

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

**Program:** Water Monitoring

**Document Type:** Report

**\*Document Date:** 4/30/2024

**\*Security:** Public

**\*Project Name:** 2024 Cyanobacteria and Cyanotoxins Beach Project

**\*Project Type:** Lakes

**\*Report Type:** Work Plan

**HUC Code:** No Selection

**Site #:** \_\_\_\_\_

**Route Name:** \_\_\_\_\_

**Document Control #** B-066-OWQ-WAP-TGM-24-W-R0

**Analysis Set #** \_\_\_\_\_

**County:** No Selection

**Cross Reference ID:** \_\_\_\_\_

2024 Cyanobacteria and Cyanotoxin Monitoring Work

**Comments:** Plan in Selected Lakes in Indiana

**Redaction Reference ID:** \_\_\_\_\_



# **2024 Cyanobacteria and Cyanotoxin Monitoring Work Plan in Selected Lakes in Indiana**

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April 30, 2024

B-066-OWQ-WAP-TGM-24-W-R0

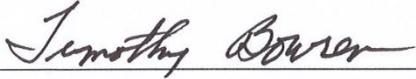
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## Approval Signatures



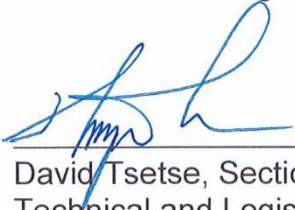
Ali Meils, Section Chief  
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4/18/2024  
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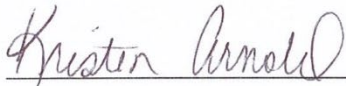
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IDEM Quality Assurance staff reviewed and approves this work plan.



Quality Assurance Staff  
IDEM Office of Program Support

4/24/2024  
Date

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## Work Plan Organization

This work plan is an extension of the existing Indiana Department of Environmental Management (IDEM) Office of Water Quality (OWQ) Watershed Assessment and Planning Branch (WAPB), [July 2023 Quality Assurance Project Plan \(QAPP\)](#) for Indiana Surface Water Programs. Per the United States Environmental Protection Agency (U.S. EPA) Guidance on Systematic Planning using the Data Quality Objectives (DQO) Process (U.S. EPA 2006) and the U.S. EPA Guidance for Quality Assurance Project Plans (U.S. EPA 2002), the work plan establishes criteria and specifications pertaining to a specific water quality monitoring project usually described in the following four QAPP groups and associated elements.

### **Group A. Project Management Planning**

- A.1. Title and Approval Sheet
- A.2. Table of Contents
- A.3. Distribution List
- A.4. Project Organization
- A.5. Problem Definition and Background
- A.6. Project Description
- A.7. Quality Objectives and Criteria for Measurement Data
- A.8. Special Training and Certification
- A.9. Documents and Records

### **Group B. Data Generation and Acquisition**

- B.1. Sampling Process Design (Experimental Design)
- B.2. Sampling Methods
- B.3. Sample Handling and Custody
- B.4. Analytical Methods
- B.5. Quality Control
- B.6. Instrument or Equipment Testing, Inspection, and Maintenance
- B.7. Instrument or Equipment Calibration and Frequency
- B.8. Inspection and Acceptance of Supplies and Consumables
- B.9. Nondirect Measurements
- B.10. Data Management

### **Group C. Assessment and Oversight**

- C.1. Assessments and Response Actions
- C.2. Reports to Management

### **Group D. Data Validation and Usability**

- D.1. Data Review, Verification, and Validation
- D.2. Verification and Validation Methods
- D.3. Reconciliation with User Requirements

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## List of Acronyms

ADDA	4 <i>E</i> ,6 <i>E</i> -3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid
AIMS	Assessment Information Management System
CFR	Code of Federal Regulations
CLP	Contract Laboratory Program
CRQL	Contract Required Quantification Limit
CWA	Clean Water Act
CAAS	Cyanotoxin Automated Assay System
DO	Dissolved oxygen
DQA	Data quality assessment
ELISA	Enzyme-linked immunosorbant assay
GPS	Global Positioning System
HUC	Hydrologic Unit Code
IAC	Indiana Administrative Code
IC	Indiana Code
IDNR	Indiana Department of Natural Resources
MDL	Method detection limit
mg/L	Milligram per liter
µg/l	Micrograms per liter
µmho/cm	Micromho per centimeter
ml	Milliliter
NHD	National Hydrography Dataset
NTU	Nephelometric Turbidity Unit(s)
QA	Quality assurance
QAC	Quality assurance coordinator
QAM	Quality assurance manager
QAO	Quality assurance officer
QA/QC	Quality assurance and quality control
QC	Quality control
QAPP	Quality assurance project plan
RL	Reporting limit
RPD	Relative percent difference
SM	Standard methods
SS	Stainless steel
SU	Standard units
U.S.	United States
U.S. EPA	United States Environmental Protection Agency
WHO	World Health Organization

## **Program Objective**

The IDEM OWQ is responsible for sampling and assessing Indiana's surface water pursuant to the Clean Water Act Section 305(b). IDEM's development of a monitoring program for lakes provides warnings to the public when cyanobacteria are present in large enough quantities and cyanotoxins are in high enough concentrations to render recreational water contact unsafe.

### **A. Project Management**

#### **A.1. Project Organization and Schedule**

(QAPP Element A4)

The IDEM WAPB staff will sample designated Indiana Department of Natural Resources (IDNR) managed swimming beaches on lakes. Staff will evaluate samples for cyanobacteria cell counts and concentrations of the cyanotoxins microcystins, cylindrospermopsin (if producing species are present), anatoxin-a, and saxitoxin during the summer recreational season (May through August). Sample collection may occur outside of this timeframe at the request of IDNR. Collection of water chemistry samples will also take place for the following nutrient parameters: ammonia nitrogen, nitrate plus nitrite, total Kjeldahl nitrogen, and total phosphorous.

Sampling will take place at the following reservoirs or lakes: Worster Lake at Potato Creek State Park, Sand Lake at Chain O'Lakes State Park, Lake James at Pokagon State Park, Lake James at Pokagon State Park Inn, Salamonie Reservoir at Lost Bridge West State Recreation Area, Mississinewa Reservoir at Miami State Recreation Area, Cecil M. Harden Lake at Raccoon State Recreation Area, Monroe Lake at Paynetown and Fairfax State Recreation Areas, Hardy Lake at Hardy Lake State Recreation Area, Whitewater Lake at Whitewater Memorial State Park, Deam Lake at Deam Lake State Recreation Area, Starve-Hollow Lake at Starve-Hollow State Recreation Area, Brookville Reservoir at Mounds and Quakertown State Recreation Areas, Summit Lake at Summit Lake State Park, Cagles Mill Lake at Lieber State Recreation Area, Kunkel Lake at Ouabache State Park, Ferdinand State Forest Lake at Ferdinand State Forest, Lake Lincoln at Lincoln State Park, and Patoka Lake at Newton-Stewart State Recreation Area.

Sampling will occur at selected sites on these lakes monthly unless cyanobacteria cell counts exceed 100,000 cells/ml. Excessive cell counts will initiate biweekly sampling. If a high cell count is due to picocyanobacteria and picocyanobacterial cells comprise 80% of the sample, a follow-up visit is not necessary. Toxin concentrations that exceed the Human Recreation Advisory thresholds in Table 1 will initiate weekly sampling. Additionally, sampling for field parameters and cyanotoxin analysis will happen at the Dog Park Lake at Fort Harrison State Park before it opens on March 1<sup>st</sup>, monthly from May through June, and then biweekly from July 1<sup>st</sup> through October 31<sup>st</sup>, or until the lake closes. The addition of sites and analyses may occur depending on time and staffing constraints.

## **A.2. Background and Project Description**

(QAPP Elements A5, A6)

The presence of sufficient numbers of cyanobacteria in surface waters serves as an indicator of possible cyanotoxin production. Exposure to high counts of cyanobacterial cells and high cyanotoxin concentrations may lead to various illnesses in humans and animals. Exposure is through contact with or ingestion of surface water. Indiana has yet to establish cyanobacteria or cyanotoxin water quality criteria or standards for full body contact recreational uses to protect human health. However, the World Health Organization (WHO) guidelines are in common use for this purpose. The U.S. EPA published “Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin” in 2019. Indiana will use a combination of WHO and U.S. EPA recommended values described in Table 1. Dog Park Lake values are based on suggestions from the California Environmental Protection Agency’s Office of Environmental Health Hazard Assessment and the Oregon Health Authority.

Cyanotoxins will be analyzed using Gold Standard Diagnostics enzyme linked immunosorbant assay (ELISA) test kits (microcystins, cylindrospermopsin, anatoxin-a, and saxitoxin) in the IDEM WAPB Algae Laboratory. The Cyanotoxin Automated Assay System (CAAS) performs automated sample processing and reading. If samples need manual processing, samples will be read with the Chromate Reader. In addition to cyanobacteria sampling, water chemistry samples will be collected for ammonia nitrogen, nitrate plus nitrite as nitrogen, total Kjeldahl nitrogen, and total phosphorous. Staff will record the following field parameters: pH, DO, DO percent saturation, temperature, turbidity, and specific conductivity. Observations of atmospheric and lake or beach conditions will also be recorded for each sampling event. At the Dog Park Lake, staff will record field measurements and collect grab samples for cyanotoxin analysis. For Ferdinand State Forest Lake, Lake Lincoln, and Patoka Lake, IDNR staff will collect cyanobacteria and cyanotoxin samples and deliver samples to the IDEM WAPB Algae Laboratory. Staff will enter all results into the WAPB Assessment Information Management System (AIMS) database and post results on [www.algae.IN.gov](http://www.algae.IN.gov).

## **A.3. Quality Objectives and Criteria**

(QAPP Element A7)

Guidance on Systematic Planning Using the Data Quality Objective Process (U.S. EPA 2006) describes a planning tool for environmental data collection activities. The process provides a basis for balancing decision uncertainty with available resources. This work plan contains the products of the process which include the data quality objectives and measurement quality objectives. U.S. EPA recommends the Data Quality Objective Process for all significant environmental data collection projects. The process is a seven-step systematic planning process used to clarify study objectives, define the appropriate types of data, and establish decision criteria on which to base the final use of the data. Numbers 1. through 7. outline the data quality objectives process outputs.

### **1. State the Problem**

The waters in the previously identified recreational lakes either have a documented history of or the potential of supporting cyanobacteria blooms which may present a human and animal health hazard.

### **2. Identify the Goals of the Study**

This project will provide information regarding the nature and extent of cyanobacteria populations in the selected swimming areas, and will measure microcystins, cylindrospermopsin, anatoxin-a, and saxitoxin concentrations. By routine monitoring of cyanobacteria and cyanotoxins in lakes, IDEM determines whether conditions are present to pose an unreasonable health risk to swimmers or dogs. Steps are taken to adequately warn the public of potential risks.

### **3. Identify Information Inputs**

Collect composite water samples at each swimming beach's sampling locations for cyanobacteria identification and enumeration, and cyanotoxin analyses. Identify and enumerate cyanobacterial cells in each sample using microscopy. Analysis for each cyanotoxin requires a specific immunoassay test kit: Gold Standard Diagnostics ADDA ELISA for microcystins and Gold Standard Diagnostics ELISA for cylindrospermopsin, anatoxin-a, and saxitoxin.

Measure field parameters (pH, DO, DO percent saturation, temperature, turbidity, and specific conductance) at each site during each sampling event.

Collect water samples for ammonia nitrogen, nitrate and nitrite, total Kjeldahl nitrogen, and total phosphorous.

Include visual field observations, weather conditions, and lake or beach conditions.

For the Dog Park Lake, only take field measurements and collect cyanotoxin grab samples.

### **4. Define the Boundaries of the Study Area**

The study areas consist of the designated swimming beaches at Worster Lake at Potato Creek State Park, Sand Lake at Chain O'Lakes State Park, Lake James at Pokagon State Park, Lake James at Pokagon State Park Inn, Salamonie Reservoir at Lost Bridge West State Recreation Area, Mississinewa Reservoir at Miami State Recreation Area, Cecil M. Harden Lake at Raccoon State Recreation Area, Monroe Lake at Paynetown and Fairfax State Recreation Areas, Hardy Lake at Hardy Lake State Recreation Area, Whitewater Lake at Whitewater Memorial State Park, Deam Lake at Deam Lake State Recreation Area, Starve-Hollow Lake at Starve-Hollow State Recreation Area, Brookville Reservoir at Mounds and Quakertown State Recreation Areas, Summit Lake at Summit Lake State Park, Cagles Mill Lake at Lieber State Recreation Area, Kunkel Lake at Ouabache State Park, the Dog Park Lake at Fort Harrison State Park,

Ferdinand State Forest Lake at Ferdinand State Forest, Lake Lincoln at Lincoln State Park, and Patoka Lake at Newton-Stewart State Recreation Area.

Sampling sites are listed in Table 2 and can be viewed in Figures 1 through 20.

Sites will be sampled monthly from May through August. Excessive cell counts ( $\geq 100,000$  cells/ml) will initiate biweekly sampling. Toxin concentrations that exceed the Human Recreation Advisory thresholds in Table 1 will initiate weekly sampling.

The Dog Park Lake at Fort Harrison State Park will be sampled before it opens on March 1<sup>st</sup> and monthly from May through June. Biweekly sampling will start from July 1<sup>st</sup> through October 31<sup>st</sup>, or until the lake closes. Detection of any toxin will initiate weekly sampling with the exception of cylindrospermopsin. Lab method detection limit studies in early 2024 led to lowering the reporting limit for cylindrospermopsin from 0.15  $\mu\text{g/l}$  to 0.10  $\mu\text{g/l}$ . Due to limited staff and resources, concentrations at or above 0.20  $\mu\text{g/l}$  cylindrospermopsin will initiate weekly sampling at the Dog Park Lake.

Sampling activities will not be conducted during hazardous weather conditions (e.g., thunderstorms or heavy rain in the vicinity) or when unexpected physical barriers to accessing the site exist. In the case of hazardous weather conditions, staff may wait until it is safe to enter the water to collect samples. If the beach at the sampling location is flooded, staff will collect samples from where they are able to and make notations on the field sampling sheet. The field crew chief will make the final determination as to whether or not a site is safe to enter.

## **5. Develop the Analytic Approach**

Compare cyanobacteria cell counts to the WHO and U.S. EPA guidelines. Through the partnership with IDNR, recreational advisory signs are posted when the cyanobacteria cell counts are at or above 100,000 cells/ml.

Table 1 summarizes the cyanotoxin exposure thresholds. U.S. EPA guidelines recommend posting recreational advisories when microcystin concentrations are at or above 8  $\mu\text{g/l}$  and IDNR issues an additional caution advisory indicating children and the immunocompromised should not swim (U.S. EPA 2019). WHO guidelines recommend prohibiting swimming when microcystin concentrations are at or above 20  $\mu\text{g/l}$ . IDNR will use the WHO recreational guideline value of 6  $\mu\text{g/l}$  for a caution advisory for cylindrospermopsin concentrations and U.S. EPA recommended cylindrospermopsin limit of 15  $\mu\text{g/l}$  for beach closures. Using the State of Ohio recommended thresholds for anatoxin-a and saxitoxin, IDNR will post a caution advisory when concentrations are at or above 8  $\mu\text{g/l}$  and 0.8  $\mu\text{g/l}$  respectively (State of Ohio 2020). The California Environmental Protection Agency's Office of Environmental Health Hazard Assessment and the Oregon Health Authority action levels are used for microcystin, anatoxin-a, cylindrospermopsin, and saxitoxin exposure for dogs. Swimming is prohibited at the Dog Park Lake when concentrations are at or above 0.8  $\mu\text{g/l}$  microcystin, 1.0  $\mu\text{g/l}$  cylindrospermopsin, 0.4  $\mu\text{g/l}$  anatoxin-a, and 0.05  $\mu\text{g/l}$  saxitoxin (Linville 2017). An

advisory will be issued if microcystin is detected at or above 0.4 µg/L or cylindrospermopsin is detected at or above 0.5 µg/l, which is half of the respective closing limit.

**Table 1. Exposure Thresholds**

Exposure Reference Values (µg/l)	Microcystin	Cylindrospermopsin	Anatoxin-a	Saxitoxin
Human Recreation Advisory	8	6	8	0.8
Human Recreation Prohibited	20	15	30	3
Dog Recreation Advisory	0.4	0.5	–	–
Dog Recreation Prohibited	0.8	1.0	0.4	0.05

The reporting limits for anatoxin-a and saxitoxin are the same as the respective closure thresholds.

**6. Specify Performance or Acceptance Criteria**

Good quality data are essential for minimizing decision error. By minimizing both sampling design error and laboratory error, the public can place more confidence in the cell counts and cyanotoxin concentrations reported through this program. Use program-specific controls to minimize the introduction of errors. Controls for field sampling include the collection of field duplicates and blanks, and field audits. Laboratory controls include thorough verification of species identifications and cyanotoxin analyses. Take and store photographs of cyanobacterial identifications. Staff will be trained on how to collect samples, operate field and lab equipment, and perform required quality assurance and quality control (QA/QC) procedures. QA/QC review reports are developed as a final step of QA process and data assessment. The data and their usability identified in the QA/QC review reports are used in the decision making for water quality assessments.

**7. Develop the Plan for Obtaining Data**

Composite sampling at each beach swimming area ensures a better representation of the cyanobacteria populations in the swimming area. Staff will wade in to about waist high and stand for a minute to let the water and sediment settle before taking the sample using the integrated water column sampler (IDEM 2023a). Collect samples in the morning and afternoon when cyanobacteria are believed to be the most prevalent. At the Fort Harrison Dog Park Lake, collecting samples near the shore reflects dogs’ drinking habits.

**A.4. Specialized Training or Certification and Staffing Requirements**

(QAPP Element A8)

Written standard operating procedures (SOPs) are available to WAPB staff. Experienced peers train staff on how to collect surface water samples, operate field and lab equipment, and

perform required quality assurance and quality control (QA/QC) procedures. Staff training and SOP techniques are based on proven U.S. EPA sampling methods and recommendations for containers, preservation techniques, and holding times as cited in the Code of Federal Regulations (CFR) (CFR 2024).

Assign two staff to each field sampling run. If necessary, one staff can sample. Senior staff will provide training and review for IDEM staff and summer interns on how to collect surface water samples, operate field and lab equipment, and perform QA/QC procedures.

Select WAPB staff received training in algal identification and enumeration from staff at PhycoTech in St. Joseph, Michigan. A representative from Eurofins Abraxis™ (now known as Gold Standard Diagnostics) trained select WAPB staff on the use of the Eurofins Abraxis™ CAAS instrument. Staff trained in cyanobacteria identification and enumeration, and use of the Gold Standard Diagnostics CAAS will perform these tasks in the IDEM WAPB Algae Laboratory.

Targeted Monitoring, Technical and Logistical Services, and Probabilistic Monitoring Sections staff share data handling. Sampling staff will input field observations and field data into the AIMS database. Sampling staff will perform QA/QC of all field data entry. Chemists from the Technical and Logistical Services Section will validate the data through QA/QC procedures cited in Section D of this work plan.

## B. Data Generation and Acquisition

### B.1. Sampling Process Design (Experimental Design)

(QAPP Element B1)

The Targeted Monitoring Section chief will select the initial sample site locations based on the availability of a public swimming beach at a state or federally managed recreation area. Table 2 lists the selected sampling sites with associated lake name, AIMS L-site, county name, HUC12, and latitude and longitude of each sampling site. Figures 1 through 20 depict all sampling sites for each lake. The Trimble Juno™ Series Global Positioning System, with an accuracy of 1-3 meters, determined the final coordinates for each site during the project’s presurvey phase.

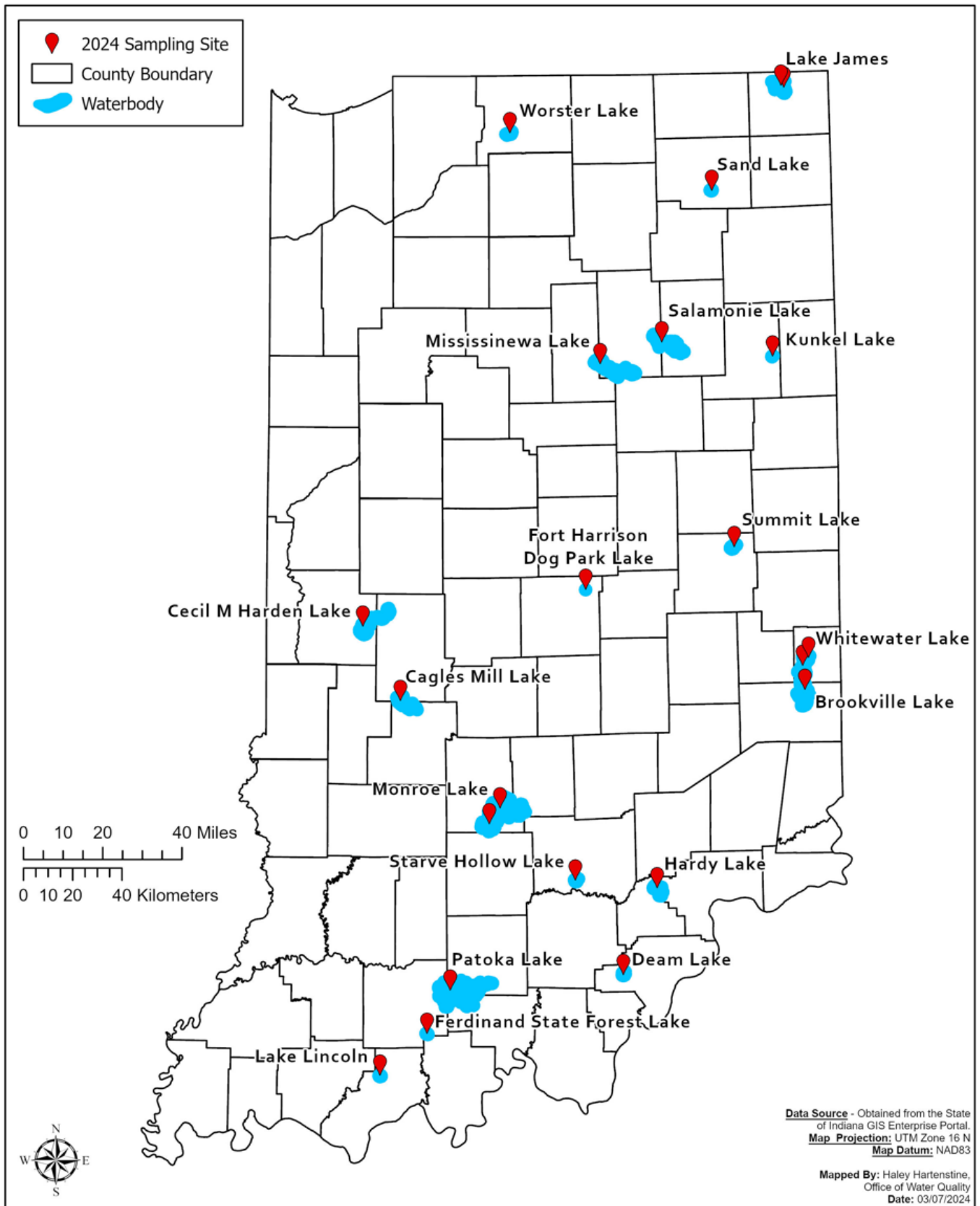
**Table 2. Sampling Sites**

Site #	Lake Name	AIMS L-site	County	HUC12	Latitude	Longitude
1	Whitewater	GMW-07-0001	Union	050800030712	39° 36' 54.9"	-84° 57' 58.1"
2	Hardy	WEM-06-0001	Scott	051202070604	38° 46' 55.2"	-85° 41' 53.8"
3	Cecil M Harden	WLV-12-0002	Parke	051201081208	39° 44' 48.9"	-87° 04' 33.5"
4	Monroe - Paynetown	WEL-07-0001	Monroe	051202080702	39° 04' 49.1"	-86° 25' 51.1"
5	Monroe - Fairfax	WEL-07-0003	Monroe	051202080703	39° 01' 20.5"	-86° 28' 53.9"

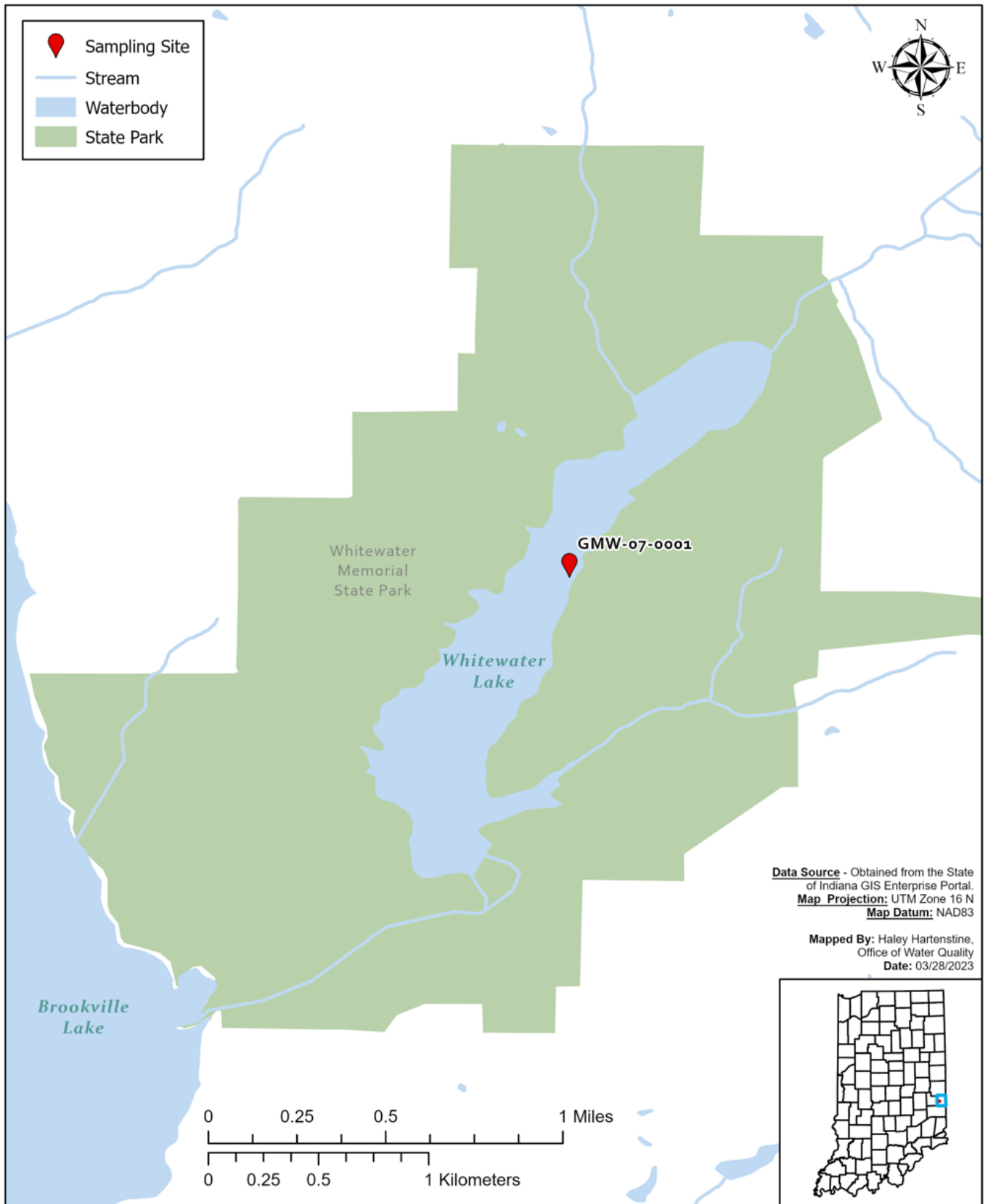
Site #	Lake Name	AIMS L-site	County	HUC12	Latitude	Longitude
6	Mississinewa	WMI-06-0001	Wabash	051201030605	40° 42' 08.9"	-85° 56' 08.0"
7	Worster	UMK-01-0005	St Joseph	071200010104	41° 33' 03.2"	-86° 21' 41.8"
8	Salamonie	WSA-04-0001	Huntington	051201020405	40° 46' 46.1"	-85° 38' 14.9"
9	Sand	LMJ-16-0024	Noble	040500011601	41° 19' 50.0"	-85° 23' 00.9"
10	Lake James - Pokagon SP	LMJ-08-0033	Steuben	040500010803	41° 42' 35.5"	-85° 02' 10.7"
11	Lake James - Potawatomi Inn	LMJ-08-0078	Steuben	040500010803	41° 42' 09.3"	-85° 01' 21.7"
12	Brookville - Quakertown	GMW-07-0005	Union	050800030715	39° 35' 10.9"	-84° 59' 41.5"
13	Brookville - Mounds	GMW-07-0004	Franklin	050800030717	39° 29' 53.2"	-84° 59' 12.6"
14	Deam	OSK-07-0001	Clark	051401010702	38° 27' 58.5"	-85° 51' 40.8"
15	Starve-Hollow	WEM-09-0007	Jackson	051202070905	38° 48' 50.4"	-86° 04' 50.3"
16	Kunkel	WUW-06-0005	Wells	051201010604	40° 43' 12.4"	-85° 06' 21.0"
17	Fort Harrison Dog Park	WWU-09-0027	Marion	051202010904	39° 52' 39.4"	-86° 01' 02.5"
18	Summit	WED-01-0001	Henry	051202040102	40° 01' 33.0"	-85° 18' 28.6"
19	Cagles Mill	WWE-05-0015	Putnam	051202030512	39° 28' 29.1"	-86° 53' 54.5"
20	Ferdinand State Forest	OLP-04-0010	Dubois	051402010402	38° 15' 21.8"	-86° 46' 35.1"
21	Lake Lincoln	OLP-09-0012	Spencer	051402010905	38° 06' 13.2"	-86° 59' 41.4"
22	Patoka	WPA-01-0016	Orange	051202090106	38° 24' 50.5"	-86° 40' 4.4"



Figure 1. Map of all sampling sites



**Figure 2. Whitewater Lake Site Map**



**Figure 3. Hardy Lake Site Map**

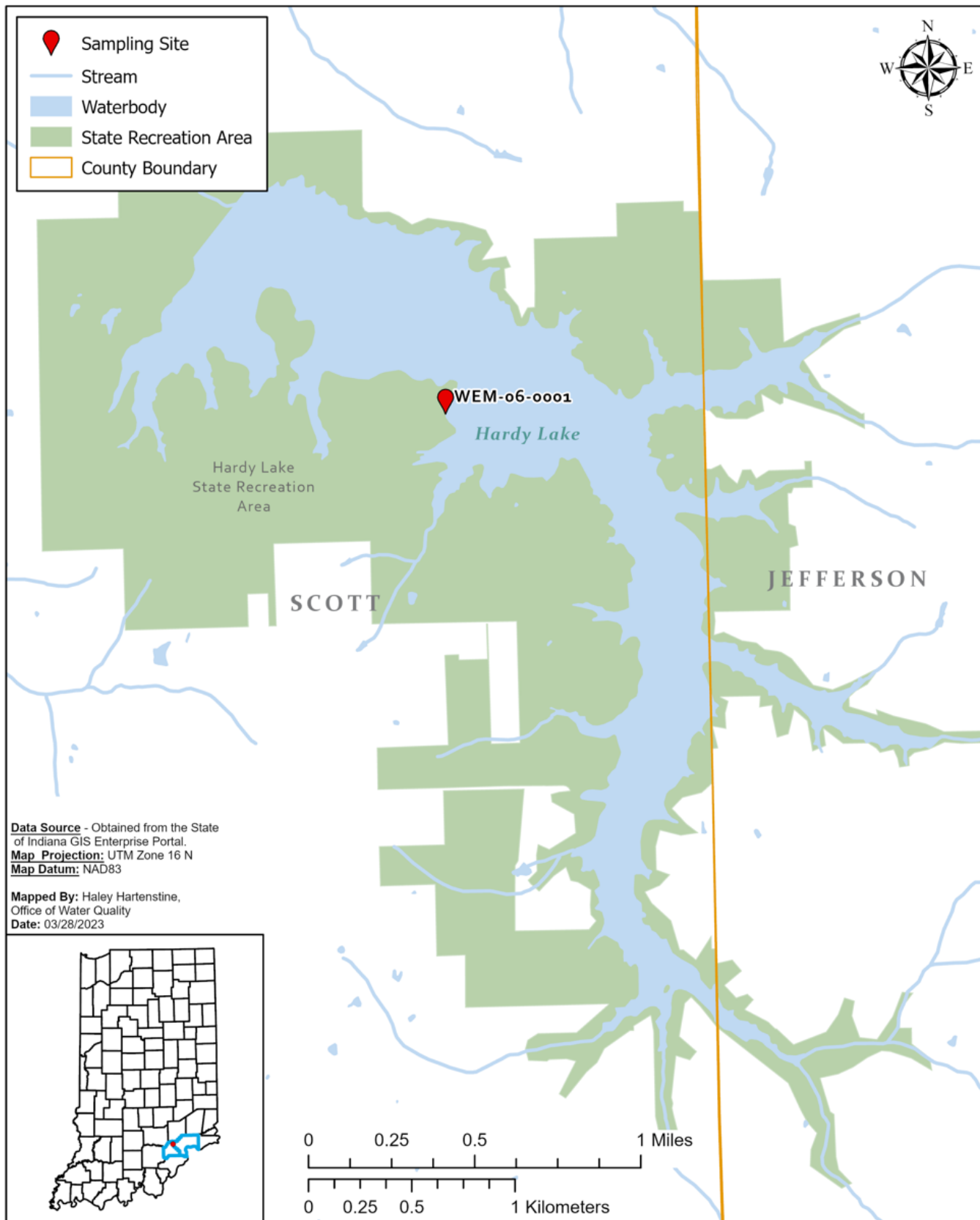


Figure 4. Cecil M. Harden Lake Site Map

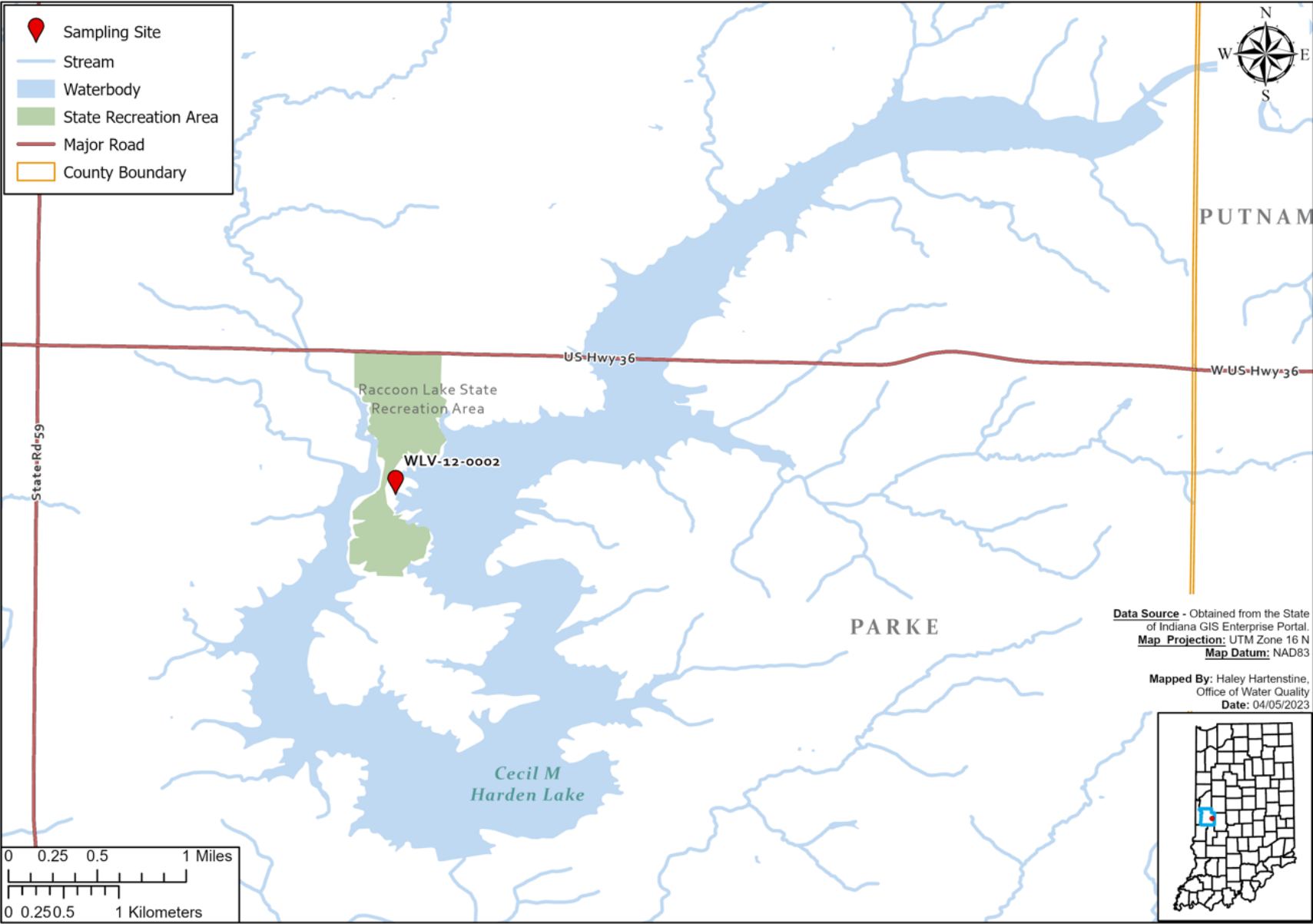


Figure 5. Monroe Lake Site Map

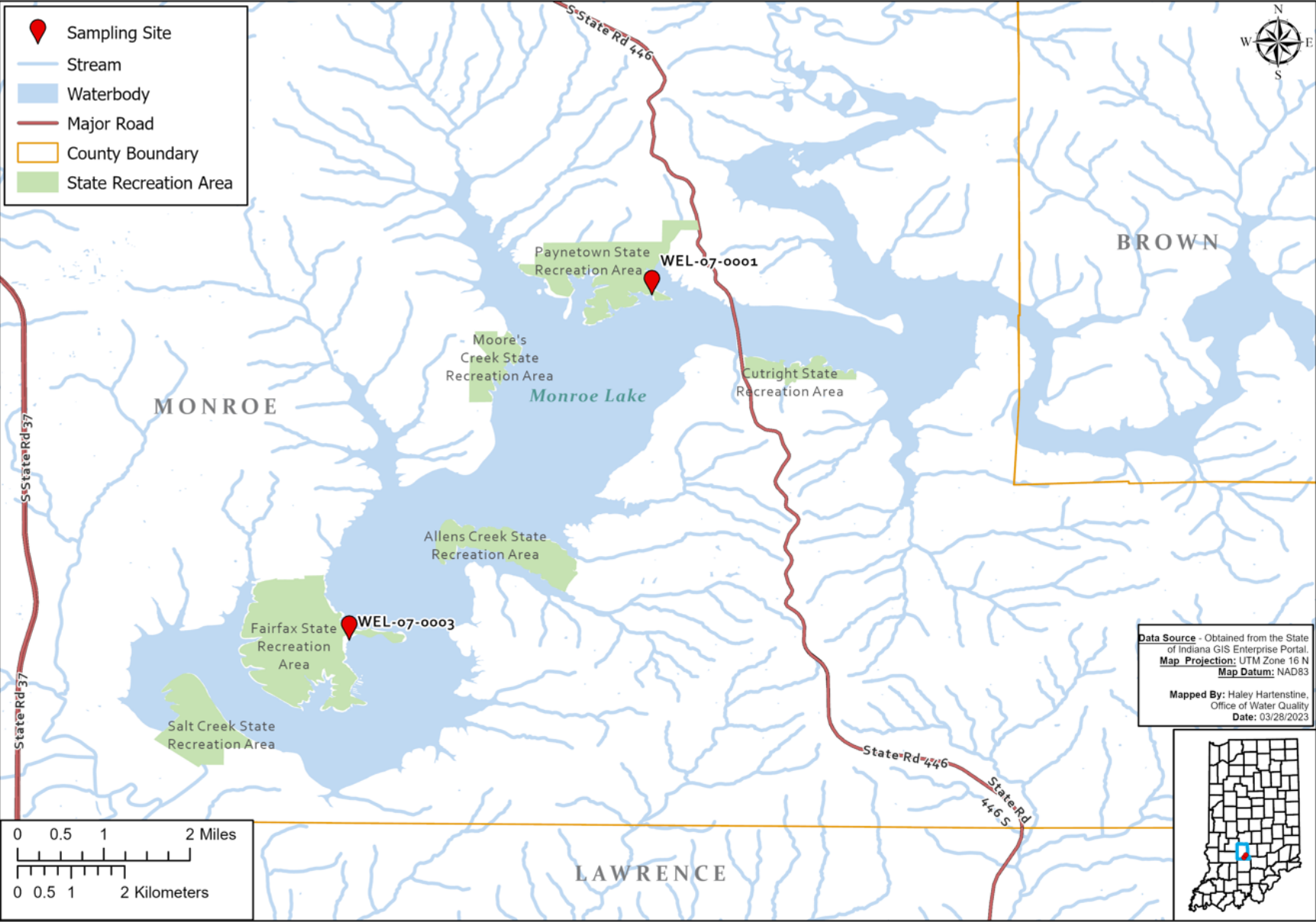


Figure 6. Mississinewa Lake Site Map



Figure 7. Worster Lake Site Map

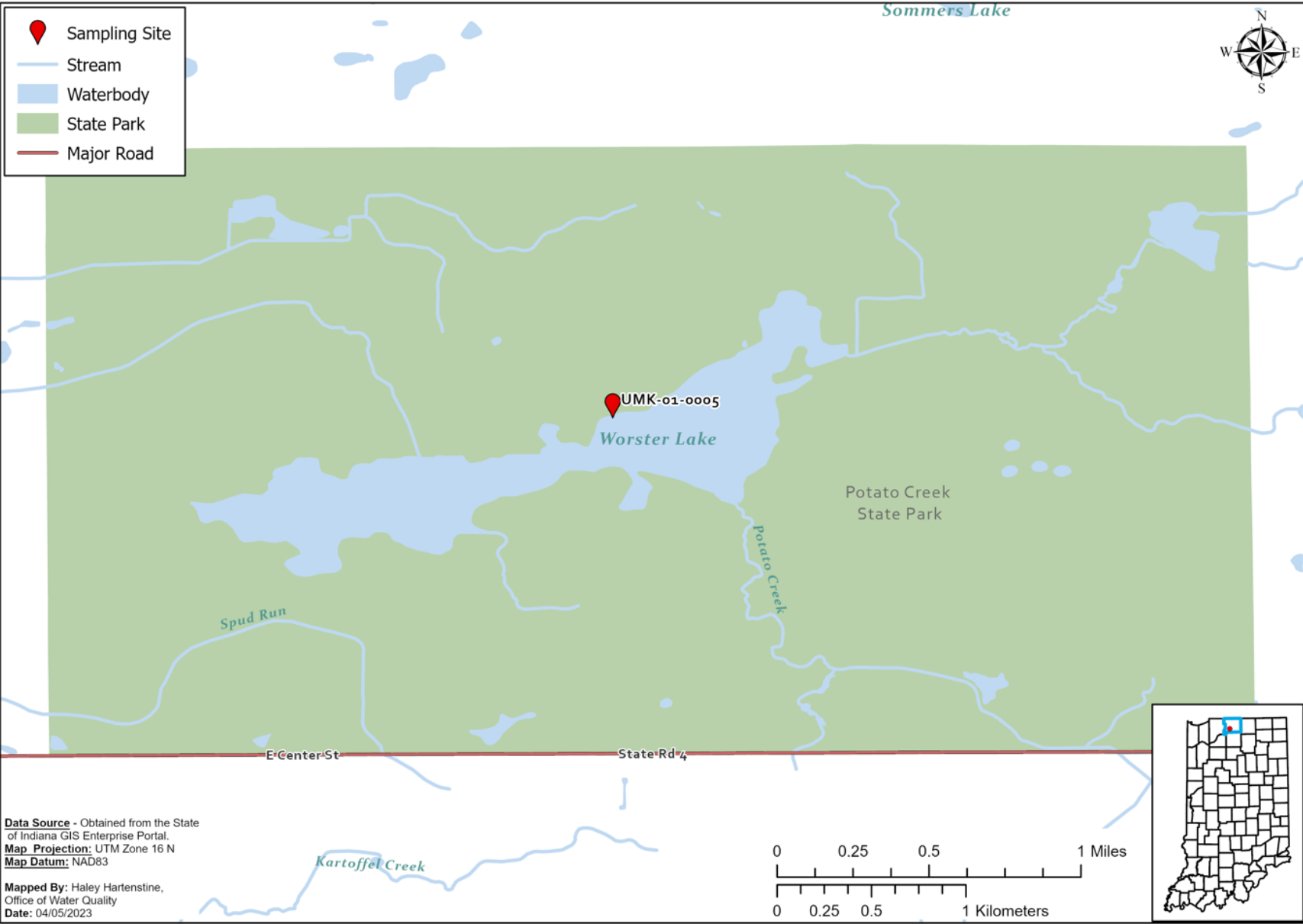


Figure 8. Salamonie Lake Site Map

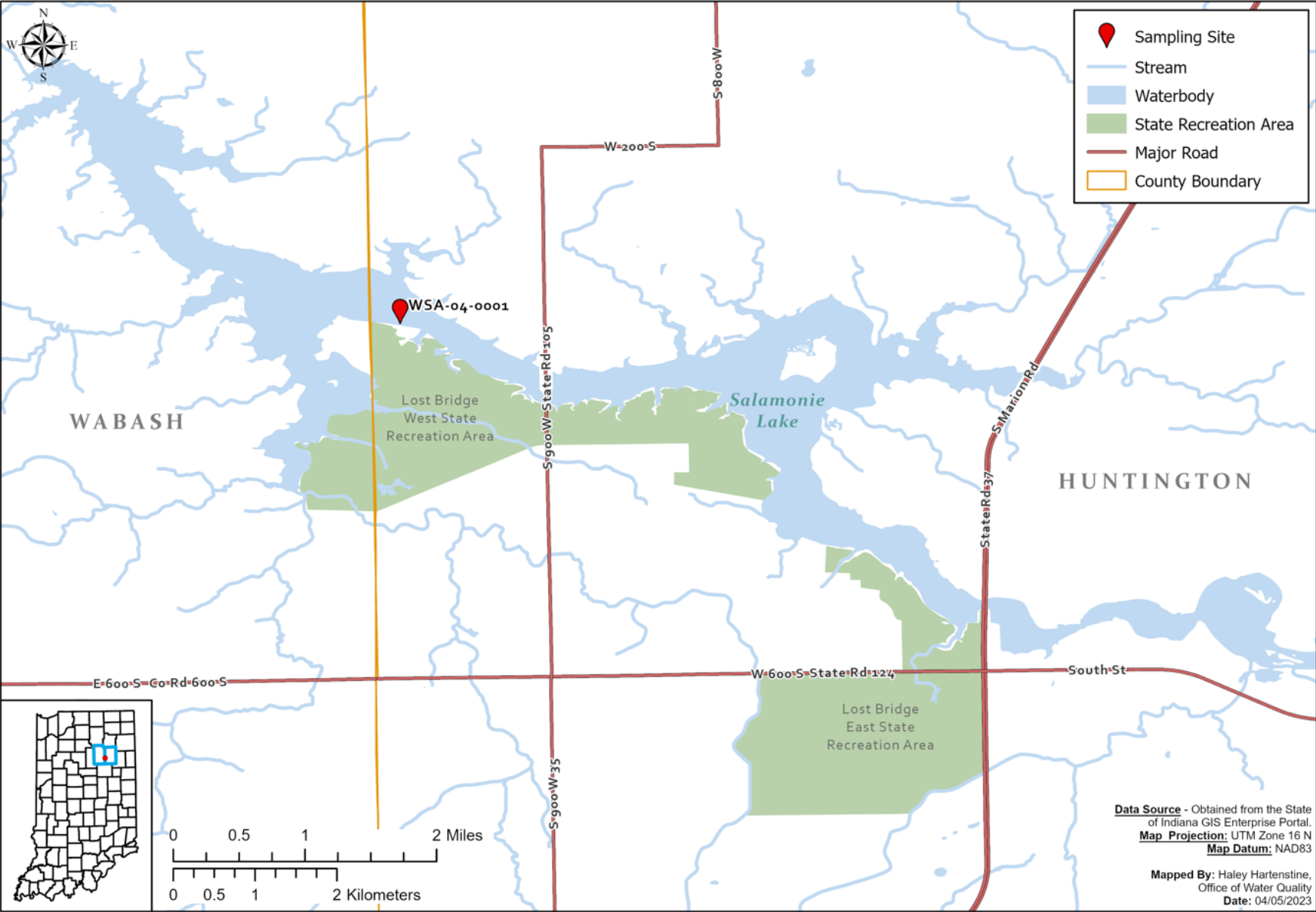




Figure 9. Sand Lake Site Map

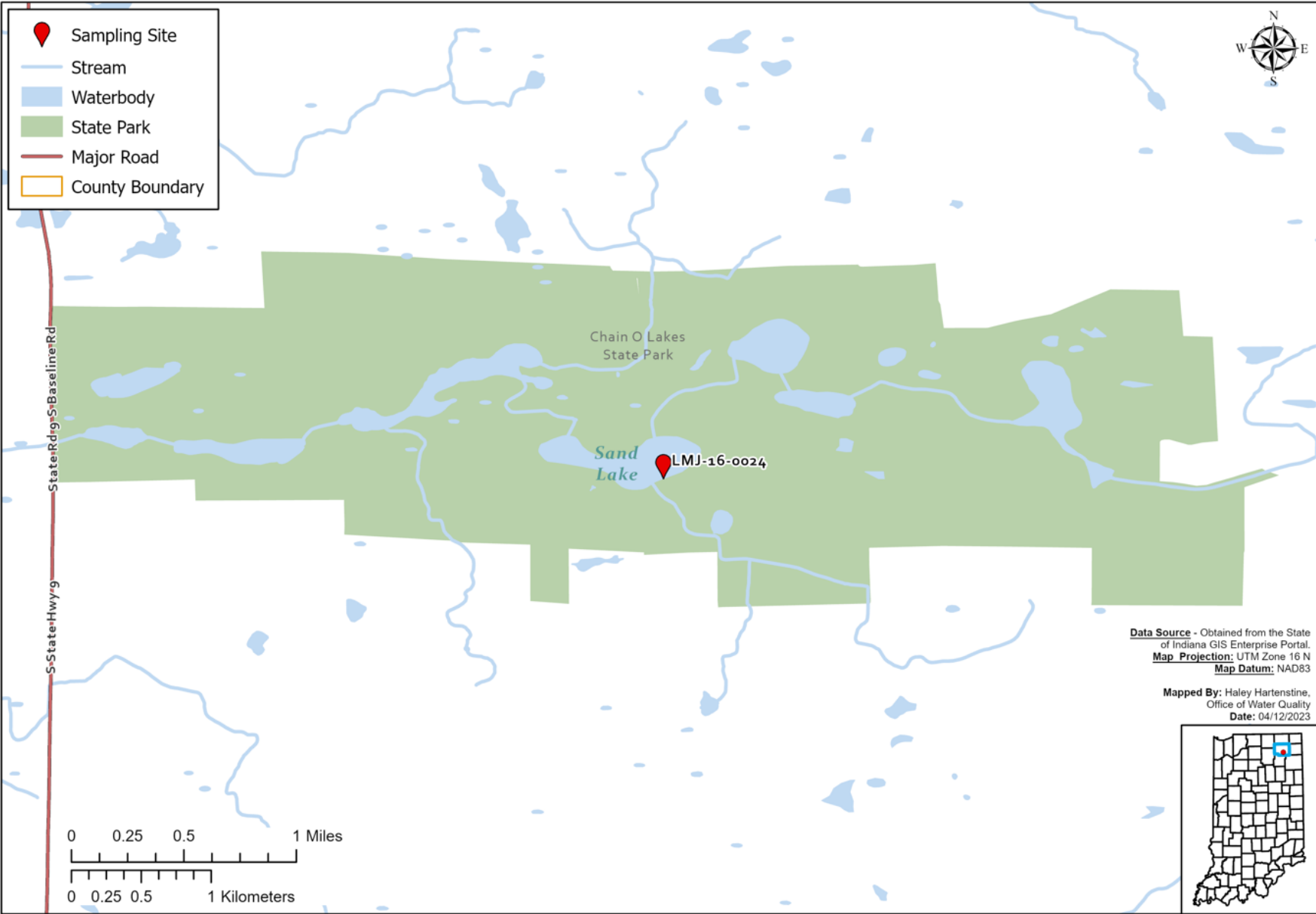


Figure 10. Lake James Site Map

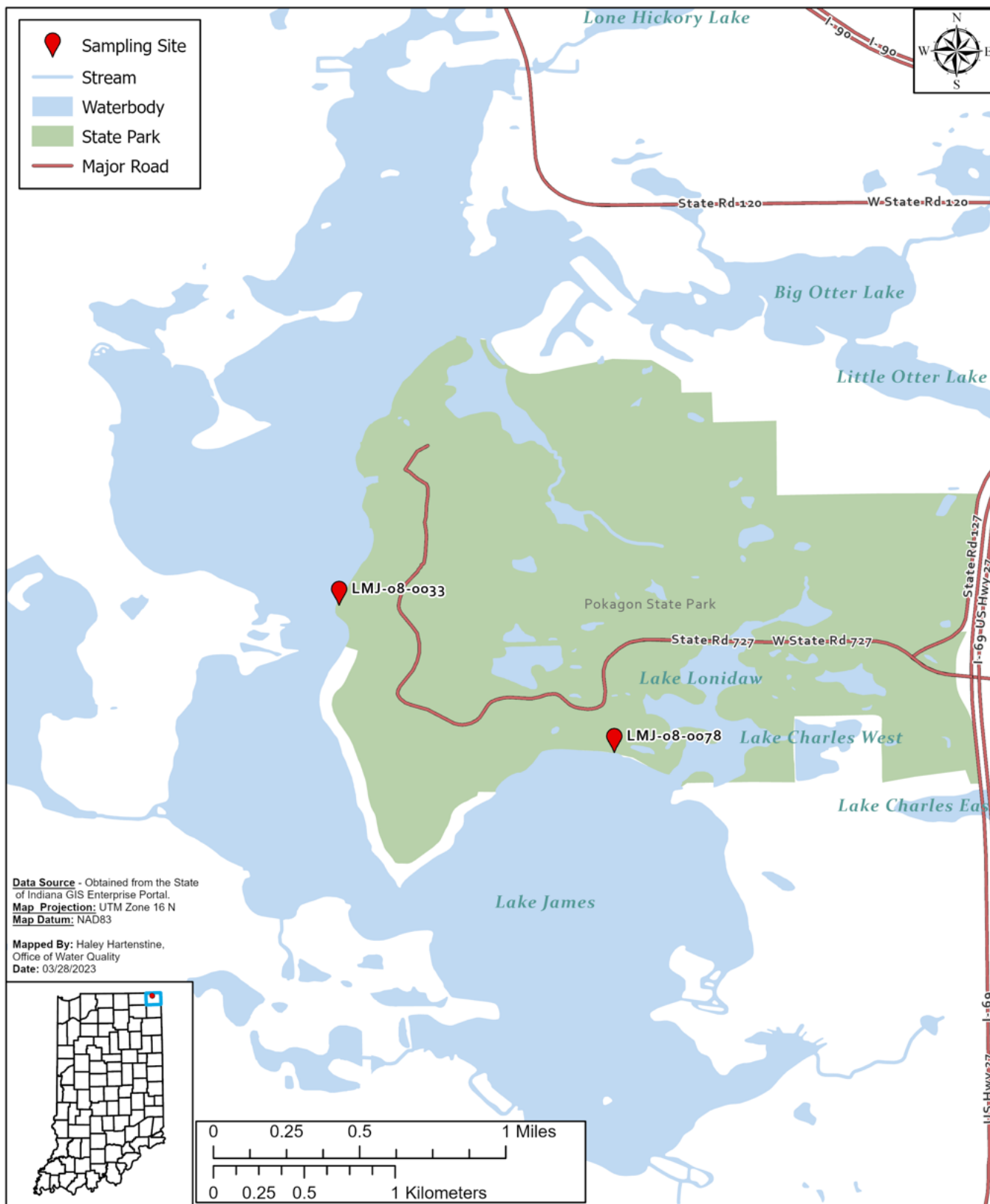


Figure 11. Brookville Lake Site Map

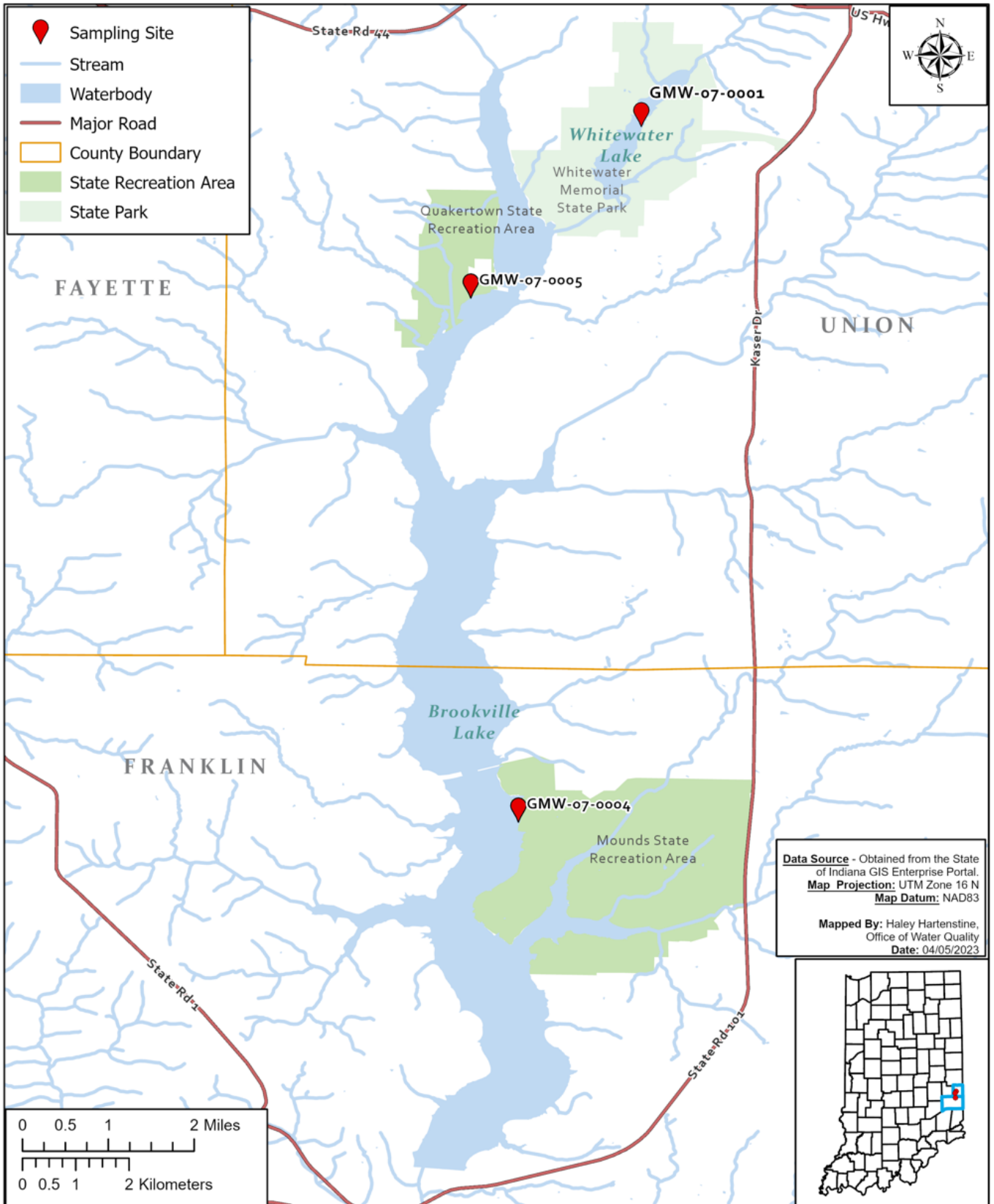


Figure 12. Deam Lake Site Map

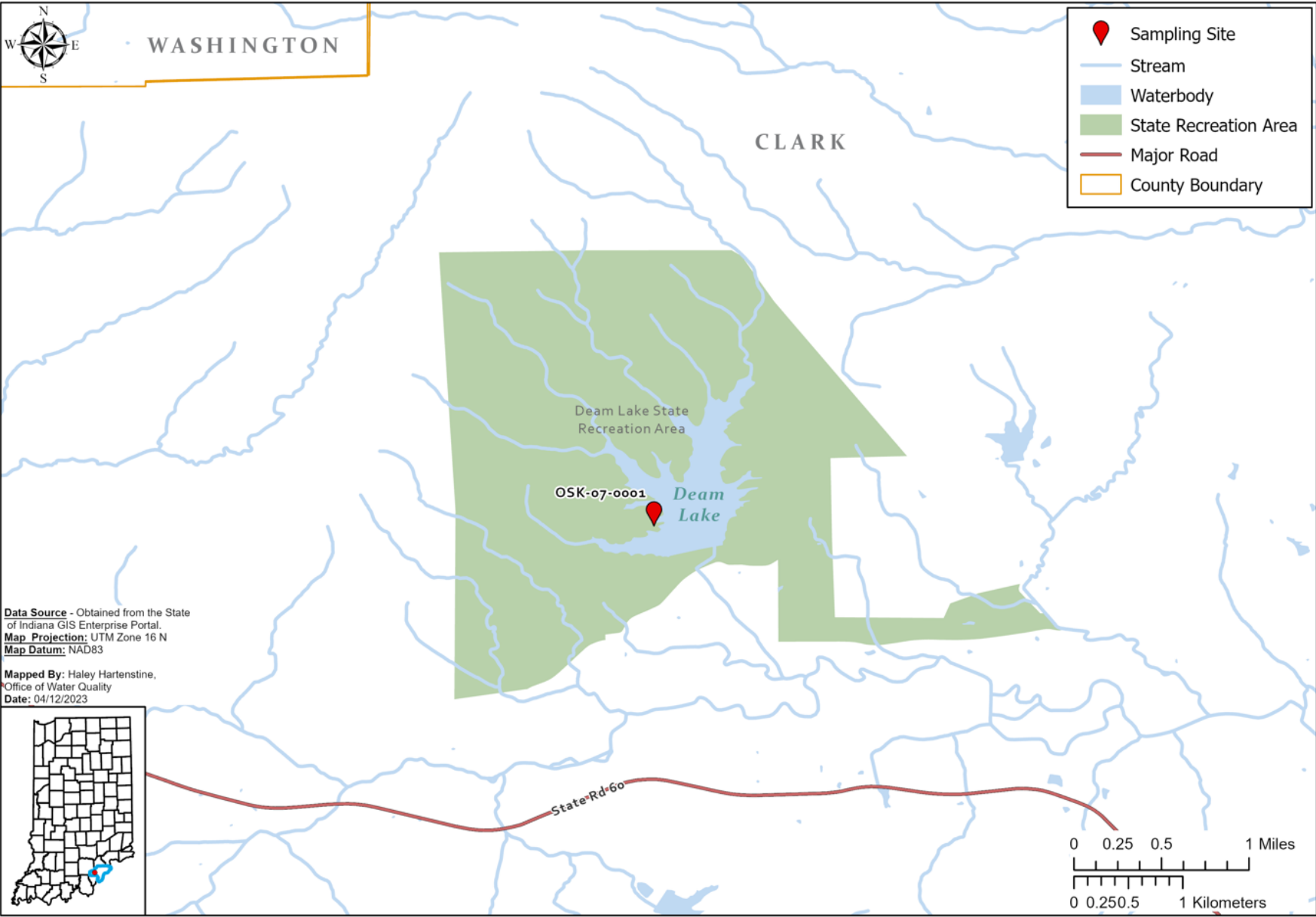


Figure 13. Starve Hollow Lake Site Map

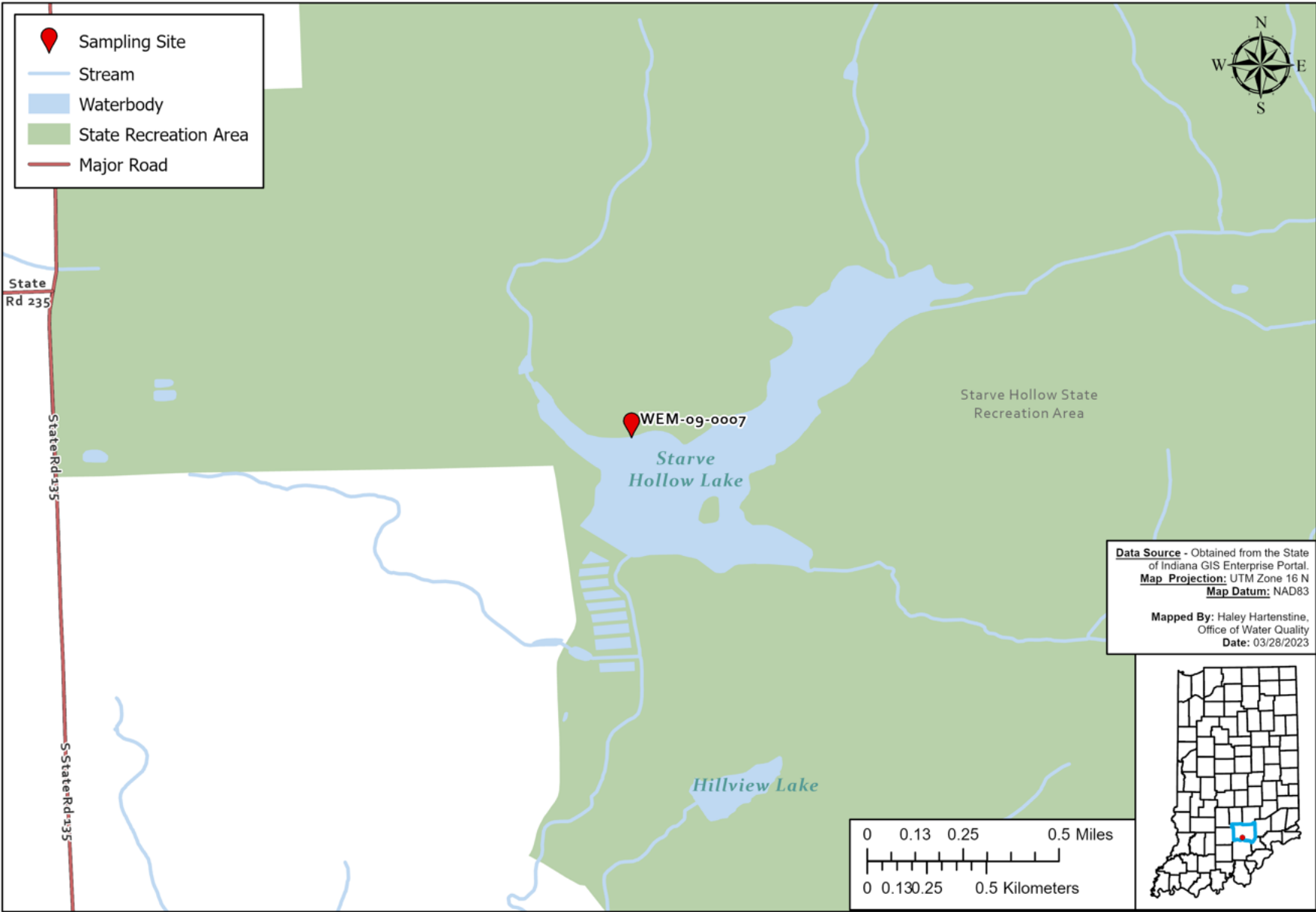
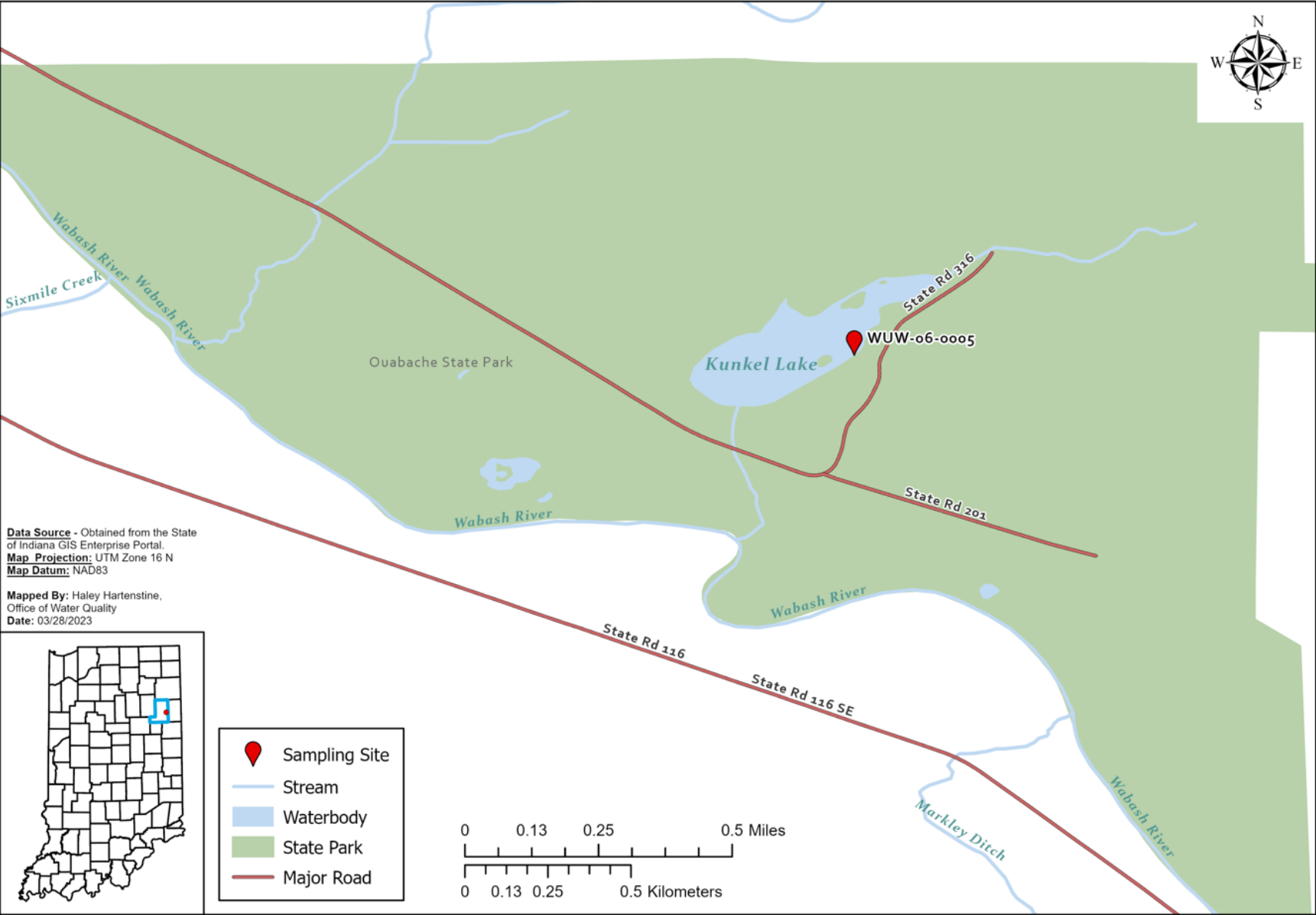


Figure 14. Kunkel Lake Site Map



**Figure 15. Fort Harrison Dog Park Lake Site Map**

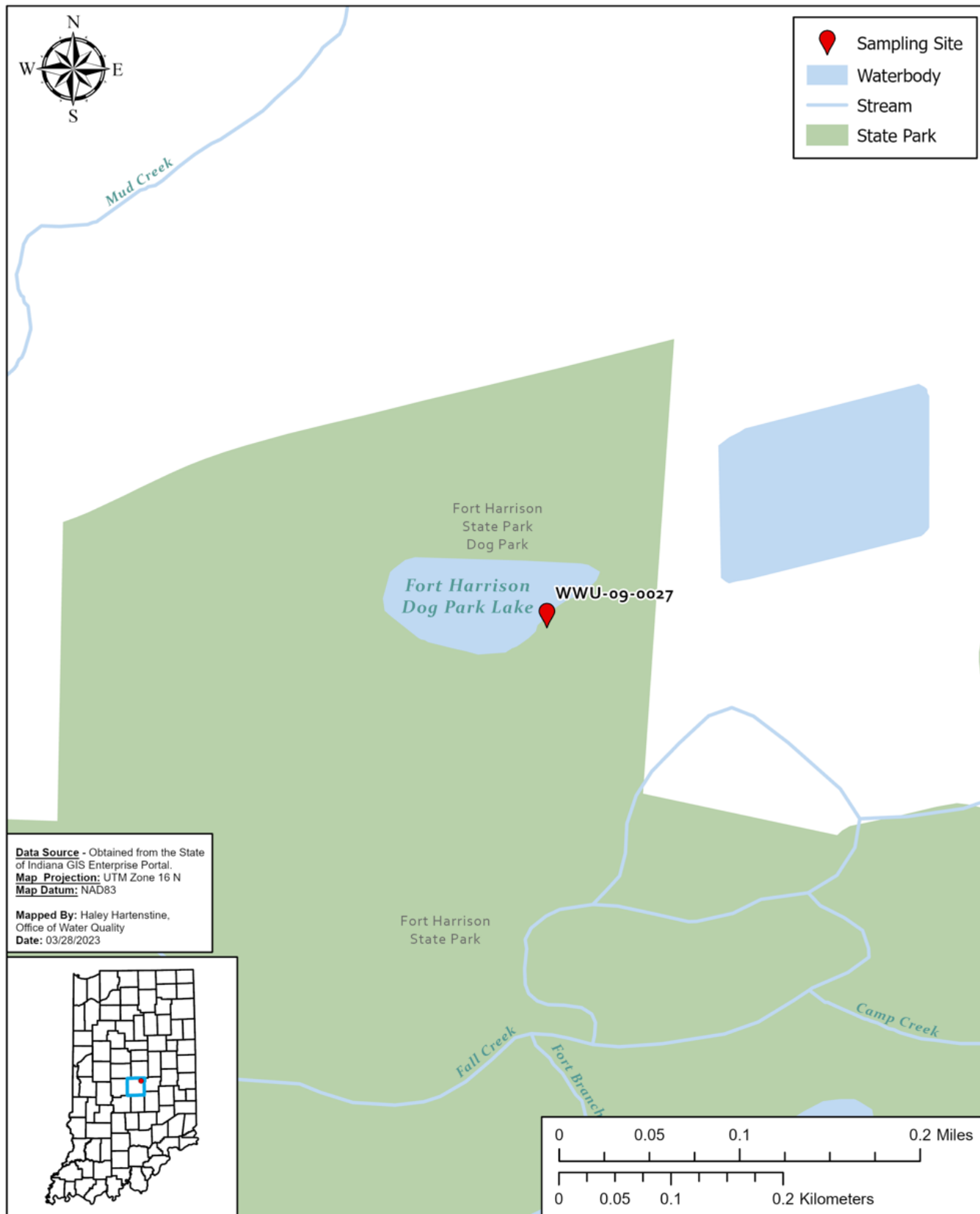


Figure 16. Summit Lake Site Map

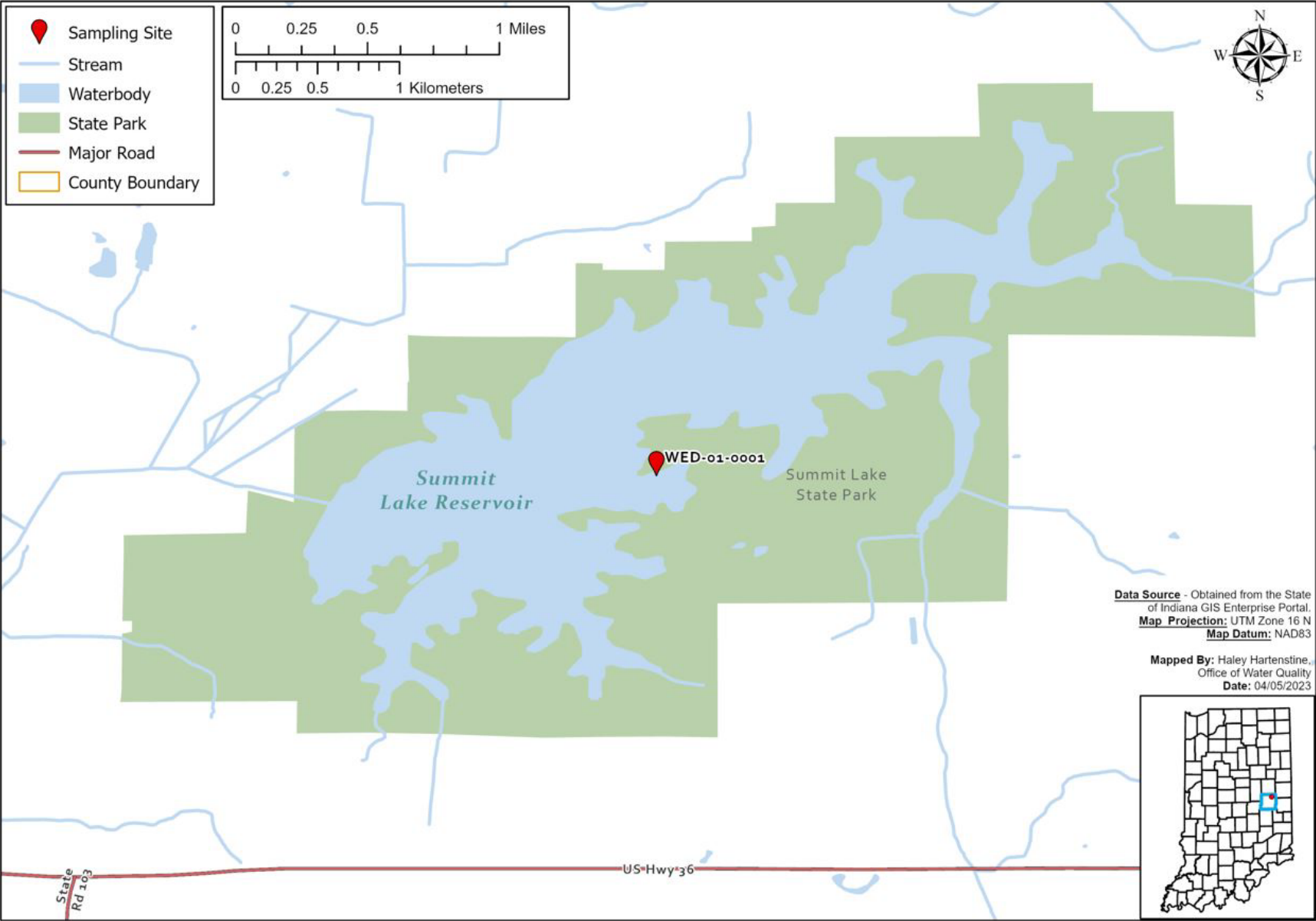




Figure 17. Cagles Mill Lake Site Map

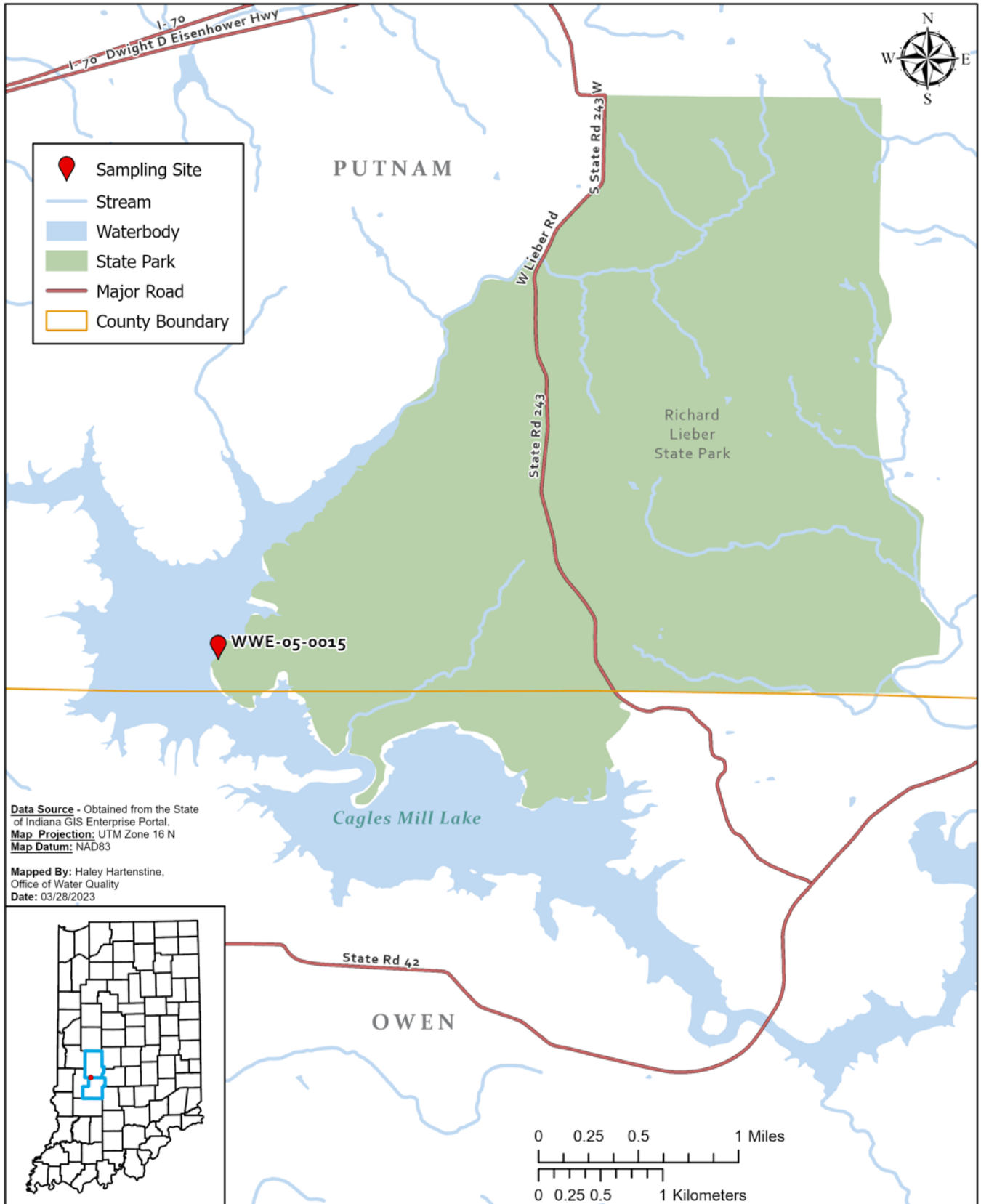


Figure 18. Ferdinand State Forest Lake Site Map

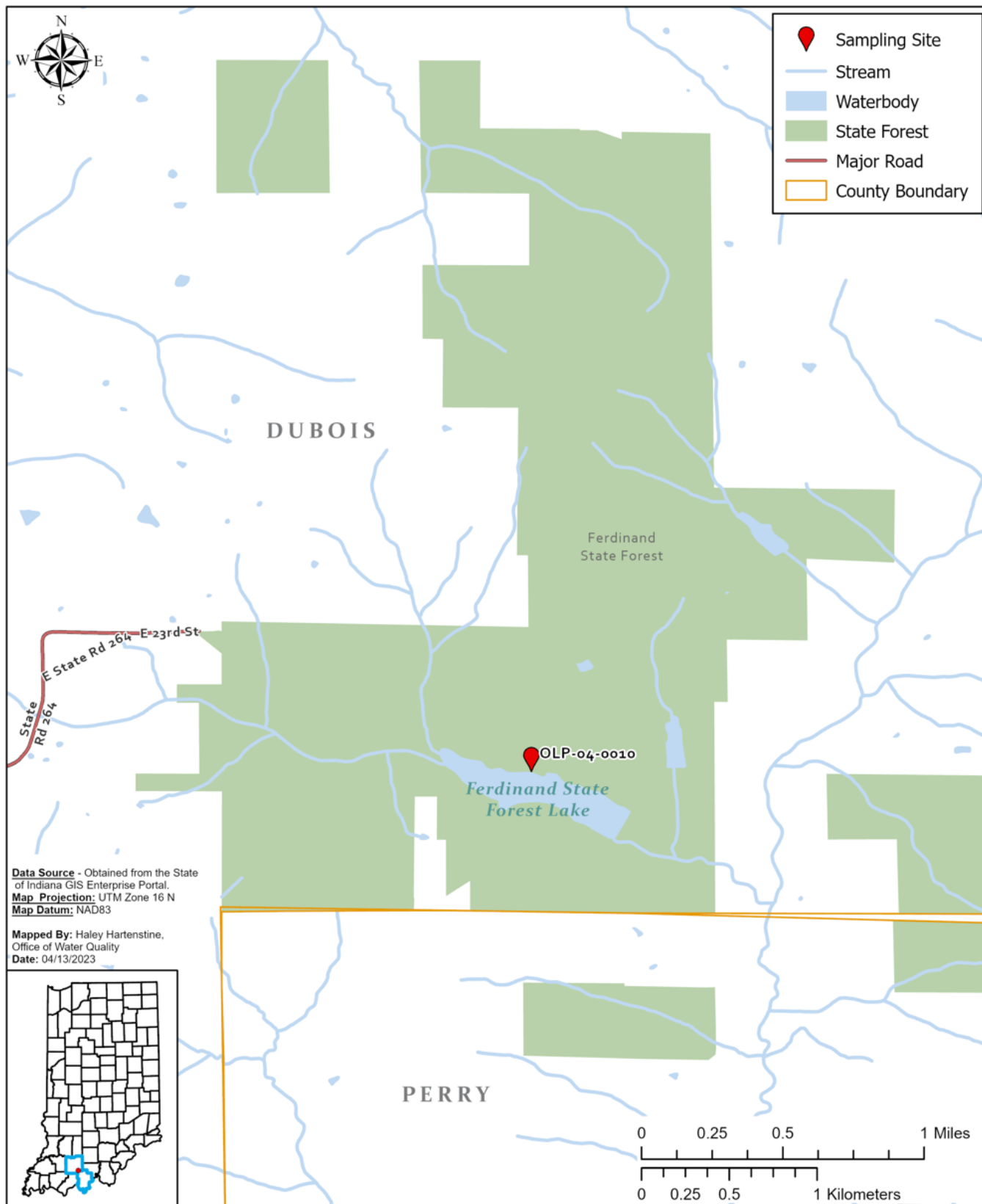


Figure 19. Lake Lincoln Site Map

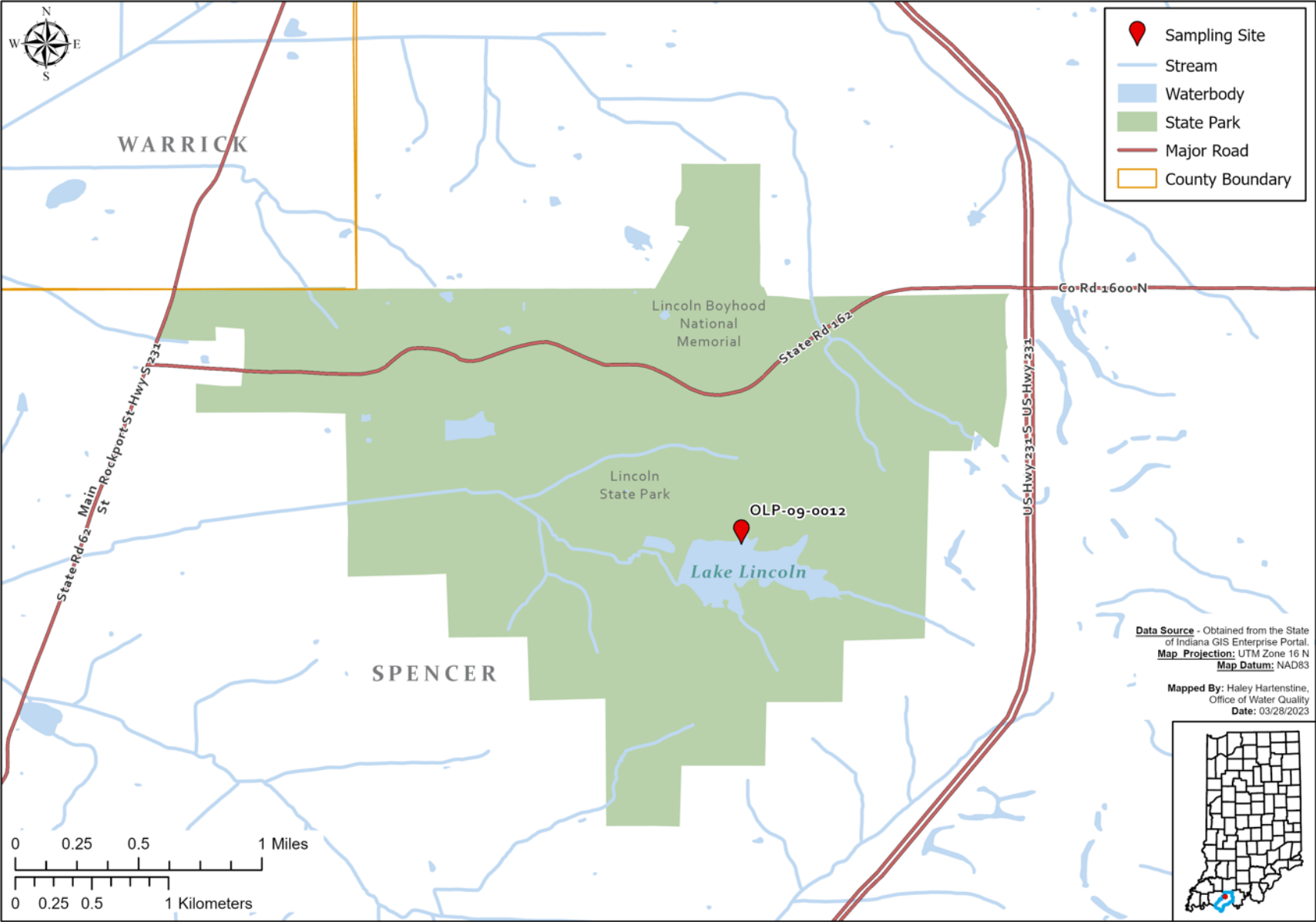
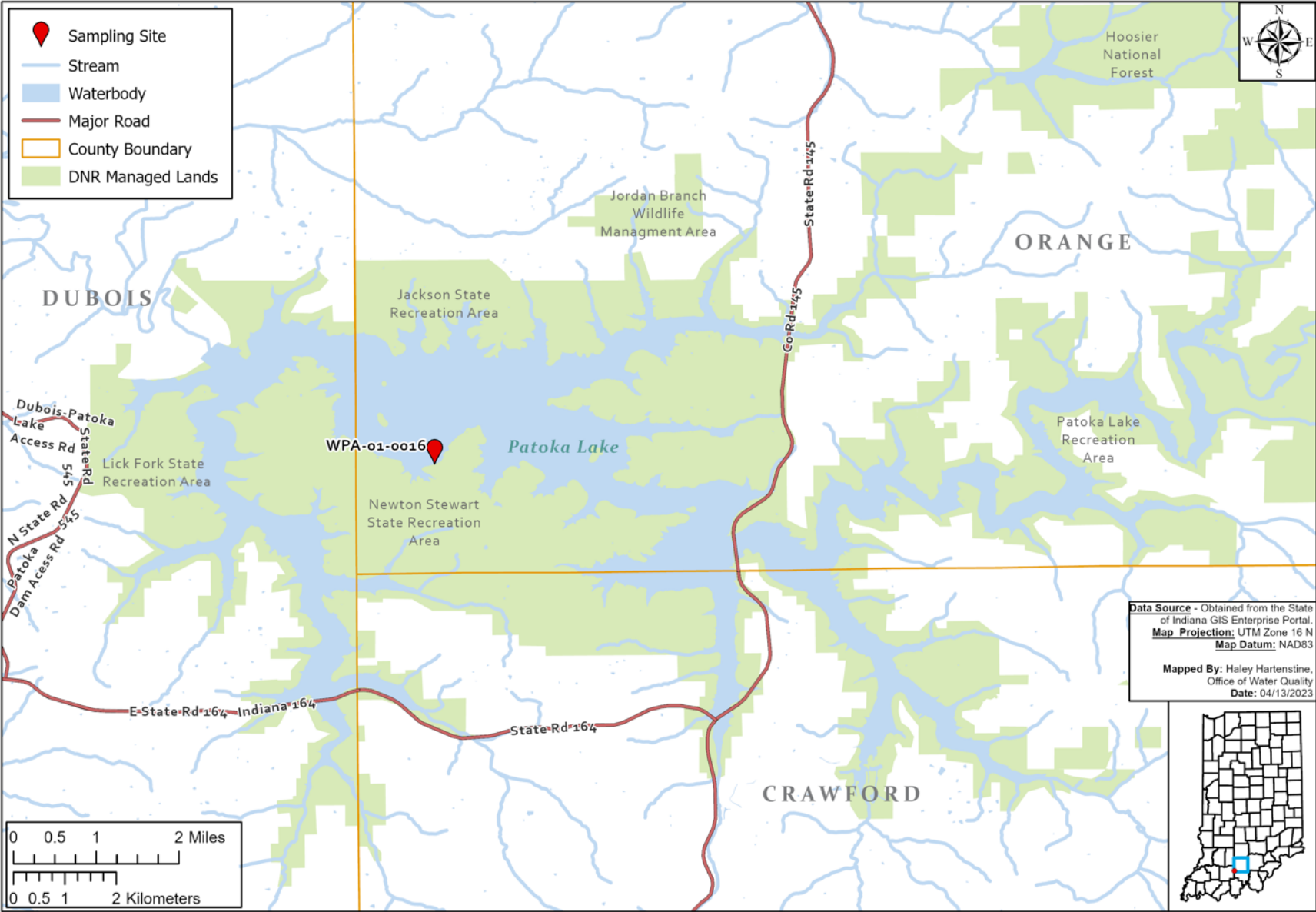


Figure 20. Patoka Lake Site Map



## **B.2. Sampling Methods and Sample Handling**

(QAPP Elements B2, B3)

### **1. Collecting Surface Water Samples for Cyanobacteria and Cyanotoxin Analysis**

Staff will wade into the water body to a level about waist high and stand still for a minute to let the water and sediment settle before taking a sample using the integrated water column sampler. Collect samples from three locations (each side and the middle portion) at each beach swimming area. Combine the samples in the churn to form a composite sample. Take samples for cyanobacteria identification and enumeration, and cyanotoxin analysis from the composite sample. Details of sampling equipment and methods are in the Collecting Surface Water Samples for Cyanobacteria and Cyanotoxin Analysis TSOP (IDEM 2023a).

At the Fort Harrison Dog Park Lake, collect grab samples near the shore to reflect dogs' drinking habits. Staff will check the pH of the buffered cyanotoxin vial to make sure it is between 5 to 7 due to sample storage requirements for anatoxin-a.

### **2. Water Chemistry Sampling**

Staff will collect nutrient samples, record water chemistry field measurements, and note lake or beach conditions on the IDEM Stream Sampling Field Data Sheet. Water chemistry sampling will adhere to the Water Chemistry Field Sampling Procedures TSOP (IDEM 2020). Collection of nutrient samples will diverge from the TSOP in that the nutrient sample bottles will be filled from the composite sample in the churn.

### **3. Field Parameter Measurements**

Measure DO, pH, water temperature, specific conductance, and DO percent saturation with a data sonde each time water samples are collected. Measure field parameters directly from the lake water.

## **B.3. Analytical Methods**

(QAPP Element B4)

### **1. Laboratory Procedure for Cyanobacteria Identification and Enumeration**

Cyanobacteria cells are identified and quantified for each sample using a microscope with brightfield Nomarski differential interference contrast and epifluorescence capabilities. These processes are detailed in the Cyanobacteria Identification and Enumeration TSOP (IDEM 2023b).

### **2. Laboratory Procedure for Microcystins, Cylindrospermopsin, Anatoxin-a, and Saxitoxin Analysis**

Samples are analyzed for microcystins, cylindrospermopsin, anatoxin-a, and saxitoxin concentrations using Gold Standard Diagnostics ELISA test kits. The Determination of Cyanobacteria Toxins in Ambient and Drinking Water by ELISA TSOP contains procedures for sample preparation and analysis for each cyanotoxin (IDEM 2021a).

### 3. Nutrient Parameters Measurements

Pace Analytical Services, Inc. (Indianapolis, Indiana) will process nutrient samples in accordance with preapproved test methods and within allotted time frames. A chain-of-custody form accompanies each sample set through the analytical process. Table 3 identifies the nutrient parameters, respective test methods, and quantification limits.

**Table 3. Nutrient Parameters Test Methods**

Parameter	Method	Limits of Quantification	Units	Preservative	Holding Times
Ammonia Nitrogen	U.S. EPA 350.1	0.1	mg/L	H <sub>2</sub> SO <sub>4</sub> < pH 2	28 days
Nitrogen, Nitrate and Nitrite	U.S. EPA 353.2	0.1	mg/L	H <sub>2</sub> SO <sub>4</sub> < pH 2	28 days
Total Kjeldahl Nitrogen (TKN)	U.S. EPA 351.2	0.5	mg/L	H <sub>2</sub> SO <sub>4</sub> < pH 2	28 days
Total Phosphorus	U.S. EPA 365.1	0.05	mg/L	H <sub>2</sub> SO <sub>4</sub> < pH 2	28 days

### 4. Field Parameter Measurements

Field measurements of DO, temperature, pH, conductivity, and turbidity will be taken each time a water sample is collected. Table 4 identifies the field parameters to measure at each sampling site, respective test methods, and quantification limits. Document field observations from each site on field sheets during each sampling run.

**Table 4. Field Parameters Test Methods**

Parameter	Method	Limits of Quantitation	Units
DO (data sonde optical)	ASTM D888-09 <sup>1</sup>	0.05	mg/L
DO % Saturation (data sonde optical)	ASTM D888-09 <sup>1</sup>	0.05	%
Turbidity (Hach™ turbidity kit)	U.S. EPA 180.1 <sup>1</sup>	0.05	NTU
Specific Conductance (data sonde)	SM 2510B	1.00	µmho/cm
Temperature (data sonde)	SM 2550B(2)	0.1	°Celsius
Temperature (field meter)	SM 2550B(2) <sup>1</sup>	0.1	°Celsius
pH (data sonde)	U.S. EPA 150.2	0.10	SU
pH (field meter)	SM 4500H-B <sup>1</sup>	0.10	SU

<sup>1</sup> Method used for field calibration check

## B.4. Quality Control and Custody Requirements

(QAPP Elements B5, B3)

### 1. Cyanobacteria Cell Count Data

AIMS will assign quality control (QC) samples for each sampling trip prior to sample collection. This includes the field blank, field duplicate, and matrix spike/matrix spike duplicate (MS/MSD) samples. IDEM taxonomists will follow procedures outlined in the Cyanobacteria Identification and Enumeration TSOP (IDEM 2023b). As with normal samples, identify and quantify cyanobacteria cells for each QC sample using a

microscope with brightfield Nomarski differential interference contrast and epifluorescence capabilities.

Rinse the nanoplankton counting chamber and coverslip thoroughly with deionized water and dry to avoid cross-contamination between samples. Analyze one field blank sample each week to ensure samples are not contaminated in the field or laboratory. This also serves as a check to ensure the coverslip and counting chamber are adequately rinsed between each sample. Analyze one field duplicate sample each week. The relative percent difference (RPD) between normal and field duplicate cell counts should be at or below 20%. The RPD between IDEM taxonomists should also be at or below 20%.

## 2. Gold Standard Diagnostics ELISA

Analytical results from the Gold Standard Diagnostics ELISA include QC checks, which can determine precision, accuracy, and completeness for each batch of samples. Archive raw data by analytical batch for easy retrieval and review. Follow chain-of-custody procedures, including date and time of sample collection; time of sample set-up for cyanotoxin analysis and the identification process; time of reading the results; and time and method of disposal. Thoroughly document any method deviations in the raw data bench sheets and records. Refer to the Determination of Cyanobacteria Toxins in Ambient and Drinking Water by ELISA TSOP for QA/QC protocols (IDEM 2021a). Collect all QA/QC samples according to the following guidelines:

Field Duplicate	1 per batch or at least 1 for every 20 samples collected ( $\geq 5\%$ )
Field Blank	1 per batch or at least 1 for every 20 samples collected ( $\geq 5\%$ )
Laboratory Blank	At least 1 laboratory blank (sterile laboratory water blanks) per analysis batch, per day, or every time the laboratory is in use
Laboratory Fortified Blank	At least 1 per analysis batch (spike solution added to sterile laboratory water blanks)
Matrix Spike/Matrix Spike Duplicate	At least 1 per analysis batch (spike solution added to field samples) Should a matrix spike value exceed the calibration range, analyze a laboratory duplicate instead.

## 3. Nutrient Data

AIMS generates a quality control (QC) sample collection schedule containing field blanks, equipment blanks, and duplicate samples for each sampling project. Use sample bottles and preservatives certified for purity. Sample collection containers for

each parameter, preservative, and holding time will adhere to U.S. EPA requirements. Collect field duplicates and MS/MSDs at the rate of one per sample analysis set or one per every 20 samples, whichever is greater. Take field blank samples, using ASTM D1193-91 Type I water, at a rate of one per sample analysis set or one per every 20 samples, whichever is greater.

For each sampling event, fill out a Chain-of-Custody Form which will accompany the sample bottles from sample collection until delivery to the contract lab. Water Chemistry Field Sampling Procedures TSOP (IDEM 2020) describes sampling procedures in more detail. QA documentation for each batch of samples consists of a Chain-of-Custody Form, bench sheets, spreadsheets of results, and the QC report generated by staff from the Technical and Logistical Services Section.

## **B.5. Field Instrument Testing and Calibration**

(QAPP Elements B6, B7)

This project will benefit from the planned upkeep of the measurement instruments, which enhances the instrument performance, and ensures accurate and precise readings. Maintain equipment logs which record equipment calibration and status.

Measurement equipment requires periodic calibration or standardization to reliably produce accurate results. Calibrate the multiparameter data sonde immediately prior to each week's sampling (IDEM 2020; IDEM 2024). Record, maintain, store, and archive calibration results and drift values in logbooks located in the WAPB calibration laboratories. The drift value is the difference between two successive calibrations. Field parameter calibrations will conform to the procedures as described in the instrument user manuals (IDEM 2024). Conduct the DO component of the calibration procedure using the air calibration method. When used in conjunction with bacteriological and water chemistry sampling, field check the unit for accuracy once during the week by comparison with a YSI EcoSense DO200A DO meter or YSI ProSolo ODO meter, as well as with a Hach™ turbidimeter and an Oakton pH meter (IDEM 2020, pages 24-26). Record field calibration values on field sheets and enter into the AIMS database. Also conduct a DO meter reading at sites where the DO concentration is 4.0 mg/L or less. Calibration of YSI Multiparameter Data Sondes TSOP documents procedures for field instrument calibration which is followed by all staff participating in data collection for this study (IDEM 2024).

## **C. Assessment and Oversight**

(QAPP Elements C1, C2)

Conduct field and laboratory performance and system audits to ensure good quality data. The field and laboratory performance includes precision measurements by relative percent difference of field and laboratory duplicates; accuracy measurements by percent of recovery of MS/MSD samples analyzed in the laboratory; and completeness measurements by the percent of planned samples actually collected, analyzed, reported, and usable for the project.



Field audits ensure sampling activities adhere to approved SOPs. WAPB management staff systematically conduct audits of all WAPB staff engaging in field sampling activities.

### **C.1. Data Quality Assessment Levels**

The samples and various types of data collected by this program are intended to meet the QA criteria and data quality assessment (DQA) levels as described in the Surface Water QAPP (IDEM 2023d, pages 107-109). Field data collected as specified in this work plan will comply with DQA Level 2 requirements. All field data are QA/QC'd for completeness, precision, and accuracy. Nutrient data submitted from Pace Analytical Services, Inc. (Indianapolis, Indiana) will adhere to DQA Level 3. Cyanotoxin data analyzed in IDEM WAPB Algae Laboratory are assigned DQA Level 4. QA/QC review reports are developed as a final step of QA process and data assessment. The data and their usability identified in the QA/QC review reports are used in the decision making for water quality assessments.

## **D. Data Validation and Usability**

(QAPP Element D1, D2)

### **D.1. Quality Assurance, Data Qualifiers and Flags**

The various data qualifiers and flags used for QA and validation of the data are found on pages 106-107 of the Surface Water QAPP (IDEM 2023d).

### **D.2. Data Usability**

The environmental data collected, and its usability are qualified and classified into one or more of the four Categories: Enforcement Capable, Acceptable Data, Estimated Data, or Rejected Data.

1. **Enforcement capable** results are DQA Level 3 or 4 data which meet all QC checks.
2. **Acceptable data** are DQA Level 2, 3, or 4 data suitable for decision making. Although a few data may be estimated or even unusable, the sample set, as a whole, has scientific and statistical integrity. Scientific and statistical decisions may be made with respect to the data quality objectives.
3. **Estimated data** may be suitable for enforcement or decision making on a case-by-case basis. Estimated data are suitable for determining future sampling needs, locating target parameters, and identifying possible contaminant levels.
4. **Rejected data** are not suitable for enforcement or for decision making.

### **D.3. Laboratory and Estimated Cost**

This project will take a minimum of four sampling days (day trips) or a maximum of eight sampling days per month. If the cell count for a site exceeds 100,000 cells/ml, sampling frequency will increase to two weeks and if toxin concentrations exceed the Human Recreation Advisory thresholds, the site will be revisited weekly until concentrations fall below threshold

levels. Sampling trips require one vehicle and two staff.

Laboratory analysis and data reporting for this project will comply with the Surface Water QAPP (IDEM 2023d), Request for Proposals 21-68153 (IDEM 2021b), and the IDEM Quality Management Plan (IDEM 2023c). IDEM WAPB Algae Laboratory staff will analyze cyanobacteria and cyanotoxin samples. Identification and enumeration of cyanobacteria samples is performed in-house and does not incur a cost per sample. Laboratory supplies to run cyanotoxin analyses using Gold Standard Diagnostics ELISA test kits will cost approximately \$30,878. Pace Analytical Services, Inc. (Indianapolis, Indiana) will perform tests on the nutrient parameters outlined in Table 3 for a total cost of \$19,156. This estimated lab cost is based on the number of samples analyzed in 2023, with a 10% contingency added for margin of error. The anticipated total budget for laboratory costs for this project is \$50,034.

#### D.4. Reference Manuals and Personnel Safety

**Table 5. Personnel Safety and Reference Manuals**

Role	Required Training/Experience	Training References	Training Notes
All staff participating in field activities	-Basic first aid and cardiopulmonary resuscitation (CPR)  -Personal Protective Equipment (PPE) Policy  -Personal flotation devices (PFD)	-A minimum of 4 hours of in-service training provided by WAPB (IDEM 2010)  -IDEM 2008  -February 29, 2000, WAPB internal memorandum regarding use of approved PFDs	- WAPB staff meeting Health and Safety Training requirements will accompany staff lacking 4 hours of in-service training or appropriate certification in the field at all times. -When working on boundary waters as defined by Indiana Code (IC) <a href="#">14-8-2-27</a> 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.
All staff performing laboratory duties	-Review relevant safety procedures  -Review safety data sheets annually	-OWQ Laboratory Safety Plan (IDEM 2021c)  -IDEM Hazard Communication (HazCom) Plan (IDEM 2019)	

## References

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- U.S. EPA 2019. [Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin](#). EPA 822-R-19-001. U.S. EPA, Office of Water, Health and Ecological Criteria Division, Washington, D.C.
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- IDEM 2020. [Water Chemistry Field Sampling Procedures](#). B-015-OWQ-WAP-XXX-20-T-R0. OWQ, WAPB, Indianapolis, Indiana.
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- Clesceri, L.S., Greenburg, A.E., Eaton, A.D., 2017. SM-Standards Methods for the Examination of Water and Wastewater 23<sup>rd</sup> Edition. American Public Health Association.
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## Distribution List

Electronic Distribution Only  
(QAPP Element A3)

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