station:

- 1. Connect the shuttle to the host computer running HOBOware.
- 2. Attach a compatible logger and coupler.
- 3. Momentarily press the coupler's lever (pressing hard enough so the lever bends).
- 4. The amber LED blinks momentarily, then the green LED should glow steadily to indicate that the logger is ready to communicate with HOBOware. (If the red LED blinks instead, the logger was not found. Make sure the logger and coupler are aligned and seated properly, and that there is no dirt or strong sunlight interfering with communications.)
- 5. When finished, remove the logger from the coupler. The green LED stops glowing when you disconnect the logger or the USB cable.

Important: The Waterproof Shuttle cannot be used *as a base station* with Pendant logger models UA-001 and UA-003 (including rain gauges RG3 and RG3-M) with serial numbers less than 988278. These loggers require a BASE-U-1 for communication with the host computer.

Indicator Lights

Green "OK" LED

The green "OK" LED blinks when HOBOware recognizes it as a base station; when it finishes reading out and relaunching a logger; and when you press the coupler lever to check the shuttle's status (see "Checking shuttle status in the field" for details). Momentarily press the coupler lever to stop the blinking (pressing hard enough so the lever bends).

The green LED glows steadily when the shuttle is being used as a base station.

Amber "Transfer" LED

The amber "Transfer" LED blinks when the shuttle is reading out a logger and relaunching it. Do not remove the logger when the Transfer light is lit.

Red "Fail" LED

The red "Fail" LED blinks whenever the shuttle encounters an error condition. Refer to "Troubleshooting" for details.

All LEDs

All LEDs blink in unison when the shuttle has just been powered up, either by installing fresh batteries or (if batteries are not installed) by connecting to the computer's USB port.

Troubleshooting

This section describes problems you may encounter while using the shuttle.

Shuttle is not recognized by host computer

If HOBOware does not recognize the shuttle when you connect it to the computer, simply disconnect and reconnect the shuttle.

Red "Fail" LED blinks

The red "Fail" LED blinks (for 15 minutes, or until you press the coupler lever, pressing hard enough so the lever bends) whenever the shuttle encounters an error. There are several conditions that might cause an error:

- Shuttle is full: If the red LED blinks when you try to read out a logger, check whether all of the banks are full, as described in "Checking shuttle status in the field." Or, use HOBOware to check the shuttle's memory.
- Shuttle batteries are low: If you cannot read out any loggers at all, check the logger's status, as described in "Checking shuttle status in the field," or use HOBOware to check the shuttle's batteries. The batteries may simply need to be replaced.
- **Compatibility:** The shuttle cannot read out or relaunch loggers that were last launched from HOBOware prior to version 2.2. You will need to read out these loggers on the host computer and relaunch them in HOBOware 2.2 or greater before you can use them with the shuttle.
- Shuttle clock is not set: The shuttle has experienced a power failure that caused the clock to reset. You must use HOBOware to offload the files that are already on the shuttle, then relaunch the shuttle before you can read out another logger.
- Can't communicate with logger: Remove the logger and coupler. Inspect them and the shuttle to ensure that all are free of dirt that could block the optic communication sensor. Carefully reassemble the shuttle, coupler, and logger, and make sure they are all seated properly. Shield the shuttle from strong sunlight, if applicable, which can interfere with optic communications.
- Other logger problems: If you can read out some loggers but not others, or if you cannot read out any loggers even with fresh batteries in the shuttle, check the loggers in HOBOware. Make sure their batteries are at acceptable levels and that there is no "corrupted header" message.

Amber "Transfer" LED stays on without blinking

The amber light is magnetically activated when you press the coupler lever. If it glows steadily at any other time, the magnet in the lever may be too close to the magnetic switch in the shuttle, or another strong magnet may be present. Try bending the lever away from the coupler to reduce the magnet's effect.

LEDs do not function

If the LEDs are not functioning at all, the batteries may be completely exhausted. To test this, attach the shuttle to the host computer and check the battery level. The shuttle should be able to communicate with the host computer, blink its LEDs normally, and perform as a base station even when the batteries are missing or depleted.

Replacing the Shuttle's Batteries

The shuttle's batteries should last about one year or at least 50 complete memory fills in typical conditions. When the shuttle's batteries run low (2.2 V or less), any logger data that is already in the shuttle will remain safe, but the shuttle will not read out another logger until its batteries are replaced.

To avoid battery problems, always check the shuttle's batteries in HOBOware before going into the field, and replace them if needed. If you cannot replace the bad batteries right away, you should remove them as soon as possible to ensure that they do not leak and damage the shuttle.

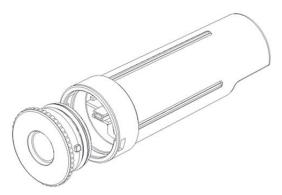
To change the shuttle's batteries:

- 1. Work over a clean surface to provide a safe platform for the disassembly.
- 2. Unscrew the center cap on the shuttle. If the cap is too tight to loosen by hand, insert a screwdriver through the lanyard hole and rotate counterclockwise until the cap is loosened.

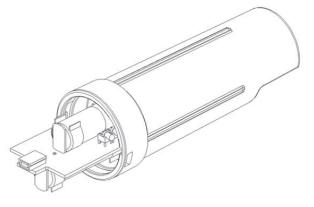
3. Use the center cap to help you carefully pull the rubber loop free of the large cap. The large cap cannot be removed while the rubber loop is in place.



4. Turn the large cap counter-clockwise slightly, then pull it off.



5. Turn the shuttle over and tap it gently. The circuit board should slide into your hand.



- 6. Remove the old batteries and install two new ones in the correct orientation. Both batteries should be turned the same way, with their positive ends facing the USB port on the board. (When the second battery makes contact, all of the shuttle's LEDs will blink in unison.)
- 7. Put the board back into the case, taking care not to bend the communication LEDs. Align the circuit board with the runners in the case. The USB port should face the open end of the shuttle, and the LEDs should show through the window on the label.
- Close the shuttle's case. Line up the tabs on the large cap with the slots on the case, press gently, and turn slightly clockwise until the large cap is closed securely.
- Replace the rubber loop and center cap. Tighten the center cap until it is just flush with the large cap, or until the O-ring is no longer visible.
- 10. Using HOBOware, offload any datafiles that are on the shuttle and launch the shuttle before going into the field again. The shuttle will not read out and relaunch loggers until the clock has been synchronized.

WANING: Do not install batteries backwards, recharge, put in fire, expose to extreme heat, or mix with other battery types, as the batteries may explode or leak. Contents of an open or leaking battery can cause chemical burn injuries.

Replace all used batteries at the same time. Recycle or dispose of batteries according to applicable federal, state, and local regulations.



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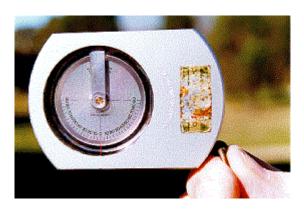
Appendix 4. Instructions for use of a Optical Reading PM-5 Clinometer

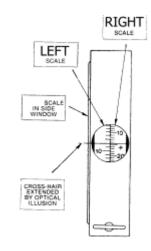
SUUNTO CLINOMETER INSTRUCTIONS:

Clinometer is a term used for any device that measures heights of trees or elevations. The Suunto clinometer is portable and accurate if used properly. *It is a sensitive instrument so be careful: do not swing the device or let it drop!*

You will use a distance tape and measure 20 meters from the base of the tree you want to determine height on. Be sure your final position allows you to see both the very top and bottom of the tree- so walk around the tree first before you go out and measure your final distance. We will measure *total height* of the tree- there are other types of height you can measure. Total height is defined as the distance form the ground level to the upper most point of the tree. Total height of a tree has many important implications including estimating overall size, competitive status, stand architecture, etc.

Once in position, hold the clinometer as shown in the right side picture below:





You will use the Percent scale- which as you look through the side 'peep' hole will probably be on the left side (it will say on the exposed side scale which side is which). When you look through the peep hole you must keep **BOTH** eyes open at all times. This will produce an optical effect that transposes the cross hair onto the tree you are looking at. If you tilt the clinometer up and down as you look through the peep hole you will notice the scales have both positive and negative numbers. You must always know whether the number is + or -.

With both eyes open tilt the clinometer till you superimpose the cross hair on the very top of the tree. Then read the proper scale and sign- this will be your upper reading. Then tilt the clinometer down till you get a number from the scale that coincides with where the tree meets the ground. Now use the equation below to get the final height:

Total height (meters)=(Top measurement – bottom measurement) X 0.20

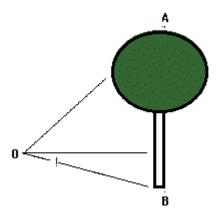
For instance in the picture below suppose the top measurement I got (20 meters away) was +100 (Measurement "A"). While the bottom measurement ("B") was

-10. Then:

Total height= (100 - (-10)) X 0.20

Total height=110 x 0.20

Total height= 22 meters



The most common mistake in measuring heights is really knowing where the true top of the tree is since sometimes side branches can be mistaken for the top- this can cause an overestimate. *Notice how in the picture above the shot for the top of the tree ("A") is through the crown of the tree!*

If you find 20 meters away from the tree is no good let your instructor know since any other distance than 20 meters will change the total height equation. For instance, if you decide 50 meters is best then the equation would be:

Total height (meters)=(Top measurement – bottom measurement) X 0.50

At 100 meters it would be:

Total height (meters)=(Top measurement – bottom measurement) X 1.00