



Christian County Planning & Zoning Commission Recommendation & Staff Report To the County Commission

Proposed Amendments to the Christian County Zoning Regulations

HEARING DATE: July 17, 2025

ENCLOSURES:

1. Amended Article 47.5 of the Zoning Regulations
2. Recharge Area Delineation Study for Smallin Civil War Cave
3. City of Ozark Presentation
4. Proposed G-1 GRPA Map

INTRODUCTION:

Article 47.5 was added to the County Zoning Regulations in November of 2024. At that same time, Article 3 was also amended to allow for the creation of certain overlay districts intended to provide increased protection in vulnerable groundwater recharge areas.

Concurrently, the City of Ozark adopted amendments to its City Code which seek to provide similar protections for vulnerable areas within their jurisdiction.

The amendment being presented for consideration here would effectively establish and delineate the boundaries of a particular GRPA to be known as “G-1”.

Because this environmental issue is a function of the natural geography in the area, the boundaries of this overlay cover properties which are located in both the City and the County similar to our adopted overlays for the Urban Service Area and Transportation.

PROCESS:

Article 47.5, Section 4-A explains the process and requirements for establishing a GRPA overlay district. Staff believes that sufficient information meeting these requirements has been submitted through the City of Ozark to consider establishing a GRPA in a vulnerable area north of the city.

The adoption of an Overlay district constitutes an amendment to the Zoning Regulation. As such, staff has followed the public notification process outlined in Sections 64.211 through 64.295 of the Revised Statutes of Missouri. The recommendation of the Planning and Zoning Commission on this matter will be presented to the County Commission for their consideration and approval.

SUMMARY OF SUPPORTING INFORMATION

A Recharge Area Delineation Study, conducted by The Ozark Underground Laboratory (OUL) in coordination with MoDNR and the City of Ozark as well as a related presentation from the City of Ozark, which are both attached to this report, provide factual information demonstrating the concern and also a defined boundary of the geographic area the proposed GRPA would apply to.

The area north of Ozark is under increasing pressure for residential and commercial development around the city. The owners of Smallin Cave brought to the city's attention that storm flows in the cave stream have carried high sediment loads and unnatural sediment deposits in the cave after precipitation events. The purpose of this study was to delineate the recharge area for Smallin Cave to assist city and county partners in their efforts to limit the detrimental impacts of land use on the cave and the Finley River.

The Ozark Underground Laboratory conducted nine groundwater traces using fluorescent tracer dyes during this study to delineate the recharge area for Smallin Cave. Seventeen sampling stations were established in and around Smallin Cave, Fielden Cave, several springs, and surface streams of interest to measure background fluorescence and detect dye from traces conducted by OUL staff. A total of 189 activated carbon samplers and 52 water samples were analyzed for the presence of the tracer dyes. One or more of the tracer dyes was detected at 9 of the 17 sampling stations monitored.

Based on the traces conducted during this study, most of the recharge to Smallin Cave is derived from the sinkhole plain located to the north and west of the cave. Dye tracing also demonstrated the existence of a shared recharge area between Smallin Cave and the spring on Parched Corn Hollow, indicating these two systems are part of a larger groundwater drainage network contributing water to the Finley River in the Ozark area.

The mapped recharge area contains a total of 1,344 acres. Currently 388 acres are within the city limits and 953 acres are in the County. Within this recharge area, the presence of karst topography and mapped sinkholes combined with increasing human development creates an increasingly vulnerable situation with respect to ground water and the people and ecology which depend on the quality of that water.

The adoption of the defined recharge area as a GRPA overlay district would allow the County to apply certain development requirements which would provide the increased protections detailed in Article 47.5 which are supportive of the findings and recommendations presented within the report.

PROPOSED AMENDMENTS TO THE CHRISTIAN COUNTY ZONING REGULATIONS

Amendment #1 Establishment of a Groundwater Recharge Protection Area Overlay District to be designated G-1

Article 3, Section 1 currently lists the various categories of zoning and overlay districts within the Christian County Zoning Regulations. This amendment is to simply add "G-1 Groundwater Recharge Protection Area" to the list of Overlay Districts.

Amendment #2 Adoption of a map for the Groundwater Recharge Protection Area Overlay District designated as G-1

Article 3, Section 9 reads as follows:

Section 9. Overlay Districts

The County may adopt overlay districts, including but not limited to overlay districts for purposes of hazard mitigation, land use planning and transportation improvements. If adopted, overlay districts shall be shown on a separate overlay district map which is referenced in this zoning ordinance and the Christian County Subdivision Regulations. The procedure for adopting or amending an overlay district boundary shall be the same as for amending the official zoning map, as specified in Article 49 of this ordinance.

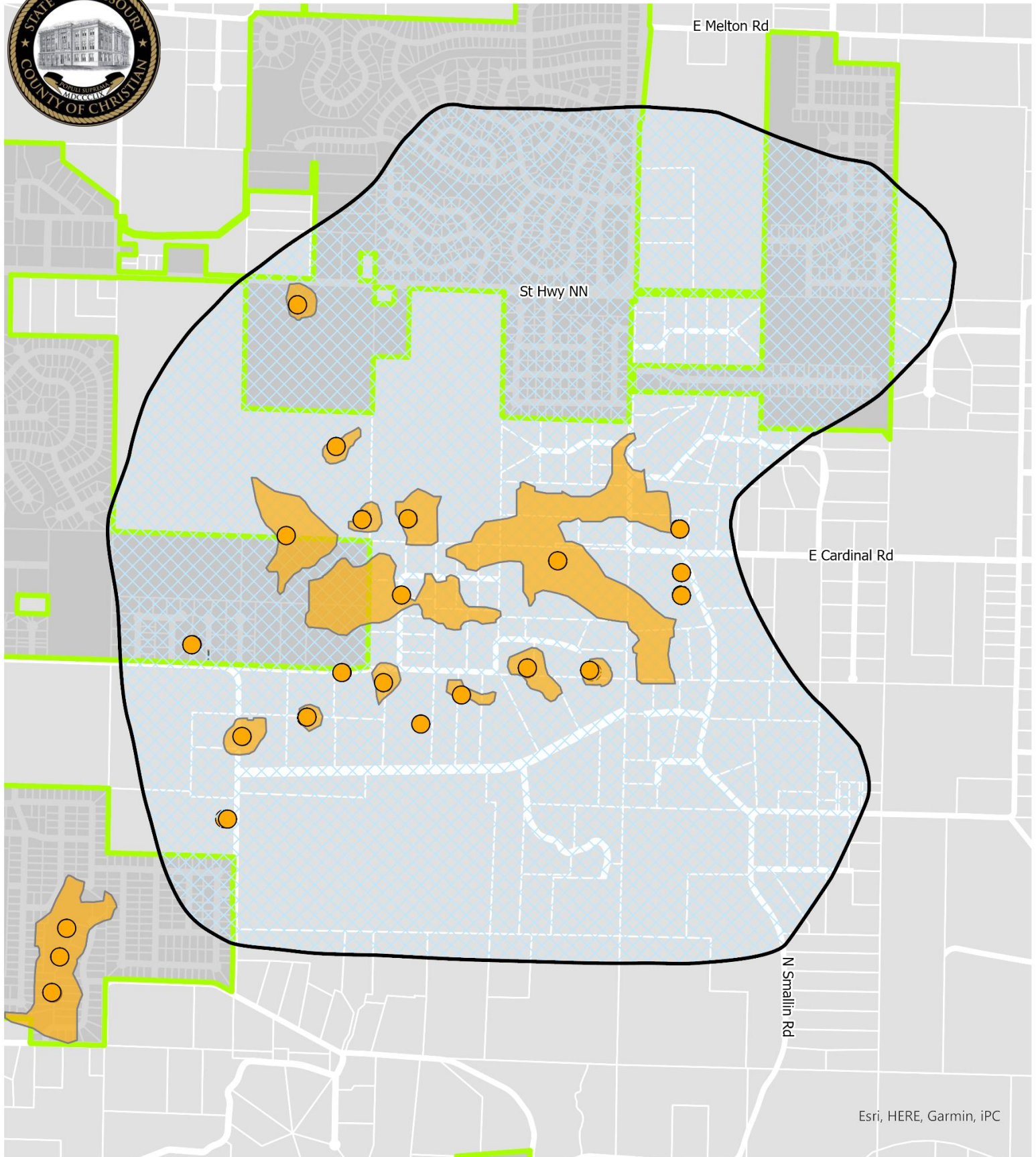
This Section calls for any adopted overlay districts to be shown on a separate map for reference to the zoning ordinance. In this case, the proposed amendment is simply the adoption of the map titled "Groundwater Recharge Protection Area G-1", a copy of which is attached to this report.

At the June 16, 2025 Planning & Zoning Commission meeting, the board reviewed the staff report and supporting information and unanimously voted to recommend amendments to the Zoning Regulations by adopting changes to Article 3, Section 1 as presented and the adoption of the map presented which delineates the boundaries of the G-1 Groundwater Recharge Protection Area.



Todd M. Wiesehan,
Director, Resource Management Department

G-1 GRPA Overlay



Adopted 7-17-2025

Legend

- GRPA Overlay
- Sinkhole Points
- Sinkhole Area
- Ozark City Limits
- Parcel

ARTICLE 47.5. GROUNDWATER RECHARGE PROTECTION AREA OVERLAY DISTRICT (GRPA)

Section 1. Statement of Intent

- A. The Groundwater Recharge Protection Area Overlay District is intended to provide increased protection of the County's water resources in designated areas where pollutants associated with urbanized development presents an increasing threat to water quality and the habitat of protected or endangered species.
- B. The intent of the Groundwater Recharge Protection Area District is to limit the introduction of pollutants in vulnerable areas from the impacts of increased human development through:
 - 1. Identification of vulnerable areas where increased protection measures are warranted.
 - 2. Application of development standards and requirements within these areas which are intended to reduce the risk of contamination.

Section 2. Recognition of Community Comprehensive Plans and Groundwater Recharge Protection Areas

- A. The Christian County Commission recognizes the adopted Comprehensive Plans that have been approved by the various incorporated communities within Christian County. Therefore, where the cooperation between Christian County and its incorporated communities is authorized by the constitution and laws of the State of Missouri, it is determined by the Christian County Commission that for the purposes of:
 - 1. Managing land development in defined areas which are known to be environmentally sensitive.
 - 2. Anticipating and avoiding the introduction of contaminants into the County's groundwater resources in areas where the risk is greatest.
 - 3. Coordinating with incorporated communities to assure that reasonable standards for development are observed.

Section 3. Definitions

Best Management Practices (BMPs)

Conservation practices or management measures which control soil loss and reduce water quality degradation mainly caused by nutrients, animal wastes, toxins, sediment in the runoff. BMPs may be either structural (grass swales, terraces, retention and detention ponds), or non-structural

(disconnection of impervious surfaces, directing downspouts onto grass surfaces and educational activities).

Buffer

A vegetated area including trees, shrubs, managed lawn areas, and herbaceous vegetation which exists or is established to protect a stream system. Alteration of this natural area is strictly limited.

Development

A change in the zoning, intensity of use or allowed use of any land, building, structure or premises for any purpose. The subdivision or severance of land. The construction, erection or placing of one or more buildings or structures on land or use of land or premises for storage of equipment or materials. Making of an addition, enlargement or alteration to a building or structure, in, on, over or under land, which has the effect of increasing the size or usability thereof. Land disturbance activities such as but not limited to site-grading, excavation, drilling, removal of topsoil or the placing or dumping of fill and installation of drainage works. The use of the term shall include redevelopment, as defined in the stormwater regulations, in all cases unless otherwise specified in these regulations.

Erosion And Sediment Control Plan

A set of plans prepared by or under the direction of a professional engineer, land surveyor, landscape architect or geologist licensed in the State of Missouri or by a Certified Professional in Erosion and Sediment Control (CPESC) indicating the specific measures and sequence to be used to control erosion and sediment on a development site before, during and after construction.

Groundwater Recharge

The hydrologic process where rainwater moves downward from the surface into subsurface areas and aquifers where groundwater is naturally stored.

Hydrogeological Assessments

A study to determine the vulnerability of a specific area, these assessments consider factors such as the geological characteristics of the subsurface, soil properties, depth to the water table, and potential contaminant sources.

Land Disturbance

Any activity that exposes soil including clearing, grubbing, grading, excavating, filling and other related activities.

Losing Stream

A losing stream is a stream or part of a stream where a significant amount of its water (at least 30% during dry conditions) flows underground into a bedrock aquifer.

Indigenous Vegetation

Any species that was present in Missouri prior to European Settlement (approximately 1735 A.D.) or any plant identified as native or indigenous on lists maintained by agencies such as the Missouri Department of Conservation or United States Department of Agriculture.

Managed Lawn Areas

Any area greater than five hundred (500) square feet where the vegetative ground cover is maintained at a uniform height of less than 5-inches.

Ordinary High-Water Mark

That line on the shore established by the fluctuations of water and indicated by physical characteristics such as clear natural line impressed on the bank, shelving, changes in the character of soil, destruction of terrestrial vegetation, the presence of litter or debris, or other appropriate means that consider the characteristics of the surrounding area.

Pollution

Any contamination or alteration of the physical, chemical, or biological properties of any waters which will render the waters harmful or detrimental to domestic, commercial, industrial, agricultural, recreational, or other legitimate beneficial uses; or to livestock, wild animals, birds, fish or other aquatic life.

Soil Erosion & Control Permit

The document issued by the County approving the Stormwater Management Plan and authorizing land disturbance activity in accordance with the Storm Water Pollution Prevention Plan (SWPPP).

Streams

Perennial and intermittent watercourses identified through site inspection and United States Geological Survey (USGS) maps and further defined and categorized as follows:

- A. Type I Streams are defined as perennial streams shown as solid blue lines on the United States Geological Survey seven and one-half minutes series topographical map and have a drainage area of greater than 50 acres.
- B. Type II Streams are defined as intermittent streams shown as dashed blue lines on the United States Geological Survey seven and one-half minutes series topographical map and have a drainage area of greater than 50 acres.
- C. Type III Streams are defined as intermittent streams or natural channels which are not shown on the United States Geological Survey seven and one-half minutes series topographical map as either blue or dashed blue lines which have drainage areas of greater than 50 acres.

United States Geological Survey (USGS) Blue Line Stream

A stream that appears as a broken or solid blue line (or a purple line) on a USGS topographic map.

Section 4. Establishment of a Groundwater Recharge Protection Area Overlay District

- A. The County or an incorporated community within Christian County may propose the creation of a GRPA in accordance with the following requirements:
 - 1. The area being considered must be at increased risk for groundwater contamination due to the presence of the following factors:
 - a. The potential for residential development at densities greater than one dwelling per three acres within the defined recharge area.
 - b. The area must contain significant areas of karst features such as mapped sinkholes or losing streams.
 - 2. The entity proposing the establishment of a GRPA shall submit a map identifying the geographic boundary of the proposed recharge area to be protected and have enacted or be prepared to enact regulations similar in nature to those which have been adopted by the County which are intended to provide increased protection from pollution within the proposed GRPA.
 - 3. Submit any hydrogeological assessments or related information which demonstrates the presence of existing pollutants or degradation of natural habitat for protected or endangered species.
 - 4. Seek a [recommendation for approval of an amendment to the Zoning Regulations, adding the GRPA boundary as a new overlay district to be approved](#) by the County Commission. ~~as part of a public hearing where property owners within the proposed boundary have been given notice via certified mail at least 15 days prior to the public hearing.~~
 - 5. [As with any other amendment to the Zoning Regulations, the procedure for adopting a new GRPA shall follow the process delineated in Article 49, Sections 3 and 4 as established and created under the authority of Sections 64.211 through 64.295 of the Revised Statutes of Missouri.](#)

Section 5. Change in Zoning Designation

- A. Changes in zoning to Commercial and Manufacturing zoning districts shall not be allowed within a designated GRPA.
- B. Following the date of adoption of any GRPA overlay district, parcels 20 acres or greater, located entirely or partially within the GRPA boundary shall be considered immediately eligible and recommended by the Planning and Zoning Commission to the County Commission for rezoning to Conservation Development District –

CD, as detailed in Article 48. For these purposes, a 20 acre or greater parcel may be created by assemblage of contiguous smaller parcels.

1. Property owners must initiate this request for zoning change through the Planning and Development Office.
2. The setbacks detailed in Section 6 of this Article shall be observed along with all other development standards listed in Article 48.

Section 6. Standards for Development Within a Groundwater Recharge Protection Area

A. Setbacks

1. In order to reduce the risk for pollutants entering and potentially contaminating the County's natural water resources, the following increased setbacks shall be observed:
 - a. Sinkholes – No new development shall be permitted closer than 50 linear feet from the mapped rim of a sinkhole.
 - b. USGS Blue Line Streams – No new development shall be permitted within 50 feet of the ordinary high-water mark of a mapped stream.

B. Erosion and Sediment Control

The following requirements related to soil erosion control shall be observed within the GRPA:

1. In General – the preserved setback/buffer areas surrounding sinkholes and streams are best preserved in their natural condition which includes indigenous vegetation, native warm season grasses and the preservation of existing trees. Areas outside the required setback/buffer may be managed lawn areas.
2. Where land disturbance requires the issuance of a Soil Erosion Control Permit – Notwithstanding the normal stormwater management plan requirements, permitted projects must also adhere to the following higher standards:
 - a. A minimum of 85% of proposed disturbed areas must be routed into properly sized sediment basins.
 - b. All disturbed areas discharging directly to a stream or sinkhole must be routed to a sediment basin.
 - c. Sediment basins shall have perforated risers wrapped in filter fabric and be sized for a 10-year storm.
 - d. Silt fence is not to be allowed as a BMP unless specifically approved for use by the County Engineer.
 - e. Work stoppage will be required if large amounts of sediment are found to be bypassing erosion control measures.

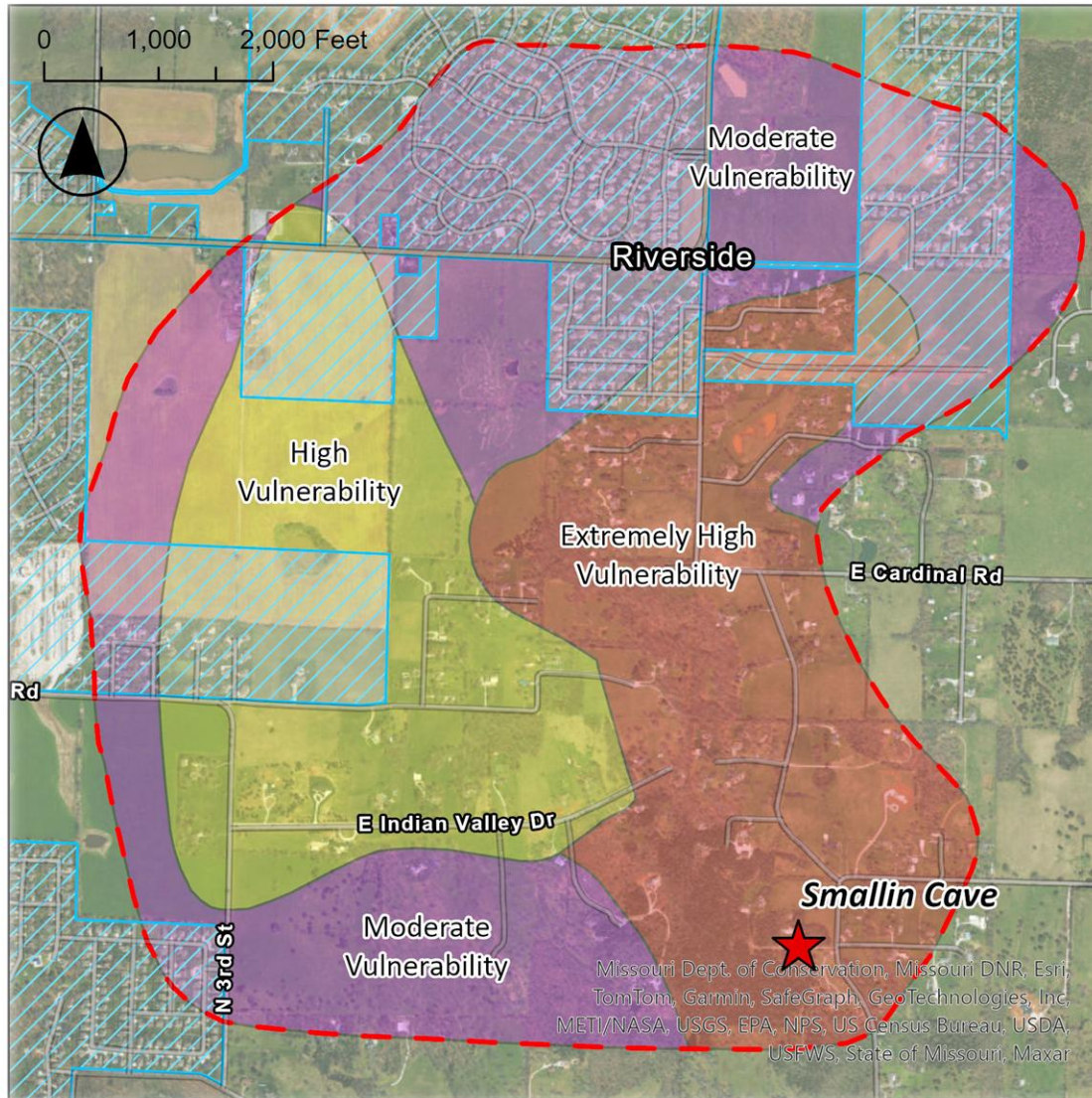
Section 7. Permitted Uses Within the Buffer Areas

- A. The following uses/activities are allowed:
 - 1. Structures or other developments in place prior to the adoption of the GRPA.
 - 2. Development which is covered by a plat recorded prior to the adoption of the GRPA.
 - 3. Permeable surfaced foot and bicycle paths
 - 4. Fencing which may also serve as protection of the buffered area
 - 5. Road and Bridge crossings
 - 6. Utilities where no practical alternatives exist as determined by the director.
 - 7. Stream restoration, stream bank restoration or restoration of indigenous vegetation in accordance with an approved plan.
 - 8. Roads, that exist on or before the date of adoption of the GRPA regulations, and associated maintenance activities.
 - 9. Modifications to stream channels or wetlands if such modifications have been approved and permitted by a Federal Agency such as the U.S. Army Corps of Engineers.

Section 8. Exceptions

- A. A developer may provide alternative Enhanced Environmental Protection Area regulation(s) when one or more of the following conditions apply:
 - 1. Topography, streams, natural rock formations, sod, vegetation or other site conditions are such that full compliance is impossible or impractical; or
 - 2. The applicability of this Article would cause safety concerns to persons or property.
- B. The County Engineer, or their designee, shall have authority to review and approve any alternative Best Management Practice.

INTERGOVERNMENT COOPERATION



Vulnerability	Total Area (Acres)	City of Ozark		Christian County	
		Area (Acres)	% Area	Area (Acres)	% Area
Moderate	596.17	264.62	44%	331.56	56%
High	348.36	99.14	28%	249.23	72%
Extremely High	387.87	27.13	7%	360.74	93%



A Recharge Area Delineation for Smallin Civil War Cave, Ozark, Missouri

July 19th, 2023

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and

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Ozark Underground Laboratory, Inc.

A contract study prepared for the City of Ozark
and partially funded by the Missouri Department of Conservation



EXECUTIVE SUMMARY

Smallin Civil War Cave (Smallin Cave) is a privately owned show-cave that offers cave tours and local history programs. The cave is also home to one of the largest populations of Bristly Cave Crayfish, *Cambarus setosus*, in Missouri. The area around Smallin Cave is under increasing pressure for residential and commercial development around the city of Ozark. Recent storm flows in the cave stream have carried high sediment loads and unnatural sediment deposits are observed in the cave after precipitation events. The purpose of this study was to delineate the recharge area for Smallin Cave to assist city and county partners in their efforts to limit the detrimental impacts of land use on the cave and the Finley River.

The Ozark Underground Laboratory (OUL) conducted nine groundwater traces using fluorescent tracer dyes during this study to delineate the recharge area for Smallin Cave. Seventeen sampling stations were established in and around Smallin Cave, Fielden Cave, several springs, and surface streams of interest to measure background fluorescence and detect dye from traces conducted by OUL staff. A total of 189 activated carbon samplers and 52 water samples were analyzed for the presence of the tracer dyes. One or more of the tracer dyes was detected at 9 of the 17 sampling stations monitored.

The OUL made flow rate measurements on two dates at Smallin Cave and at the spring on Parched Corn Hollow. In addition, Onset HOBO MX 2001 water level loggers were installed at these two locations to monitor temperature and water level fluctuations. Temperature and spring discharges responded rapidly to precipitation events, indicating close connections between the surface and the groundwater system.

Using information from the dye traces conducted in this study, a recharge area of approximately 1,344 acres (2.1 square miles) was delineated for Smallin Cave. Based on the traces conducted during this study, most of the recharge to Smallin Cave is derived from the sinkhole plain located to the north and west of the cave. Dye tracing also demonstrated the existence of a shared recharge area between Smallin Cave and the spring on Parched Corn Hollow, indicating these two systems are part of a larger groundwater drainage network contributing water to the Finley River in the Ozark area.

The shortest travel time of a tracer dye detected in Smallin Cave occurred during Trace 23-08, when rhodamine WT dye introduced into a sinkhole on Indian Valley Road was visually observed 5,490 feet away at Smallin Cave approximately 58 hours later. This produced an estimated groundwater flow rate of 2,288 feet per day. Short travel times between dye introduction sites and sampling stations are indicative of discrete recharge zones where well-developed connections exist between the surface and subsurface waters.

Groundwater quality hazards were mapped within the identified recharge area. The most significant potential groundwater hazards identified within the recharge area include insufficient erosion control measures at construction sites, poorly sited stormwater control structures, and agricultural inputs directly into sinkholes were observed in this study. Considering the open

nature of the recharge area and the rapid rate at which surface water reaches the groundwater system, the unnatural sediment issues in Smallin Cave are the result of land use practices on the surface. This can be detrimental to the cave system by destroying aquatic habitat for cave species, impairing groundwater quality, and ruining the aesthetic character that makes Smallin Cave a popular tourist attraction. The famous rimstone dams in Smallin Cave slow the water before it flows over their tops, causing the sediment carried in the water to deposit upstream of the natural dams. Flow velocities necessary to flush the sediment out of the pools are higher than the velocities that allowed for the deposition of the sediment, making it difficult for natural processes to move the sediment out of the pools once it's been deposited. The sediment deposition destroys crayfish habitat by filling the interstitial spaces in stream bed used by the Bristly Cave Crayfish and the aquatic organisms they eat.

Vulnerability classes were assigned according to land use and proximity to sinkholes and cave passages. Within the recharge area approximately 598 acres were classified as moderate vulnerability, 353 acres as high vulnerability, and 393 acres as extremely high vulnerability.

Land use issues not only affect Smallin Cave, but also adversely impact groundwater quality throughout the area and water quality in the Finley River. This study demonstrated multiple connections between major springs along the Finley River. Thus, the water quality issues that are affecting Smallin Cave are also affecting other caves and springs in the area. These systems ultimately drain to the Finley River. The OUL estimates that greater than 75% of the water entering the Finley River in the study area has passed through the groundwater system.

The Finley River is a defining feature of the city of Ozark. Efforts to protect water quality in the Finley River must focus on the protection of water quality in the caves and springs contributing water to the river, as these aquatic systems are inextricably connected. These issues should be addressed through the establishment of appropriate best management practices and ordinances to protect vulnerable groundwater quality.

Acknowledgements

This study was made possible through funding provided by the Missouri Department of Conservation and the City of Ozark. City officials were helpful in obtaining access permission from landowners, providing insights into areas of interest, and administering project oversight. Special thanks to John McCart, Assistant Director of Public Works and Environmental Resources. Several landowners were instrumental in this study by providing access to private lands for the establishment of sampling stations, dye introduction points, and spring monitoring locations. This project could not have been accomplished without the cooperation of Kevin and Wanetta Bright, owners of Smallin Civil War Cave. The Brights provided an abundance of knowledge of the Smallin Cave system and assisted with landowner access and logistical support during field days. In addition, the Brights communicated often with OUL staff to report on dye observations and stream flow conditions during the study period. Their assistance in this study is greatly appreciated.



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1.0 INTRODUCTION

The investigation described in this report was conducted in the first half of 2023 by the Ozark Underground Laboratory, Inc. (OUL) under contract with the City of Ozark (City). The investigation involved groundwater tracing with the use of fluorescent tracer dyes to delineate the recharge area of Smallin Civil War Cave (Figure 1).

Figure 1. Smallin Cave viewed from the boardwalk inside the cave.



1.1 Purpose of Study

Smallin Civil War Cave (Smallin Cave) is a privately owned show-cave that offers cave tours and local history programs. The cave is also home to one of the largest populations of Bristly Cave Crayfish (*Cambarus setosus*) in Missouri (Dr. Dave Ashley, personal communication). The Bristly Cave Crayfish is a Missouri Species of Conservation Concern due to its limited range and habitat restrictions that make it vulnerable to extinction. Threats to the species identified by the Missouri Department of Conservation include sediment

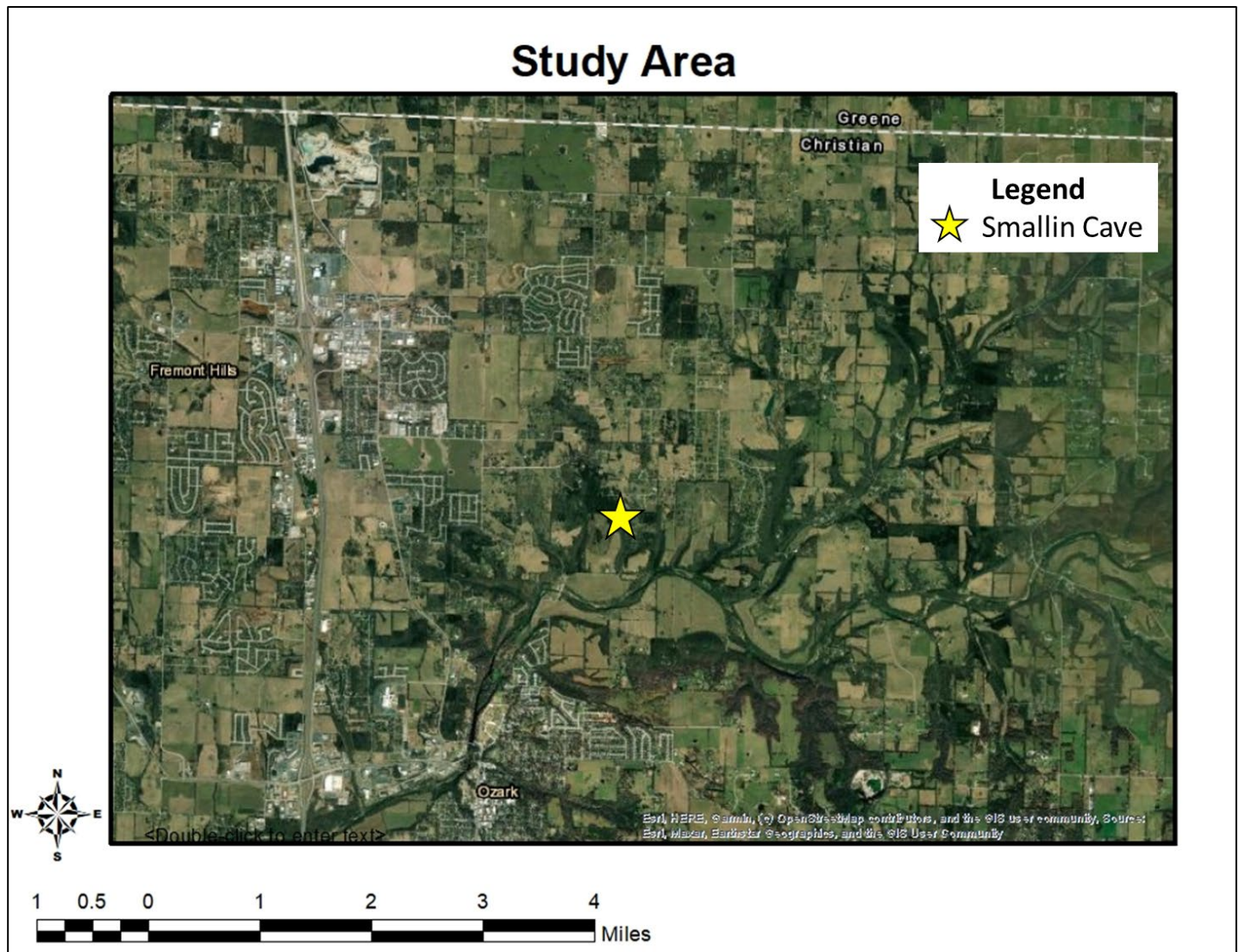
from surface excavation that can enter the groundwater system and destroy sensitive habitat necessary for the crayfish's survival. The area immediately north of Smallin Cave is under increasing pressure for residential and commercial development in northern Christian County, particularly around the city of Ozark. Recent storm flows in the cave stream have carried high sediment loads and unnatural amounts of sediment have deposited on the stream bed in the cave after precipitation events. The purpose of this study was to delineate the recharge area for Smallin Cave to assist city and county partners in efforts to limit adverse impacts of land development on the cave.

1.2 Study Area

Smallin Cave is located along the Finley River approximately 2 miles east of the city of Ozark in northern Christian County, Missouri. The elevation of the entrance of Smallin Cave is approximately 1,210 feet, and the cave runs north-northwest for approximately 5,600 feet (National Register). An intermittent stream flows from the mouth of the cave south to the Finley River. The cave stream exits the cave and under certain flow conditions discharges into the Finley River approximately 3,500 feet downstream of the cave mouth. The major surface water drainage in the study area is the Finley River, which flows southwest to the James River. The study area included Smallin Cave as well as other springs, caves, and surface tributaries along approximately 1.5 miles of the Finley River (Figure 2).

The study area is located in the Springfield Plateau physiographic region of the Ozark Plateau. The area is characterized by a thick sequence of Mississippian age strata, predominantly limestone with varying amounts of chert. Geologic mapping of the study area shows that Smallin Cave is located in the Mississippian Keokuk and Burlington limestone and chert. The Burlington and overlying Keokuk are about 100 feet thick in southwestern Missouri. The two formations are lithologically similar and are typically referred to as the Burlington-Keokuk unit. The Burlington-Keokuk is characterized by white to light buff, very coarsely crystalline, fossiliferous, crinoidal limestone with layers of chert nodules (Howe and Koenig 1961). Based on the 1970 geologic map of the Ozark Quadrangle, the contact between the Burlington-Keokuk limestone and the underlying Elsey (or Grand Falls) and Reeds Spring Formations is about 10 feet downgradient from the cave entrance. The contact is difficult to identify in the field due to the lithologic similarity between the formations. In Arkansas and Oklahoma, the Burlington-Keokuk combined with the Reeds Spring and Grand Falls formations are collectively designated as the "Boone Formation."

Figure 2. Location of the study area.



1.3 Soils

Based on the Soil Survey of Christian County, Missouri (Dodd 1985), two soil associations are present within the Smallin Cave recharge area:

- Goss-Clarksville Association.** Deep, well drained and somewhat excessively drained, gently sloping to steep soils formed in cherty residuum from limestone. This soil association in Christian County is comprised of 78% Goss soils and 12% Clarksville soils. The remaining 10% is comprised of soils of minor extent (Cedargap, Secesh, Wilderness, and shallow Gasconade). Slope, overgrazing and the hazard of erosion are the main management concerns. Pollution of groundwater by waste disposal facilities is a possibility, especially in areas that have sinkholes, such as the sinkhole plain area to the

northwest of Smallin Cave. This soil association dominates in the central and southern part of the recharge area.

- **Tonti-Wilderness Association.** Deep, moderately well drained, gently sloping and moderately sloping soils formed in loess or other silty material and cherty residuum from limestone. This soil association in Christian County is comprised of 49% Tonti soils and 41% Wilderness soils. The remaining 10% is comprised of soils of minor extent (noncherty Captina, Needleeye, Goss and Cedargap). Overgrazing and the hazard of erosion are the main management concerns. The subsoil above the fragipan ranges from 11–15 inches in Tonti and Wilderness soils. Permeability is moderate in the subsoil below the fragipan in these soils. The major soils generally are unsuitable for septic tank absorption fields. Seasonal wetness and slow permeability in the fragipan are the main concerns. This soil association dominates in the northern portion of the recharge area.

Soil series and soil complexes within these soil associations have distinctly different properties related to their ability to remove contaminants and protect underlying karst groundwater systems. Data on some important hydrogeological characteristics of the major soil series and complexes found in the Smallin recharge area are summarized in Table 1.

Table 1. Major Soil Series and Complexes and Hydrologically Important Characteristics. Data from Dodd (1985).

Soil Series/Complex	Depth to Bedrock (in.)	Depth Below Surface (in.)	Percent less than 3 inches passing #4 sieve	Permeability (in/hr)
Goss	> 60	0-6	65-90	2.0-6.0
		6-26	40-60	2.0-6.0
		26-62	45-70	0.6-2.0
Tonti	> 60	0-12	75-95	0.6-2.0
		12-22	70-90	0.6-2.0
		22-40	50-75	0.06-0.2
		40-62	45-70	0.6-2.0
Captina	> 60	0-6	95-100	0.6-2.0
		6-27	95-100	0.6-2.0
		27-46	80-95	0.06-0.2
		46-63	60-95	0.6-2.0
Needleye	> 60	0-9	95-100	0.6-2.0
		9-28	85-100	0.2-0.6
		28-60	60-90	0.06-0.2
		60-70	50-75	0.6-2.0
Gasconade	4-20	0-3	75-90	0.6-2.0
		3-17	45-55	0.2-0.6
		17	unweathered bedrock	-
Peridge	80-100 or more	0-5	95-100	0.6-2.0
		5-39	95-100	0.6-2.0
		39-64	55-100	0.6-2.0
Wilderness	> 60	0-5	60-85	2.0-6.0
		5-15	40-70	0.6-2.0
		16-31	30-60	0.06-0.2
		31-60	30-70	0.6-2.0

Water stored in soil horizons above the horizon with the lowest permeability is available for use by vegetation during the growing season. Excess amounts will slowly move through the slowly permeable unit and recharge karst groundwater supplies. As a general rule, the lower the permeability rate in the least permeable horizon of the soil the greater the amount of overland runoff to surface streams and sinkholes. Much of the infiltration of water through the soils and underlying residuum and limestone in the Ozarks follows very localized preferential flow routes called discrete recharge zones. Flow through these zones is much more rapid than indicated by the general permeability rates reported in soil surveys such as the one for Christian County (Dodd 1985).

Almost all of the surface streams in the study area are losing streams that provide substantial amounts of groundwater recharge through discrete recharge zones. These losing streams routinely have flow for only short periods of time and only after significant precipitation events. These observations indicate that the

channels of losing streams, or at least segments of the channels, are underlain by highly permeable alluvium and cavernous limestone. The same is true for sinkholes in the Smallin Cave study area. This highly permeable alluvium and cavernous limestone permits the rapid entry of water from the surface losing stream or sinkhole into the karst groundwater system. Rapid flow occurs through solutional openings in the limestone that are integrated into a flow system that conveys the water to a downgradient spring or springs.

2.0 METHODS

2.1 Dye Tracing Study

Groundwater tracing using fluorescent dyes is the most appropriate method for delineating the recharge areas of caves and springs (Aley and Aley, 1991). Groundwater tracing requires both water and dye. The owners of Smallin Cave and City partners were helpful in identifying springs and surface stream segments that may contribute water to Smallin Cave as potential dye introduction locations.

OUL staff conducted field reconnaissance work in the study area on December 21, 2022, to establish sampling stations, place activated carbon samplers, collect grab samples of water for background fluorescence analysis, and take temperature and conductivity measurements. Activated carbon samplers are continuous and accumulating samplers that adsorb and retain tracer dyes (if the dyes are present) during the entire period that they are in place in flowing water. Samplers are periodically collected, and new samplers placed at intervals appropriate to the study. After dye introduction, one collected sampler from each sampling location was analyzed. Background water samples were collected to demonstrate the absence of tracer dyes or compounds with similar fluorescence characteristics prior to any introduction of the tracer dyes. Activated carbon samplers were also collected once to determine background fluorescence.

OUL staff collected carbon samplers and grab samples of water throughout the study period to be analyzed for the presence of one or more of the tracer dyes. The analysis was conducted at the OUL's tracer dye analytical laboratory in Protom, Missouri. The analysis methods are outlined in detail in the OUL's Procedures and Criteria Document (see Appendix B).

2.2 Tracer Dyes Used

OUL conducted nine separate traces in this study using fluorescent tracer dyes. Information on each dye introduction can be found in Table 2. Four different dyes were used separately during the groundwater tracing investigations. These dyes were fluorescein, eosine, rhodamine WT (RWT), and sulforhodamine B (SRB). All of these dyes are environmentally safe and pose no risk to aquatic life, humans, or animals in the concentrations used in professionally directed groundwater tracing work (Smart and Laidlaw, 1977; Smart, 1984; Field et al., 1995). Table 3 illustrates the chemical structures of these dyes and summarizes some of their more important properties. These dyes are among the most detectable of the commonly used fluorescent tracer dyes. They can be adsorbed onto activated carbon samplers for cumulative sampling and can also be detected in water samples.

Dye introductions and dye analysis work was performed under the direction of Tom Aley, a Registered Geologist licensed in the State of Missouri. Each of these dye introductions was made specifically for this

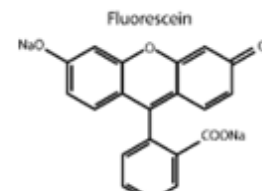
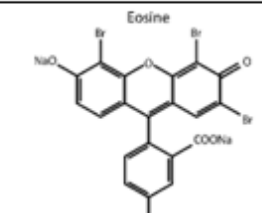
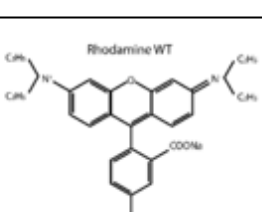
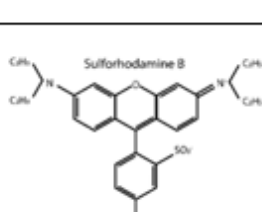
investigation. The traces are numbered sequentially with the first two digits indicating the year and the last pair of digits indicating the serial number of the trace.

Table 2. Dye introductions performed by the OUL.

Trace #	Dye Introduction Location	Dye and Quantity	Date	Easting	Northing	Approximate Elevation
23-01	Northtown Park detention basin	RWT; 3 lbs.	1/18/2023	-93.1886	37.0665	1,310
23-02	Upper Parched Corn basin	Fluorescein; 2 lbs.	1/18/2023	-93.1684	37.0818	1,340
23-03	Sunset Road sinkhole	SRB; 3 lbs.	2/7/2023	-93.2006	37.0587	1,300
23-04	West Liberty Road	Eosine; 4 lbs.	2/7/2023	-93.2145	37.0617	1,290
23-05	McGuffy Park sinkhole	RWT; 3 lbs.	3/23/2023	-93.2122	37.0511	1,290
23-06	The Rivers Subdivision	Fluorescein; 2 lbs.	3/23/2023	-93.1922	37.0762	1,350
23-07	Wacha Farms sinkhole	Eosine; 3 lbs.	5/4/2023	-93.2038	37.0689	1,320
23-08	Indian Valley sinkhole	RWT; 4 lbs.	5/5/2023	-93.2055	37.0569	1,320
23-09	Hemlock Road	Fluorescein; 100 g	5/11/2023	-93.1869	37.0546	1,220

* Dye equivalent in mixtures presented in Results section.

Table 3. Properties of tracer dyes used.

Fluorescein Dye - Also known as Acid Yellow 73 - Color Index Constitution Number 45350 - Brilliant fluorescent yellow-green dye - Most commonly used fluorescent tracer dye - Most easily detectable dye	 Chemical structure of Fluorescein, showing a xanthene core with a sulfonate group (COONa) and a sodium salt group (NaO).
Eosine Dye - Also known as Acid Red 87 - Color Index Constitution Number 45380 - Greenish to peach-colored dye (dependent on concentration)	 Chemical structure of Eosine, showing a xanthene core with two bromine atoms (Br) and a sodium salt group (COONa).
Rhodamine WT Dye - Also known as Acid Red 388 - Color Index Constitution Number not assigned - Reddish orange-colored dye - Less resistant to adsorption onto aquifer materials	 Chemical structure of Rhodamine WT, showing a xanthene core with two diethylamino groups (N(C2H5)2) and a sodium salt group (COONa).
Sulforhodamine B Dye - Also known as Acid Red 52 - Color Index Constitution Number 45100 - Reddish purple-colored dye - Moderately resistant to adsorption onto aquifer materials	 Chemical structure of Sulforhodamine B, showing a xanthene core with two diethylamino groups (N(C2H5)2) and a sulfonate group (SO3Na).

2.3 Types of Samples

Two sampling methods were employed during the project: activated carbon samplers and grab water samples. Primary reliance was placed upon the activated carbon samplers. The activated carbon samplers consisted of fiberglass screen wire packets filled with 4.25 grams of laboratory-grade activated coconut shell carbon. These samplers adsorb, retain, and concentrate the tracer dyes. When eluted in the laboratory, samples routinely yield dye concentrations one to two orders of magnitude greater than the mean dye concentrations in the water. Activated carbon samplers are continuous and accumulating samplers.

Grab samples of water provide dye concentrations at a particular point in time. Water samples were routinely collected at all sampling stations where practical and are archived. The water samples were analyzed if dye was detected in the associated activated carbon sampler, if an activated carbon sampler was lost or was not collected, if fluorescence peaks in the associated carbon sampler suggested that the water sample should be analyzed, or if the data would be useful for the study. Water samples were collected in 50-milliliter plastic sample vials.

All sample collection procedures followed the protocols found in OUL's Procedures and Criteria document found in Appendix B (Aley and Beeman, 2015).

2.4 Sampling Locations

Seventeen sampling stations were established for this project. Sampling locations consisted of points in Smallin Cave, Fielden Cave, several springs, and surface streams of interest (Figure 3). Table 4 provides summary information on all sampling station locations.

2.5 Sampling Events

Background sampling was performed at all sampling stations to detect and quantify the presence of tracer dyes or any other fluorescence with characteristics similar to tracer dyes. Background sampling was performed with activated carbon and water samples and was conducted from December 14th, 2022, through January 4th, 2023, prior to the first dye introduction on January 18th, 2023.

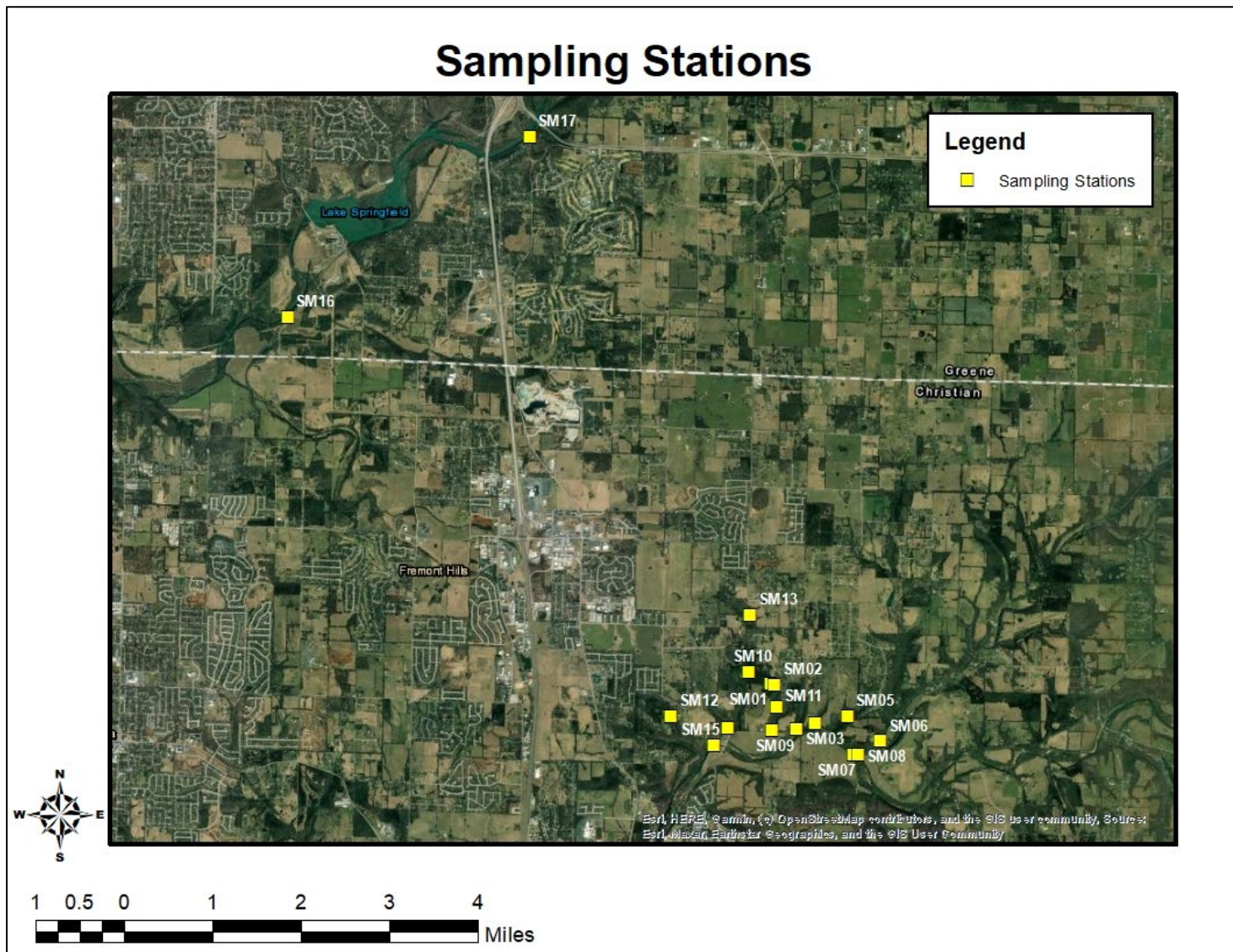
Sampling for tracer dyes was conducted following dye introductions. Sampling was performed by OUL staff. Sample collection intervals were planned to occur weekly following dye introductions when sampling locations were accessible. Sampling frequencies occasionally varied when flooding occurred.

Table 4. Dye sampling stations and their coordinates.

Station	Station Location	Easting	Northing	Approximate Elevation
SM01	Smallin Cave	-93.1881	37.0110	1,210
SM02	Fielden Cave	-93.1874	37.0509	1,200
SM03	1st Drainage East of Smallin Cave	-93.1838	37.0452	1,118
SM04	2nd Drainage East of Smallin Cave	-93.1809	37.0459	1,134
SM05	3rd Drainage East of Smallin Cave	-93.1755	37.0468	1,150
SM06	Spring on Parched Corn Hollow	-93.1702	37.0436	1,139
SM07	Finley River Upstream of Parched Corn Hollow	-93.1745	37.0418	1,116
SM08	Parched Corn Hollow	-93.1737	37.0418	1,118
SM09	Smallin Branch	-93.1879	37.0449	1,120
SM10	McClerran Spring	-93.1917	37.0525	1,211
SM11	Seep Downstream of Smallin Cave	-93.1871	37.0480	1,145
SM12	Cave Spring	-93.2044	37.0468	1,216
SM13	Jeff Cave	-93.1914	37.0600	1,251
SM14	2nd Drainage West of Smallin Cave	-93.1952	37.0454	1,123
SM15	Finley River Downstream of Riverside Park	-93.1973	37.0431	1,107
SM16	Camp Cora Spring	-93.2670	37.0988	1,127
SM17	Winoka Spring	-93.2275	37.1223	1,145

Notes: UTM X and UTM Y coordinates are given in NAD1983.

Figure 3. Sample station locations.



2.6 Dye Sampler Placement and Collection Procedures

Carbon samplers at Smallin Cave, springs, and surface streams were placed in flowing water or at locations anticipated to have flowing water following precipitation events. Packets were firmly anchored with plastic tie wire and weighted in place. A minimum of two samplers were placed at each sampling station. Placement of multiple samplers allows for the analysis of duplicate samples if needed and provides a spare in case a sampler is lost or damaged. When carbon samplers were collected, new samplers were placed. Collected samplers were placed in sterile whirl packs. The bags were labeled on the outside with the station name, date, and time of collection. Grab water samples were also collected at the same time as the activated carbon samplers and were also labelled with station name, date, and time of collection.

Samples collected in the field were maintained under refrigeration until delivery to the laboratory. Upon arrival at OUL, samplers were refrigerated at 4°C until analysis. All sampler placement, collection, and analysis work was conducted by OUL personnel and conformed to the protocol in Appendix B (Aley and Beeman, 2015).

2.7 Laboratory Analysis

Laboratory analysis was performed at the OUL in Protom, Missouri. Activated carbon samples were rinsed under a relatively strong jet of water and eluted in a standard eluting solution. Water samples were pH adjusted to raise the pH of the water to 9.5 or higher. Elutant and pH-adjusted water samples were analyzed on a Shimadzu RF-5301 spectrofluorophotometer under a synchronous scanning protocol. All dye concentrations were based on the as-sold mixtures of the dyes.

Little or no detectable fluorescence background in the general range of the tracer dyes is generally encountered in most groundwater tracing studies. However, in some locations downstream of wastewater treatment facility discharges or in industrial areas, these dyes have been found during background sampling. Background sampling was also necessary to determine if residual dye from earlier traces could be detected in the groundwater. Background sampling prior to the introduction of any tracer dyes is routinely performed to characterize this background fluorescence and to identify the existence of any tracer dyes that may be present in the area.

The OUL has established normal emission fluorescence wavelength ranges for each of the four dyes used in this project (eosine, fluorescein, RWT, and SRB). The normal acceptable range equals mean values plus and minus two standard deviations. These values are derived from actual groundwater tracing studies conducted by the OUL.

The detection limits are based upon concentrations of dye necessary to produce emission fluorescence peaks where the signal to noise ratio is 3. The detection limits are realistic for most field studies since they are based upon results from actual field samples rather than being based upon values from spiked samples in a matrix of reagent water or the elutants from unused activated carbon samplers. In some cases, detection limits may be smaller than reported if the water being sampled has very little fluorescent material in it. In some cases, detection limits may be greater than reported; this most commonly occurs if the sample is turbid due to suspended material or a coloring agent such as tannic compounds. Turbid samples are typically allowed to settle, centrifuged, or, if these steps are not effective, diluted prior to analysis.

Table 5 provides normal emission wavelength ranges and detection limits for the four dyes used in this study when analyzed on the OUL's RF-5301 spectrofluorophotometer. Detailed procedures and criteria used during the tracer study are found in the OUL's Procedures and Criteria Document found in Appendix B (Aley and Beeman, 2015).

Table 5. Normal Emission Wavelength Ranges and Detection Limits for the Four Dyes Used in this Study in Water and Activated Carbon Elutant Samples.

Dye and Matrix	Normal Acceptable Emission Wavelength Range nanometers (nm)	Detection Limit (ppb)
Fluorescein in Elutant	514.5 to 519.6	0.025
Fluorescein in Water	506.8 to 510.6	0.002
Rhodamine WT in Elutant	565.2 to 571.8	0.170
Rhodamine WT in Water	572.4 to 577.7	0.015
Eosine in Elutant	539.3 to 545.1	0.015
Eosine in Water	532.5 to 537.0	0.050
Sulforhodamine B in Elutant	575.2 to 582.0	0.008
Sulforhodamine B in Water	580.1 to 583.7	0.008

Note: Detection limits are based upon the as-sold weight of the dye mixtures used by the OUL.

The following four criteria are used by the OUL as normal criteria for determining positive dye recoveries in elutants from activated carbon samplers:

Elutant Criterion 1. At least one fluorescence peak must be present at the station in the normal range for the dye for samples analyzed by the RF-5301 (see Table 3).

Elutant Criterion 2. The dye concentration associated with the fluorescence peak must be at least 3 times the detection limit.

Elutant Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Elutant Criterion 4. The shape of the fluorescence peak must be typical of the dye for which the analysis is run. Much background fluorescence yields low, broad, and asymmetrical fluorescence peaks rather than the narrower and symmetrical fluorescence peaks typical of fluorescent tracer dyes. In addition, there must be no other factors that suggest that the fluorescence peak may not be the dye from the groundwater tracing work.

The following four criteria are used by the OUL as normal criteria for determining positive dye recoveries in water samples.

Water Criterion 1. The associated charcoal samplers for the station should contain a positive dye detection in accordance with the elutant criteria listed above. This criterion may be waived if no charcoal sampler exists.

Water Criterion 2. No factors must exist that suggest that the fluorescence peak may not be fluorescent dye from the groundwater tracing work in question. The fluorescence peak should generally be in the typical range listed in Table 3.

Water Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit.

Water Criterion 4. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station.

2.8 Vulnerability and Hazards Area Assessment

Previous investigations by the OUL have demonstrated that the vulnerability of a karst groundwater system and its associated biological community is a function of the hydrobiological characteristics of its particular groundwater system and land uses within the recharge area. The vulnerability map developed during this project qualitatively depicts risks posed to groundwater quality by various portions of its recharge area. Vulnerability mapping will help to inform city and county partners on management strategies to limit the potential impacts of development activities within the recharge area.

Lands within the recharge area are included in up to four qualitative natural resource vulnerability categories. Not all recharge areas contain lands in all four categories. The categories used in this study are consistent with criteria developed by Aley and Aley (1997) and have been used in many subsequent recharge area delineations and vulnerability assessments (e.g., Aley and Beeman 2015; Aley and Goers 2023). Vulnerability mapping for recharge area delineation studies has routinely qualitatively depicted risks posed to groundwater quality by various portions of the recharge areas.

- 1) **Low Vulnerability lands.** These are lands where the hydrobiological setting and existing and foreseeable land uses pose low risks of groundwater impacts likely to adversely affect aquatic groundwater fauna or its associated biological community. These are often upland areas remote from sinkholes or losing streams where land use does not include hazards such as urban or suburban development, concentrated or confined animal operations, or poultry operations.
- 2) **Moderate Vulnerability lands.** These are lands where the hydrobiological setting and existing and/or foreseeable land uses pose moderate risks of groundwater impacts likely to adversely affect aquatic groundwater fauna and the associated biological community. These are typically upland areas underlain by soils capable of removing many contaminants. Moderate Vulnerability lands are remote from sinkholes or losing streams and are areas where land use does not include localized groundwater contamination hazards such as suburban development utilizing on-site disposal of sewage or concentrated or confined animal operations (including poultry).
- 3) **High Vulnerability lands.** These are lands where the hydrobiological setting and existing and/or foreseeable land uses pose high risks of groundwater impacts likely to adversely affect aquatic groundwater fauna and the associated biological community.
- 4) **Extremely High Vulnerability lands.** These are lands where the hydrobiological setting and existing and/or foreseeable land uses pose extremely high risks of impacts likely to adversely affect aquatic groundwater fauna and the associated biological community.

Localized land use activities likely to create significant adverse groundwater quality impacts were located by field reconnaissance assisted by evaluation of satellite imagery. Potential threats to water quality in the recharge area consist of non-point and point sources, identified as hazards. Hazard areas are point sources for the potential entry of contaminated water into a cave or spring system of conservation concern. Potentially hazardous land use activities evaluated in this study include those in the following categories:

- A. agricultural and forestry;
- B. sewage disposal facilities or concentrated housing served by on-site sewage systems;
- C. landfills, dumps, and salvage yards;
- D. industrial sites;
- E. transportation routes, including pipelines;
- F. petroleum storage sites;
- G. other chemical storage sites;
- H. other types of sites or facilities.

The identified hazard features and designated vulnerability areas in the delineated recharge area are discussed in Section 4.0.

2.9 Sinkhole Analysis

To better evaluate discrete recharge in the study area, a sinkhole analysis was conducted using high-resolution digital elevation models (DEMs) and ArcGIS Pro software. Data obtained from the Missouri Spatial Data Information Service (MSDIS) includes Light Detection and Ranging (LiDAR) DEM data, known and probable sinkhole and sink area locations developed by the Missouri Department of Natural Resources, as well as losing stream segments identified by the Missouri Department of Natural Resources. The LiDAR data used in this analysis is from February 2017, resulting in DEMs with a 3-foot cell size.

Verification of all individual sinkholes within the recharge area is beyond the scope of this project. Ozark Underground Laboratory followed the methodology of Doctor and Young (2013) to identify closed depressions that may represent sinkholes. The first step in the process used the Fill tool in ArcGIS Pro to recondition the DEM to fill all sinks to their spill elevation. Then the original DEM was subtracted from the filled DEM, generating a fill-difference layer. All values greater than 10 cm were extracted to a new layer, reclassified to an integer-type layer, and the pixels were converted into polygons that represent the boundaries of the possible sinks.

3.0 RESULTS

The OUL was contracted to conduct a minimum of three dye traces in this study. Ultimately, nine dye introductions were made in this study over the course of five months. Seventeen sampling stations were established in and around Smallin Cave and nearby caves, springs, and surface streams to measure background fluorescence and detect dye from traces conducted by OUL staff. A total of 189 activated carbon samplers and 52 water samples were analyzed for the presence of the tracer dyes. One or more of the tracer dyes were detected at 9 of the 17 sampling stations monitored.

The following sections of this report provide details on each of the traces and information from sampling stations where the tracer dyes were subsequently detected. Traces are identified by two numbers separated by a hyphen, with the first number indicating the year the trace was conducted and the second number indicating the sequence the traces were conducted in that calendar year.

3.1 Trace 23-01. Northtown Park Detention Basin Rhodamine WT (RWT) Trace.

At 08:45 on January 18th, 2023, three pounds of rhodamine WT dye mixture containing 21% dye equivalent was introduced at the weir structure of the stormwater detention basin in the Northtown Park development. Figure 5 shows the location of the dye introduction. The purpose of this trace was to determine if there was a connection between the detention basin discharge and Smallin Cave. Recent rainfall caused discharge from the detention basin at the time of introduction. Flow at the time of introduction was approximately 15-20 gallons per minute (gpm) and flow entered the groundwater system within 10 feet downstream of the concrete apron of the basin's weir structure.

Table 6 shows maximum RWT dye mixture concentrations in activated carbon samplers from all sampling stations where dye from Trace 23-01 was detected. Maximum values are used as a way of broadly displaying dye observations among all sites; however, all dye concentrations measured during the entire study are included in the tables in Appendix A.

Figure 4 shows the locations of sampling stations where RWT dye from Trace 23-01 was detected. RWT dye was first detected two days post dye introduction in a grab sample of water and elutant from a carbon sampler collected on January 20th, 2023, at SM06 at a concentration of 1.82 ppb. (See Appendix A). Straight-line distance from the dye introduction site was 7,975 feet to the nearest dye detection sampling station at SM06, indicating groundwater flow rates of at least 3,988 feet per day.

Table 6. Maximum RWT dye mixture concentrations from trace 23-01 detected at sampling stations.

Station Number and Name	Elevation (ft)	Maximum concentration in carbon samplers (ppb)	Days Post Introduction	Distance from Dye Introduction Point (ft)
SM01. Smallin Cave	1210	82.5	30	5,490
SM06. Spring on Parched Corn Hollow	1139	618	16	7,975
SM08. Parched Corn Hollow	1118	14	16	10,140
SM09. Smallin Branch	1120	39.5	30	7,815
SM11. Seep Downstream Smallin Cave	1145	14	51	6,710

Figure 4. Sampling stations where RWT from Trace 23-01 was detected. The diagrammatic arrows indicate overall straight-line groundwater flow paths.

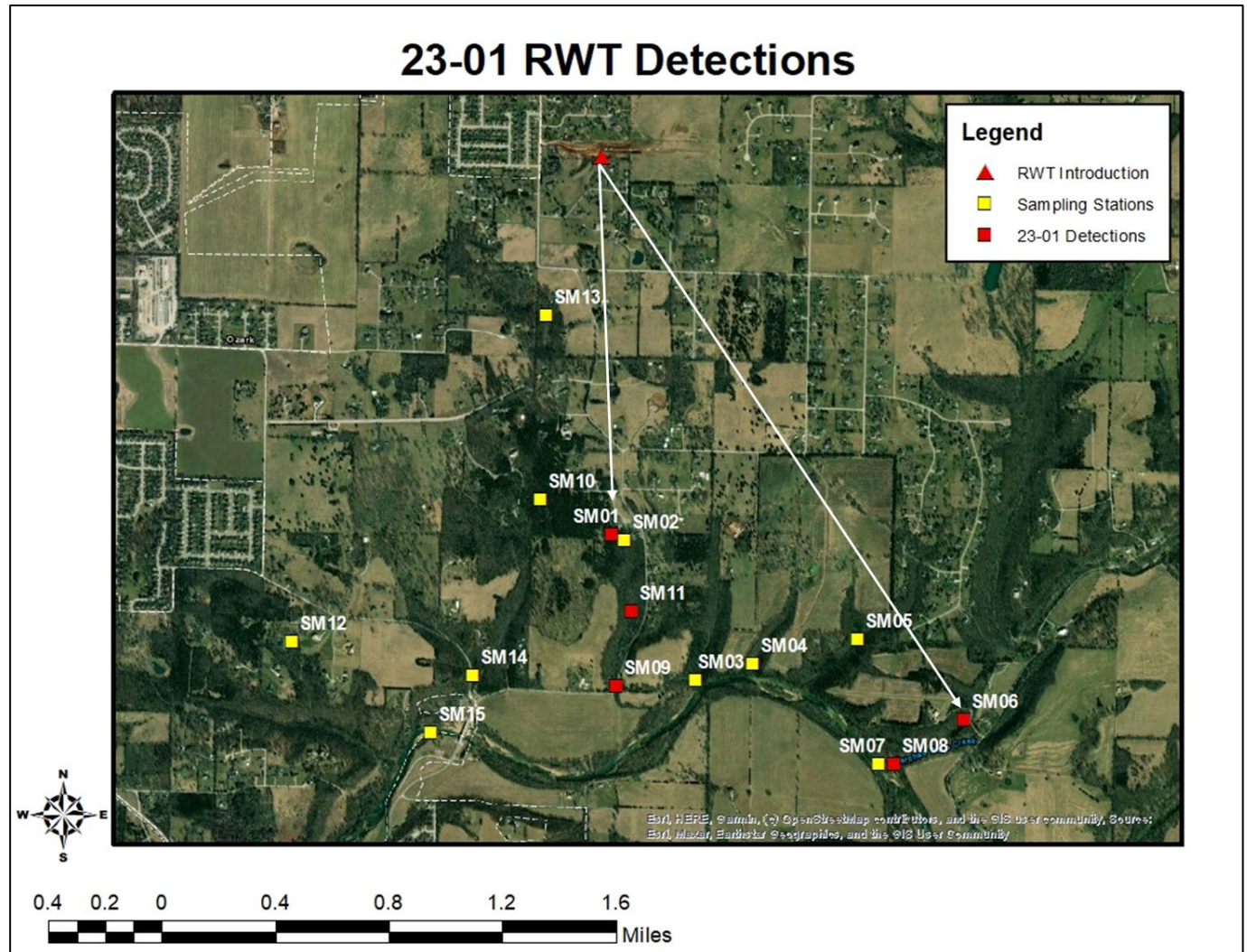


Figure 5. RWT dye introduced into the weir structure of a stormwater detention basin in the Northtown Park development.



3.2 Trace 23-02. Sinkhole in Upper Parched Corn Hollow Basin Fluorescein Trace.

At 10:40 on January 18th, 2023, two pounds of fluorescein dye mixture containing 70% dye equivalent was introduced into a sinkhole located in a residential yard in an unnamed tributary to Parched Corn Hollow. The purpose of this trace was to determine the eastern boundary of the recharge area contributing water to Smallin Cave. The sinkhole had been covered with a concrete slab; however, a small crack allowed for the introduction of dye. There was no surface flow at the time of introduction, but water could be heard flowing beneath the concrete slab covering the sinkhole. Approximately 125 gallons of water was introduced along with the dye using a garden hose from a nearby residence.

Table 7 shows maximum fluorescein dye mixture concentrations in activated carbon samplers from all sampling stations where dye from Trace 23-02 was detected. Fluorescein dye was first detected in a grab sample of water and elutant from a carbon sampler collected on February 3rd, 2023, at SM06 in the spring on Parched Corn Hollow (see Appendix A). Dye from this trace was detected in two grab samples of water collected at SM06 on February 3rd and February 10th, 2023, at concentrations of 0.011 ppb and 0.035 ppb, respectively.

Figure 6 shows the locations of sampling stations where fluorescein dye mixture from Trace 23-02 was detected. First detection of fluorescein dye occurred 16 days post dye introduction. Straight-line distance between the dye introduction location and SM06 was approximately 14,200 feet, indicating groundwater flow rates of at least 888 feet per day to SM06. The first samples were not collected for two weeks after the Trace

23-02 dye introduction, so it is likely groundwater flow rates were higher than calculated. Straight-line distance from the dye introduction site to the furthest dye detection sampling station at SM08 was 14,625 feet (2.77 miles).

Table 7. Maximum fluorescein dye mixture concentrations from trace 23-02 detected at sampling stations.

Station Number and Name	Elevation (ft)	Maximum concentration in carbon samplers (ppb)	Days Post Introduction	Distance from Dye Introduction Point (ft)
SM06. Spring on Parched Corn Hollow	1139	31.1	16	14,200
SM08. Parched Corn Hollow	1118	1.91	30	14,625

Figure 6. Sampling stations where fluorescein dye from Trace 23-02 was detected. The diagrammatic arrows indicate overall straight-line groundwater flow paths.

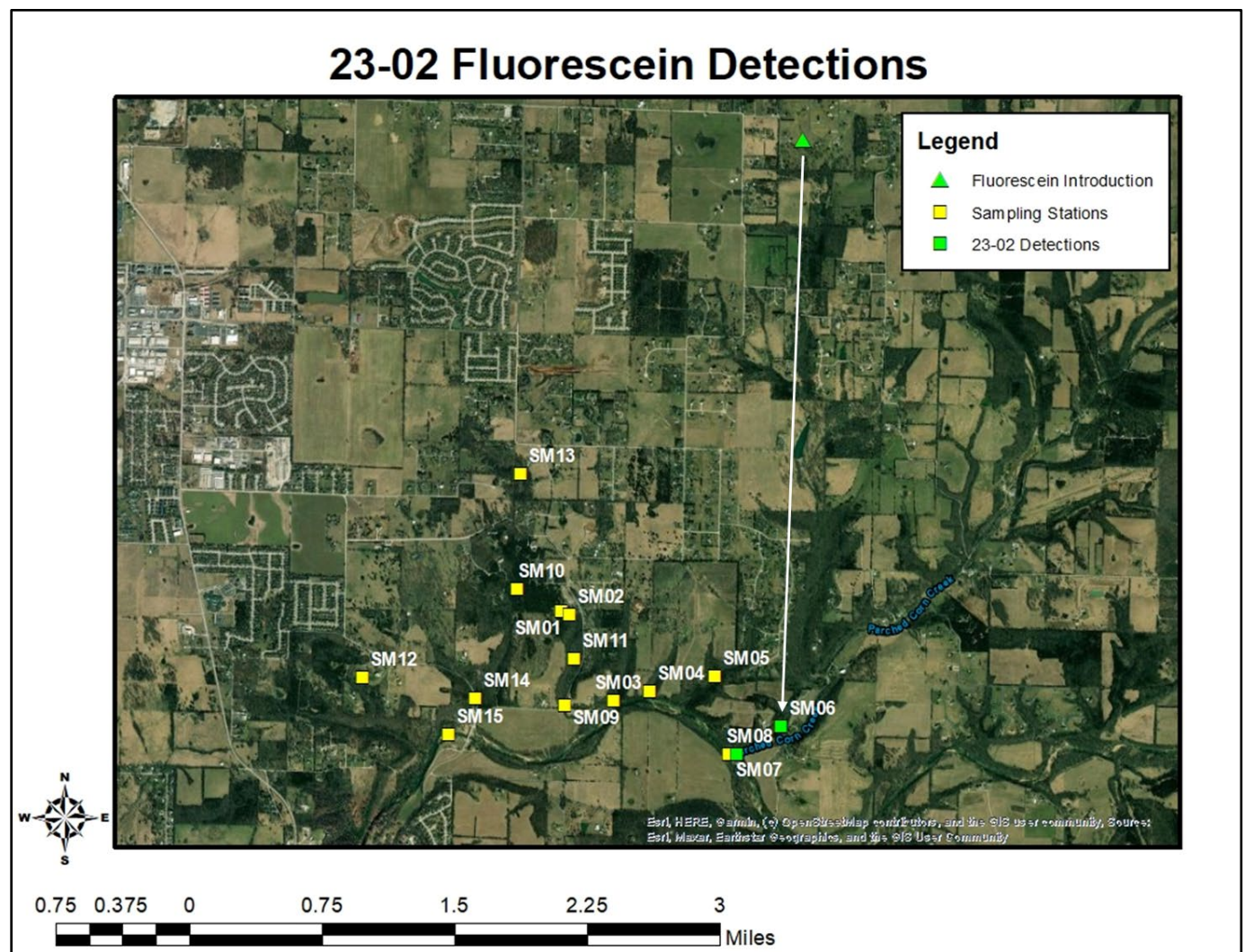


Figure 7. Flushing water behind Trace 23-02 with a garden hose. The small space between the tree and the slab covering the sinkhole allowed for the fluorescein introduction.



3.3 Trace 23-03. Sunset Road Sinkhole Sulforhodamine B Trace

At 08:35 on February 7th, 2023, 3 pounds of sulforhodamine B (SRB) dye mixture containing 35% dye equivalent was introduced as a dry set beneath a road culvert under Sunset Road. The purpose of this trace was to determine if sinkholes along Sunset Road contribute water to Smallin Cave. The culvert drained into a grass-bottomed sinkhole immediately south of the road. The culvert was dry at the time of introduction. The dry set was achieved by placing the SRB dye into a shallow disposable baking pan immediate beneath the culvert outlet so that storm flow would mobilize the dye.

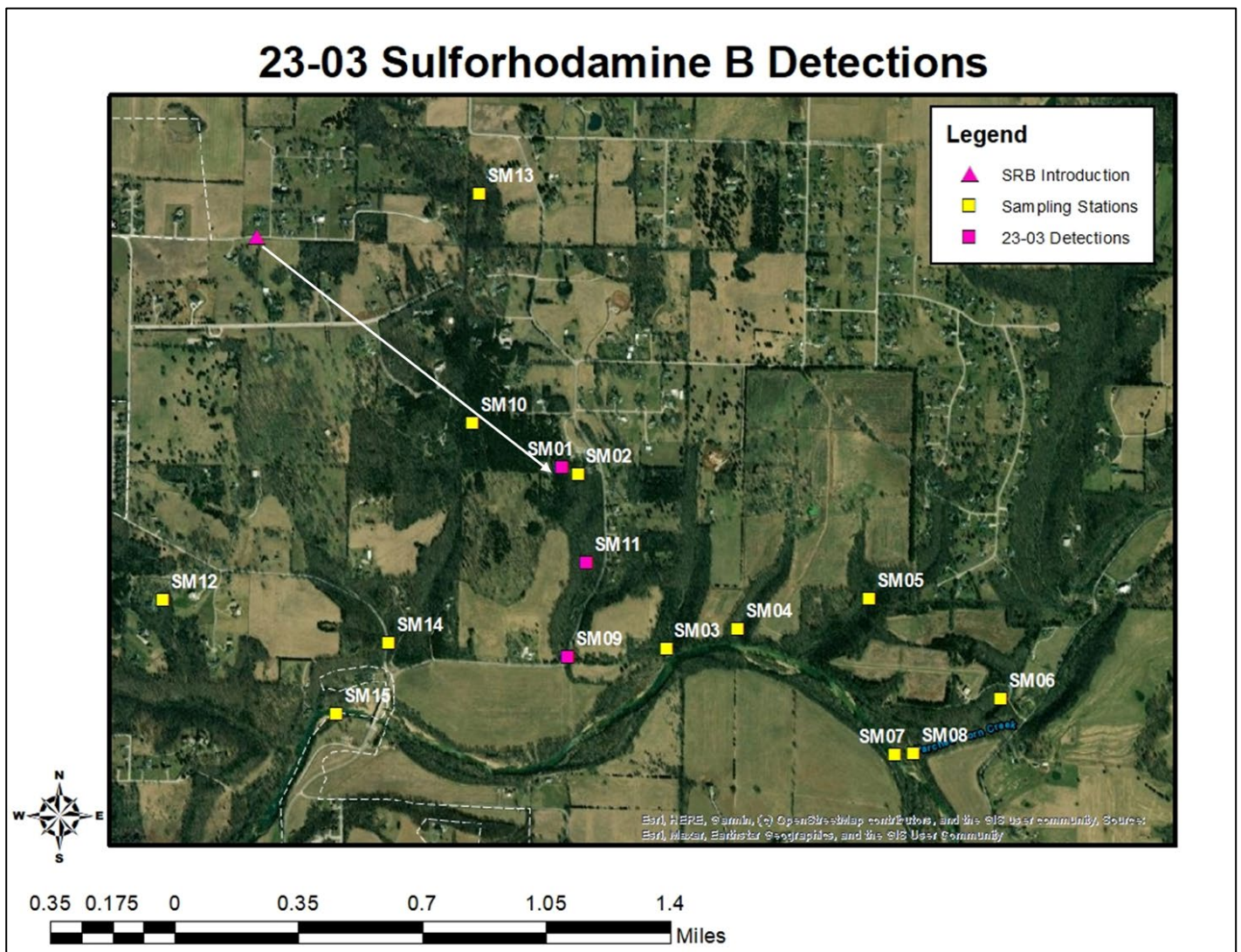
Table 8 shows the maximum SRB dye mixture concentrations in the activated carbon samplers where dye from Trace 23-03 was detected. Complete sampling results are found in the tables in Appendix A. No SRB was detected in grab samples of water during the study. SRB dye introduced in Trace 23-03 was first detected in elutant from a carbon sampler on February 24th, 2023, at Smallin Cave (SM01), in Smallin Branch (SM09), and in the seep downstream of Smallin Cave (SM11). First detection of SRB dye occurred seven days post introduction.

Figure 8 shows the location of sampling stations where SRB dye from Trace 23-03 was detected. The straight-line distance from the SRB introduction point on Sunset Road was 4,540 feet to the nearest detection station at SM01 and 6,125 to the furthest detection station at SM09. Based on the detection times and distances, the groundwater flow rate at the time of the trace was at least 649 feet per day.

Table 8. Maximum SRB dye mixture concentrations from Trace 23-03 detected at sampling stations.

Station Number and Name	Elevation (ft)	Maximum concentration in carbon samplers (ppb)	Days Post Introduction	Distance from Dye Introduction Point (ft)
SM01. Smallin Cave	1210	60.8	17	4,540
SM09. Smallin Branch	1120	21.4	17	6,125
SM11. Seep Downstream Smallin Cave	1145	14.6	17	5,545

Figure 8. Sampling stations where SRB dye from Trace 23-03 was detected. The diagrammatic arrow indicates an overall straight-line groundwater flow path.



3.4 Trace 23-04. West Liberty Road Eosine Trace

At 08:55 on February 7th, 2023, four pounds of eosine dye mixture containing 96% dye equivalent was introduced into a culvert on West Liberty Road that drains into an unnamed tributary to the James River. The purpose of this trace was to determine the northern boundary of the recharge area contributing water to Smallin Cave. Water flow in the culvert was approximately 3-5 gpm at the time of introduction.

Figure 9 shows where eosine dye was introduced for Trace 23-04. No eosine dye from Trace 23-04 was detected during this study. It is the OUL's conclusion that the dye introduction point does not contribute water to any of the points sampled.

Figure 9. Eosine dye introduction location for Trace 23-04.

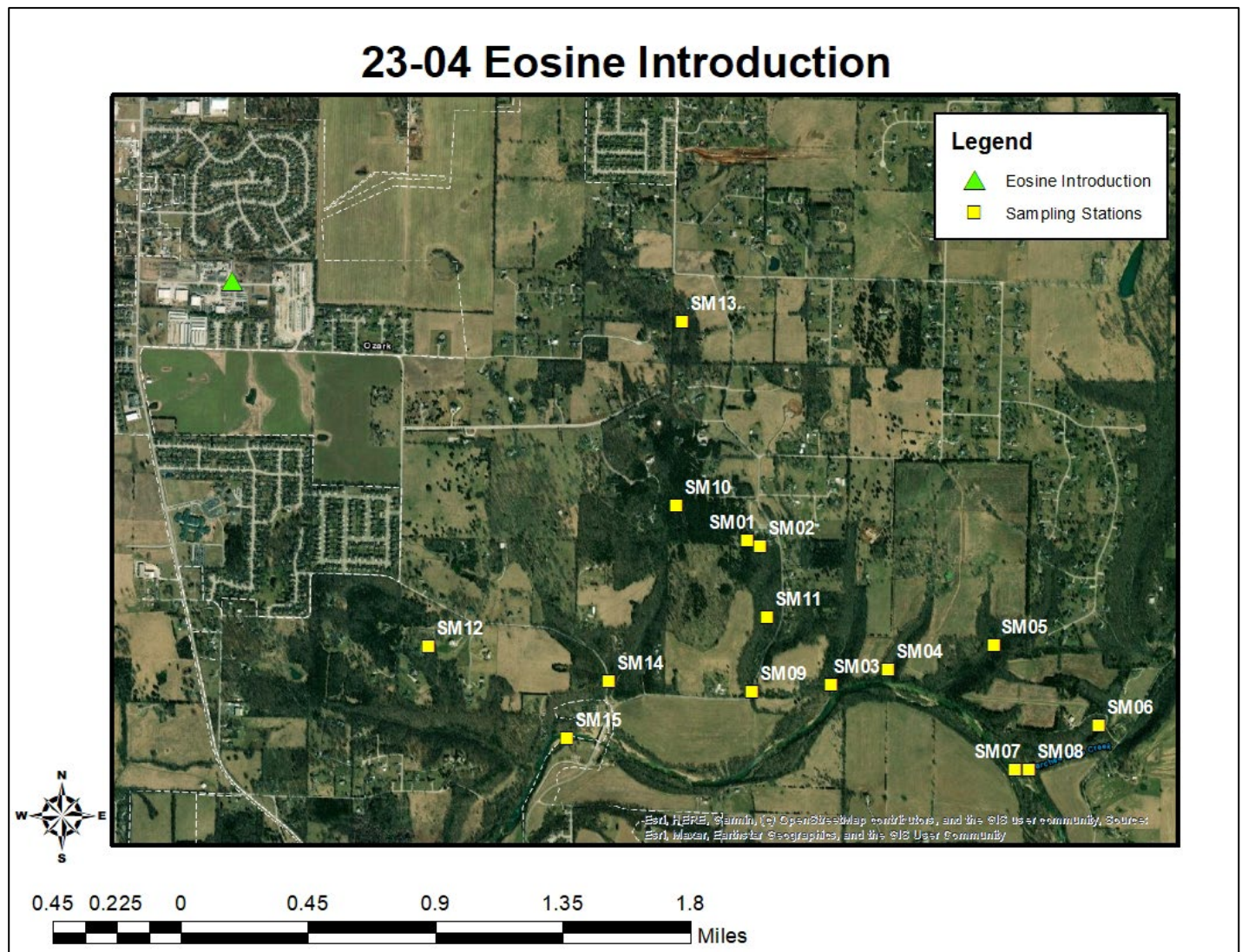


Figure 10. Eosine dye introduced into West Liberty Road culvert for Trace 23-04.



3.5 Trace 23-05. McGuffey Park Sinkhole Rhodamine WT Trace

At 12:50 on March 23, 2023, three pounds of RWT dye mixture containing 21% dye equivalent was introduced as a dry set into a sinkhole basin in McGuffey Park in anticipation of forecast precipitation that evening. The purpose of this trace was to determine the western boundary of the recharge area contributing water to Smallin Cave. The dry set was achieved by placing the RWT dye into a shallow disposable baking pan at the bottom of the sinkhole depression so that storm flow would mobilize the dye. There was no flow at the time of introduction.

Table 9 shows maximum RWT dye mixture concentrations in activated carbon samplers from all sampling stations where dye from Trace 23-05 was detected. RWT dye was first detected in a grab sample of water and elutant from a carbon sampler collected on March 30th, 2023, at SM12 in Cave Spring (see Appendix A). Dye from this trace was detected in two grab samples of water collected at SM12 on March 30th and April 6th, 2023, at concentrations of 0.097 ppb and 0.116 ppb, respectively. Dye from Trace 23-05 was not detected at any other sampling station.

Figure 11 shows the locations of sampling stations where RWT dye from Trace 23-05 was detected. First detection of RWT dye occurred 7 days post dye introduction. Straight-line distance between the dye introduction location and SM12 was approximately 2,795 feet, indicating groundwater flow rates of at least 399 feet per day to SM12.

Table 9. Maximum RWT dye mixture concentrations from Trace 23-05 detected at sampling stations.

Station Number and Name	Elevation (ft)	Maximum concentration in carbon samplers (ppb)	Days Post Introduction	Distance from Dye Introduction Point (ft)
SM12. Cave Spring	1216	1,800	7	2,795

Figure 11. Sampling stations where RWT from Trace 23-05 was detected. The diagrammatic arrow indicates an overall straight-line groundwater flow path.

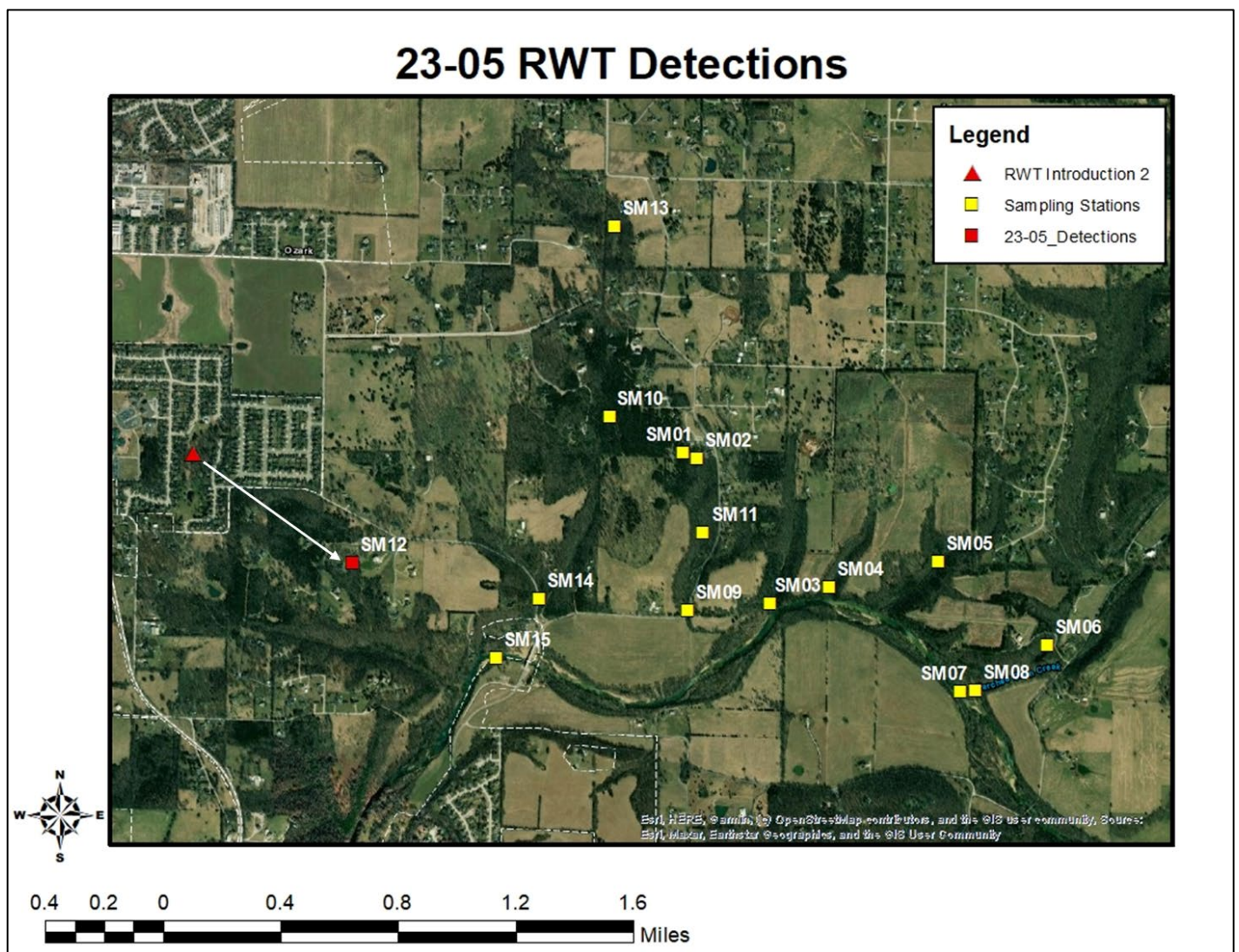


Figure 12. Dry set introduction of RWT into McGuffey Park sinkhole.



Figure 13. Mobilization of RWT dye by storm flows approximately 12 hours after introduction.



3.6 Trace 23-06. The Rivers Subdivision Fluorescein Trace.

At 13:14 on March 23rd, 2023, two pounds of fluorescein dye mixture containing 70% dye equivalent was introduced into a stormwater culvert under 13th Avenue in The Rivers subdivision. The purpose of this trace was to determine the northernmost boundary of the area contributing water to Smallin Cave. The fluorescein was introduced as a dry set in anticipation of precipitation forecasted that evening. The dry set was achieved by placing the powdered dye mixture into the stream substrate in the road culvert. Surface flow from this location runs through a stormwater conveyance in The Rivers and Waterford subdivisions and later flows into an unnamed tributary to the James River. There was no flow at the time of introduction. Visual observation at 19:30 on March 23rd, 2023, verified that the dye was mobilized by stormflow six hours after introduction.

Figure 14 shows where fluorescein dye was introduced for Trace 23-06 in relation to sampling stations. No fluorescein dye from Trace 23-06 was detected during this study. It is the OUL's conclusion that the dye introduction point does not contribute water to any of the points sampled.

Figure 14. Fluorescein dye introduction location for Trace 23-06.

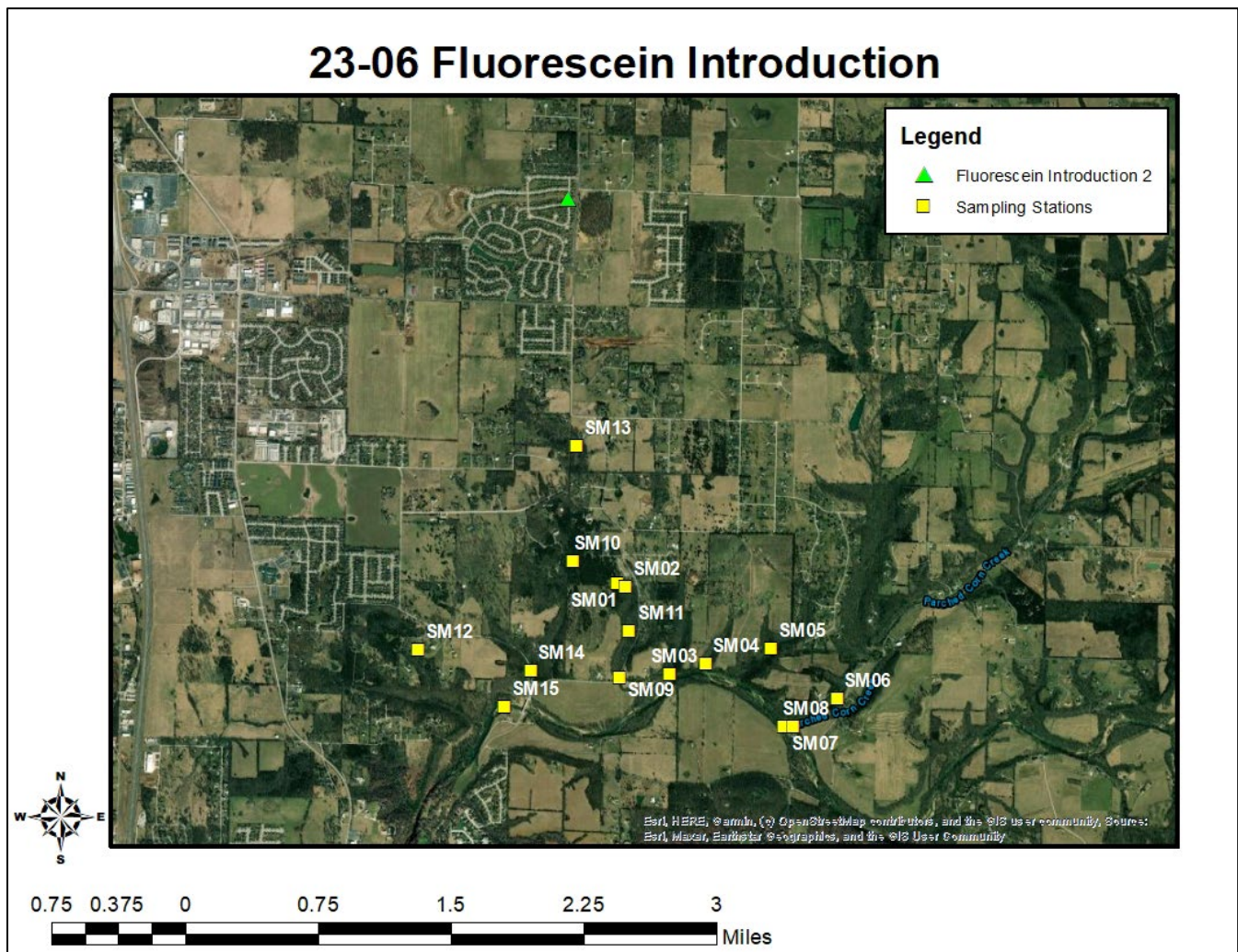


Figure 15. Mobilization of fluorescein dye by storm flows six hours post-introduction for Trace 23-06.



3.7 Trace 23-07. Wacha Farms Eosine Trace.

At 15:55 on May 4th, 2023, three pounds of eosine dye mixture containing 96% dye equivalent was introduced as a dry set into a sinkhole in an alfalfa field on Wacha Farms. The purpose of this trace was to determine the northernmost boundary of the area contributing water to Smallin Cave. There was no flow at the time of introduction. The dry set was achieved by pouring the eosine dye mixture into the sinkhole in anticipation of storm flow forecasted for that evening. Approximately 1.75 inches of rain fell in the area overnight after the introduction.

Table 10 shows maximum eosine dye mixture concentrations in activated carbon samplers from all sampling stations where dye from Trace 23-07 was detected. Eosine dye was first detected in grab samples of water and elutant from carbon samplers collected on May 11th, 2023, at SM01, SM06, SM09, and SM11 (see Appendix A). Dye from this trace was also detected at stations SM07, SM08, and SM15 on May 19th, 2023. These sites were not sampled on May 11th due to high water from recent storm flows.

Figure 16 shows the locations of sampling stations where eosine dye from Trace 23-07 was detected. First detection of eosine dye occurred 7 days post dye introduction. Straight-line distance between the dye introduction location and the furthest detection station from the introduction site at SM06 was approximately 13,410 feet, indicating groundwater flow rates for first dye arrival of at least 1,916 feet per day to SM06.

Table 10. Maximum eosine dye mixture concentrations from Trace 23-07 detected at sampling stations.

Station Number and Name	Elevation (ft)	Maximum concentration in carbon samplers (ppb)	Days Post Introduction	Distance from Dye Introduction Point (ft)
SM01. Smallin Cave	1210	1,660	7	7,970
SM06. Spring on Parched Corn Hollow	1139	255	7	13,465
SM07. Finley River Upstream of Parched Corn	1116	0.437	15	13,040
SM08. Parched Corn Hollow	1118	4.52	15	13,150
SM09. Smallin Branch	1120	194	7	10,085
SM11. Seep Downstream Smallin Cave	1145	279	15	8,765
SM15. Finley River Downstream Riverside Park	1107	0.962	15	9,610

Figure 16. Sampling stations where eosine dye from Trace 23-07 was detected. The diagrammatic arrow indicates an overall straight-line groundwater flow path.

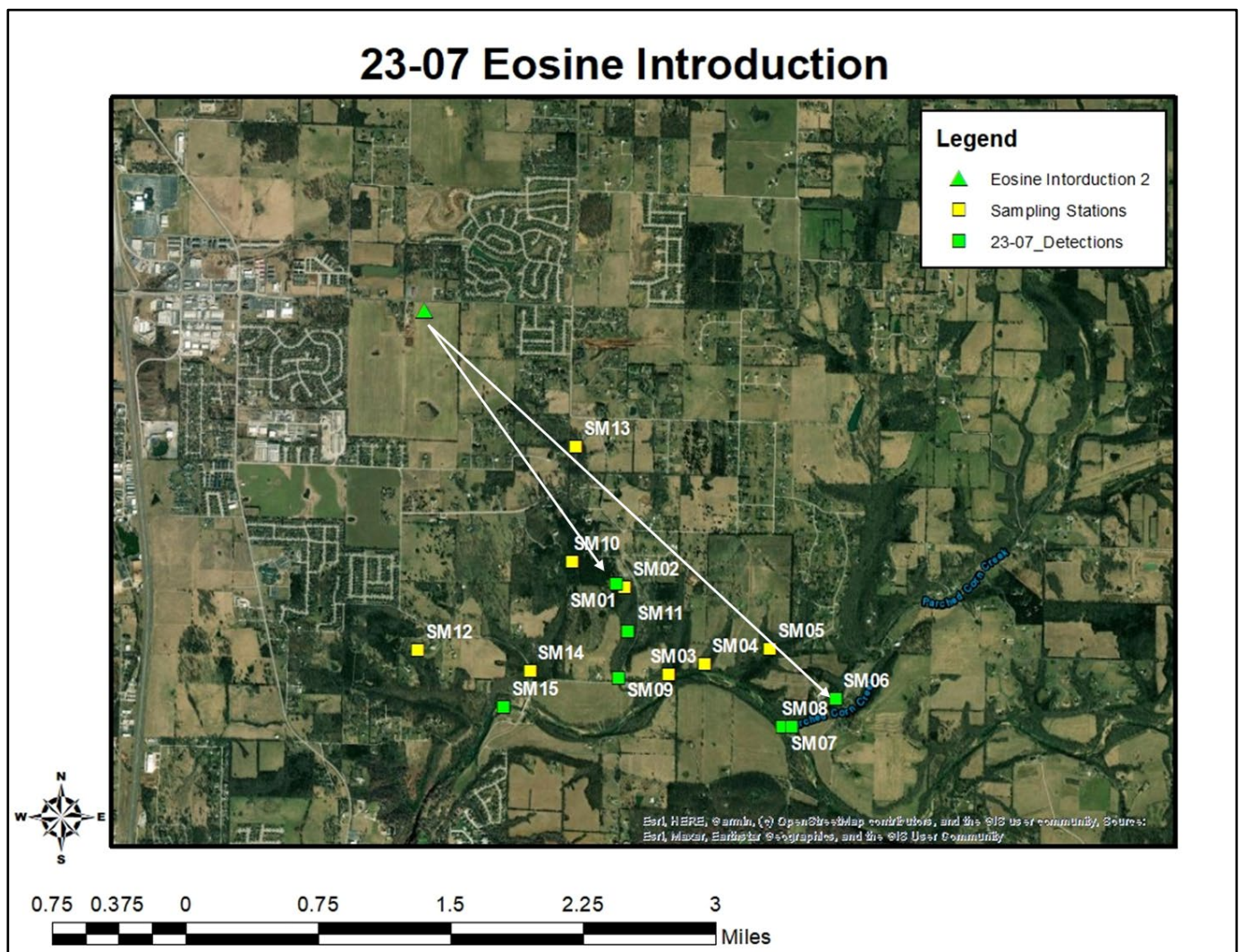


Figure 17. Trace 23-07 dye introduction location into a sinkhole in an alfalfa field at Wachsa Farms.



3.8 Trace 23-08. Indian Valley Sinkhole RWT Trace.

At 07:45 on May 5th, 2023, four pounds of RWT dye mixture containing 21% dye equivalent was introduced into a sinkhole on a residential property on Indian Valley Road. The purpose of this trace was to determine the western extent of the area that contributes water to Smallin Cave. There was no flow at the time of introduction. The dye was introduced directly into the sinkhole along with approximately 240 gallons of water from a garden hose from the nearby residence. Approximately 1.75" of rain fell in the area overnight before the introduction.

Table 11 shows maximum RWT concentrations in activated carbon samplers from all sampling stations where dye from Trace 23-08 was detected. RWT dye was first detected in grab samples of water and elutant from carbon samplers collected on May 11th, 2023, at SM01, SM06, and SM09 (see Appendix A). Dye from this trace was also detected at stations SM08 and SM11 on May 19th, 2023. Station SM09 was not sampled on May 11th due to high water from recent storm flows.

Figure 18 shows the locations of sampling stations where RWT dye from Trace 23-08 was detected. First detection of RWT dye occurred by visual observation at station SM01 (Smallin Cave) approximately 58 hours post dye introduction. Straight-line distance between the dye introduction location and SM01 was approximately 5,490 feet, indicating groundwater flow rates of at least 2,288 feet per day to Smallin Cave.

Table 11. Maximum RWT dye mixture concentrations from Trace 23-08 detected at sampling stations.

Station Number and Name	Elevation (ft)	Maximum concentration in carbon samplers (ppb)	Days Post Introduction	Distance from Dye Introduction Point (ft)
SM01. Smallin Cave	1210	13,000	6	5,490
SM06. Spring on Parched Corn Hollow	1139	614	6	11,460
SM08. Parched Corn Hollow	1118	7.07	14	10,880
SM09. Smallin Branch	1120	2,220	6	6,810
SM11. Seep Downstream Smallin Cave	1145	1,250	14	6,335

Figure 18. Sampling stations where RWT dye mixture from Trace 23-08 was detected. The diagrammatic arrow indicates an overall straight-line groundwater flow path.

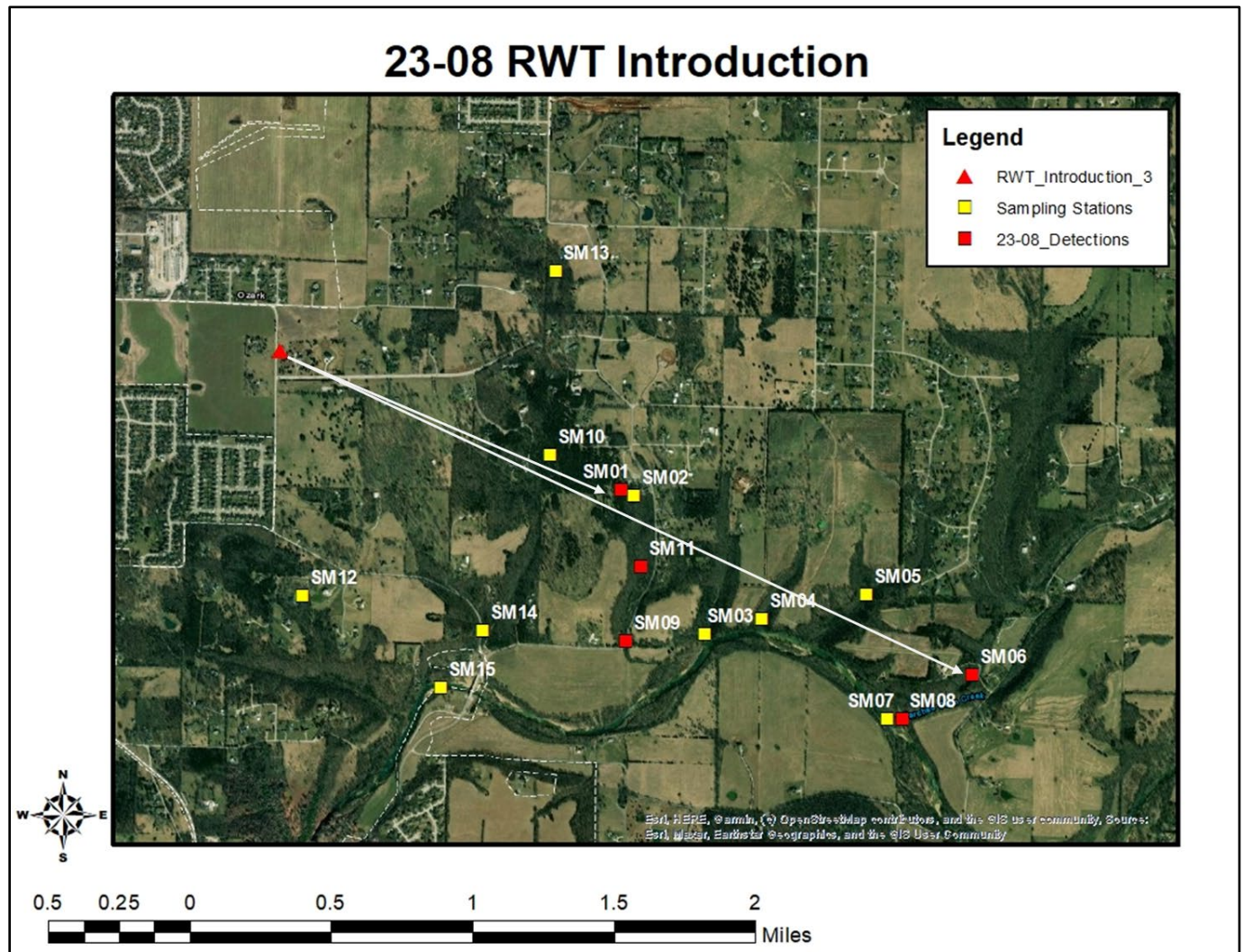
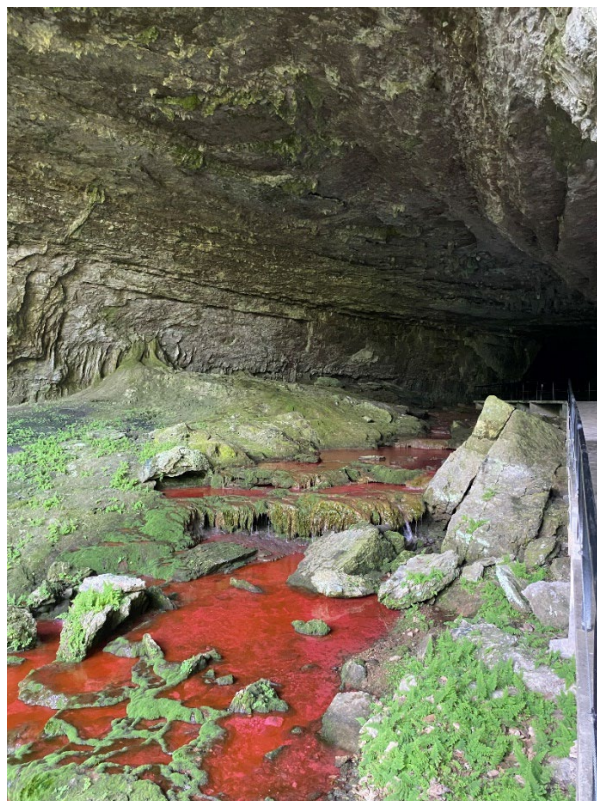


Figure 19. Trace 23-08 dye introduction location in a sinkhole on residential property on Indian Valley Road.



Figure 20. RWT dye from Trace 23-08 at Smallin Cave approximately 58 hours post introduction.



3.9 Trace 23-09. Hemlock Road Fluorescein Trace.

At 10:22 on May 11th, 2023, 100 grams of fluorescein dye mixture containing 70% dye equivalent was introduced into a losing stream reach flowing through a culvert under Hemlock Road. The purpose of this trace was to determine the source of sedimentation in Fielden Cave following storm flow events near the cave. At the time of introduction, the target stream had a high sediment load and flow was estimated to be 10-15 gpm. A solution consisting of fluorescein dye and one liter of water was introduced directly into the water flowing through the culvert. The stream flow disappeared approximately 75 feet downstream of the road.

Table 12 shows maximum fluorescein concentrations in activated carbon samplers from all sampling stations where dye from Trace 23-09 was detected. Fluorescein dye was first detected in grab samples of water and elutant from carbon samplers collected on May 19th, 2023, at SM02, SM11 and SM15 (see Appendix A).

Figure 21 shows the locations of sampling stations where fluorescein dye from Trace 23-09 was detected. First detection of fluorescein dye occurred eight days post introduction at stations SM02 (Fielden Cave), SM11, and SM15. Straight-line distance between the dye introduction location and SM02 was 1,345 feet, indicating groundwater flow rates of at least 168 feet per day to Fielden Cave.

Table 12. Maximum fluorescein dye mixture concentrations from Trace 23-09 detected at sampling stations.

Station Number and Name	Elevation (ft)	Maximum concentration in carbon samplers (ppb)	Days Post Introduction	Distance from Dye Introduction Point (ft)
SM02. Fielden Cave	1200	3,490	8	1,345
SM11. Seep Downstream Smallin Cave	1145	71	8	2,375
SM15. Finely River Downstream Riverside Park	1107	0.629	8	5,175

Figure 21. Sampling stations where fluorescein dye mixture from Trace 23-09 was detected. The diagrammatic arrow indicates an overall straight-line groundwater flow path.

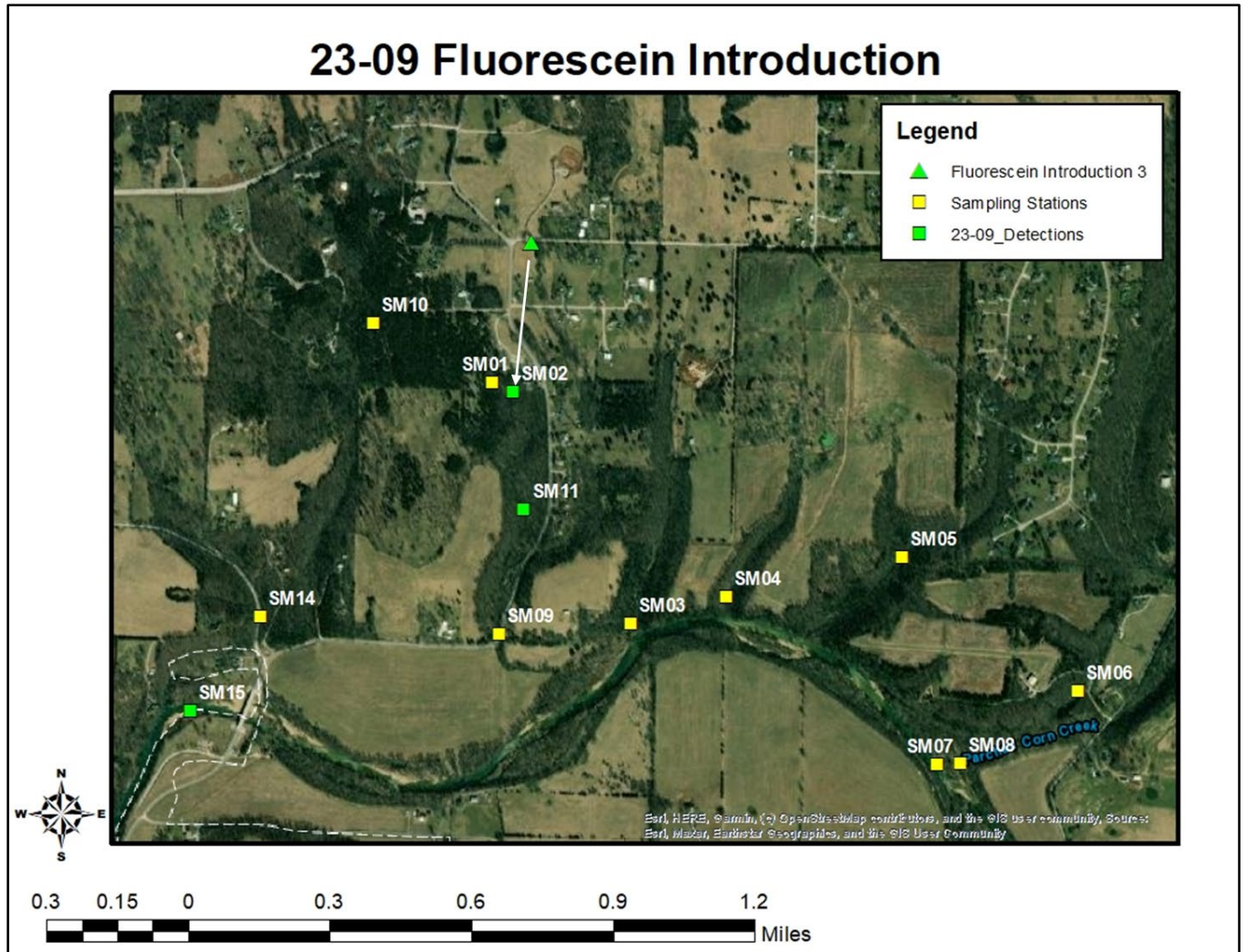


Figure 22. Fluorescein dye introduction at Hemlock Road into a losing stream. The stream disappears 75 feet downstream of the introduction point. Note the abundance of sediment suspended in the water.



3.8 Recharge Area Delineation

The recharge area for a cave, spring, or other karst feature is the land area that contributes water to that feature. Often, groundwater recharge areas in karst landscapes do not coincide with surface topography watersheds. Dye tracing was used to delineate the recharge area for Smallin Cave to determine the size and characteristics of the area that contributes water to the cave and map areas likely to be vulnerable to impacts from development and other landscape disturbances.

Figure 23 shows groundwater flow paths identified through nine traces conducted during this study. Dye from four different traces was detected at Smallin Cave and dye from four traces was detected at the spring on Parched Corn Hollow. Three dye introductions were detected at both Smallin Cave and the spring on Parched Corn Hollow, indicating connections exist between the two cave systems. In addition to the dye detections at Smallin Cave and the Parched Corn spring, dye from one trace was detected at Fielden Cave and dye from one trace was detected at Cave Spring.

Figure 23. A diagrammatic depiction of all groundwater flow paths identified from dye tracing in this study.

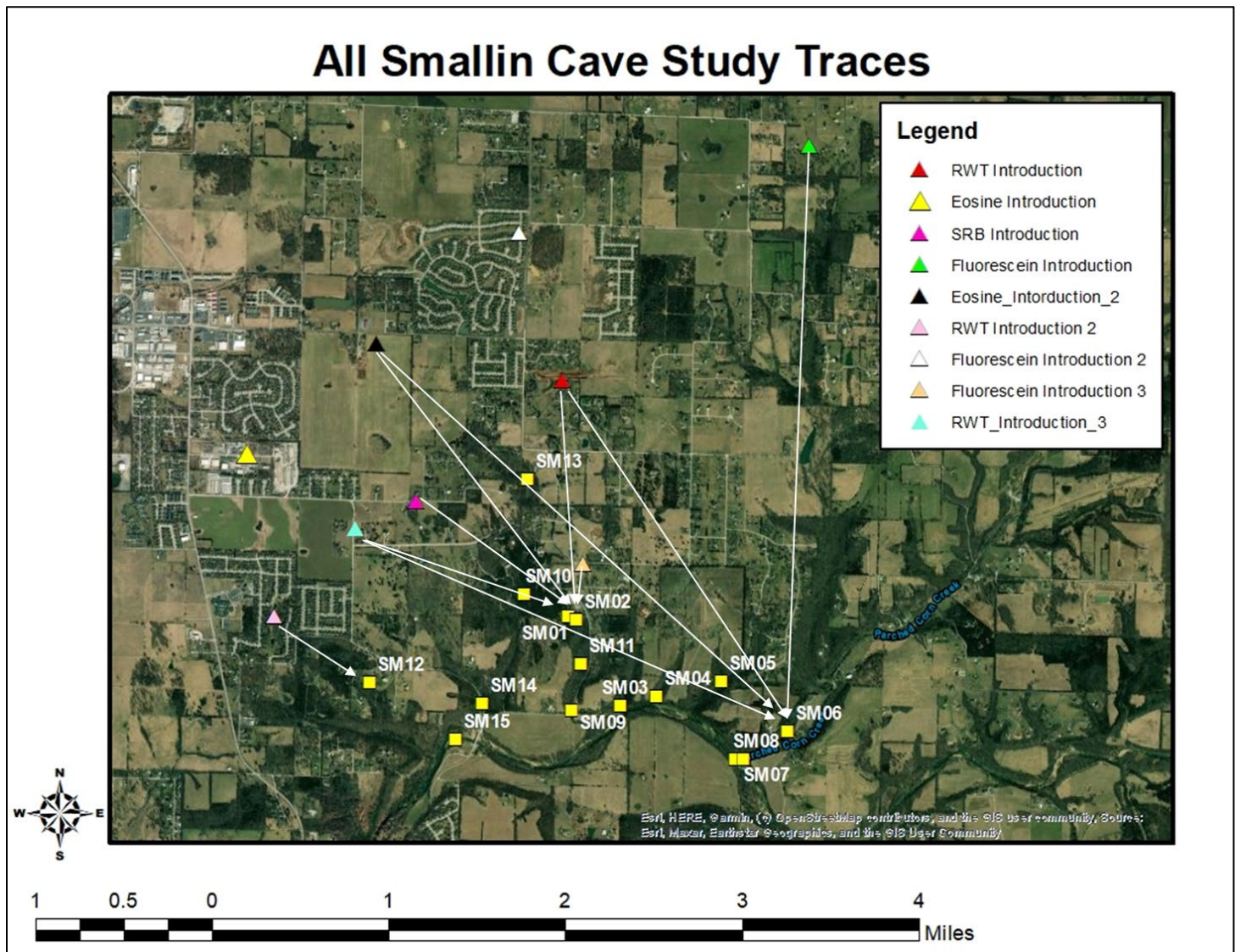


Figure 24 shows the delineated recharge area for Smallin Cave based on results from the nine dye traces conducted during this study. The recharge area for Smallin Cave encompasses approximately 1,344 acres (2.1 square miles). The recharge area was determined by incorporating all points where tracer dye introductions were detected at Smallin Cave and excluding topographic basins where dye was not detected during the respective traces in those areas.

Based on the traces conducted during this study, most of the recharge to Smallin Cave is derived from the sinkhole plain located to the north and west of the cave. Dye introductions were located on the periphery of this sinkhole plain to determine the extent of the area that contributes water to Smallin Cave. The Missouri Department of Natural Resources' (MODNR) Geological Survey had identified 23 sinkholes in the vicinity of Smallin Cave. The scope of this study did not allow for dye tracing at every sinkhole in this area; however, the dye tracing results from sinkholes on the boundaries of the sinkhole plain indicate all sinkholes within this area

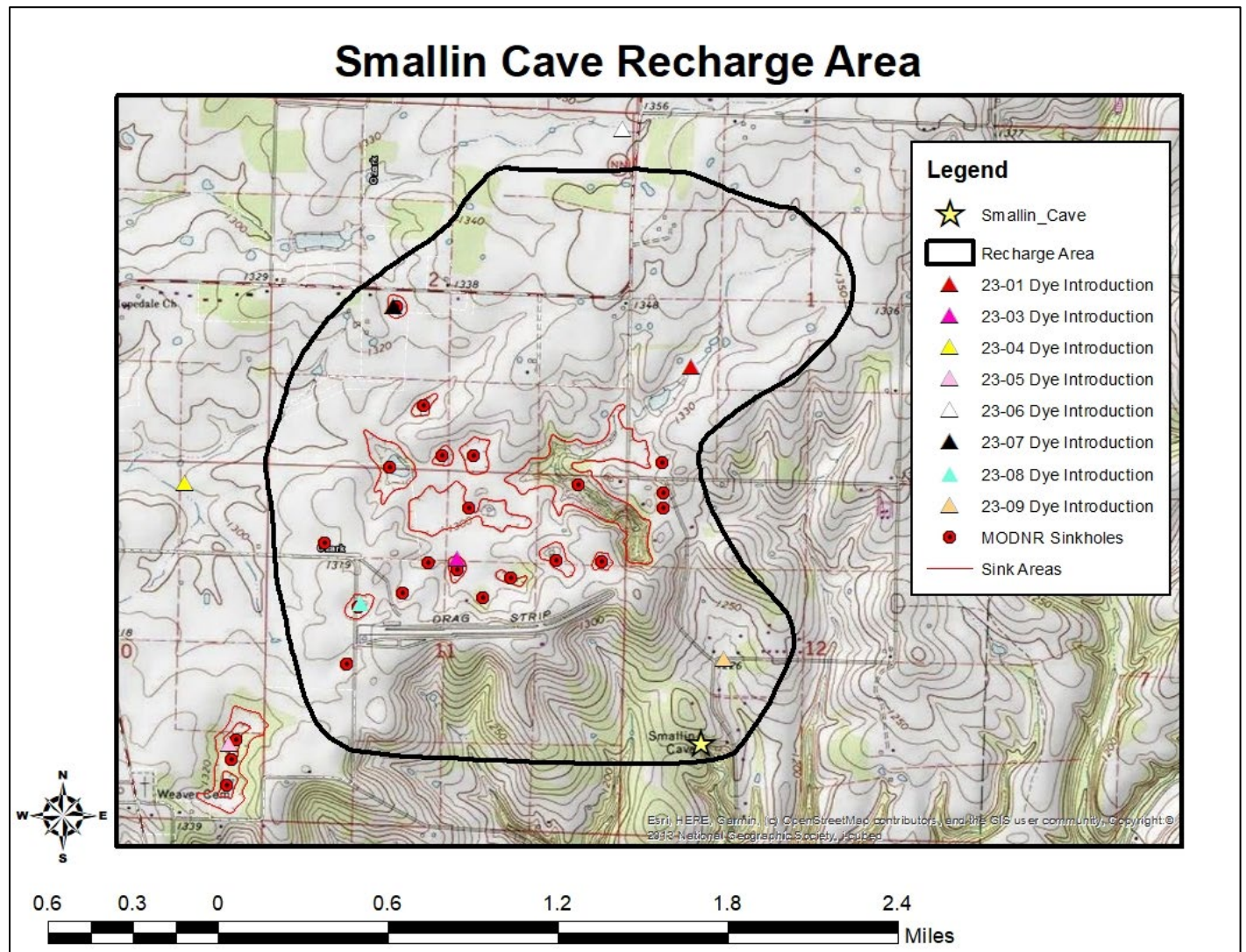
contribute water to Smallin Cave. Twenty of the 23 known sinkholes identified by MODNR in the vicinity of Smallin Cave were included in the recharge area. There are several additional sinkholes in the recharge area that have not been identified by MODNR which likely contribute water to Smallin Cave, including the location of the stormwater detention basin in Northtown Park where Trace 23-01 was conducted.

Two dye traces were conducted in surface streams during this study (Trace 23-04 and 23-06). None of the dye used in these traces was detected in Smallin Cave. Therefore, the topographic basins of these streams were excluded from the Smallin Cave recharge area. While no losing stream segments were identified in these basins, it is possible that unidentified sinkholes in the drainages may contribute water to Smallin Cave. The identification of these features would require further dye tracing in the area.

Dye for Trace 23-02 was introduced into a sinkhole in the upper Parched Corn Hollow basin. This dye was detected in the spring on Parched Corn Hollow but not at Smallin Cave. No dye traces were conducted within the stream channel or in other known sinkholes in the Parched Corn Hollow basin, so it is currently unclear whether some portions of this basin could contribute water to Smallin Cave. None-the-less, there was a general west-to-east trend in groundwater flow paths identified in most of the dye traces and lacking any additional data to the contrary, the entire Parched Corn Hollow drainage was excluded from the Smallin Cave recharge area.

Dye for Trace 23-05 was introduced into a sinkhole in McGuffey Park just east of State Highway NN. The dye was detected at Cave Spring, west of Smallin Cave, but not in Smallin Cave. Therefore, the known sinkholes in the vicinity of McGuffey Park were excluded from the recharge area for Smallin Cave.

Figure 24. Recharge area delineation for Smallin Cave.

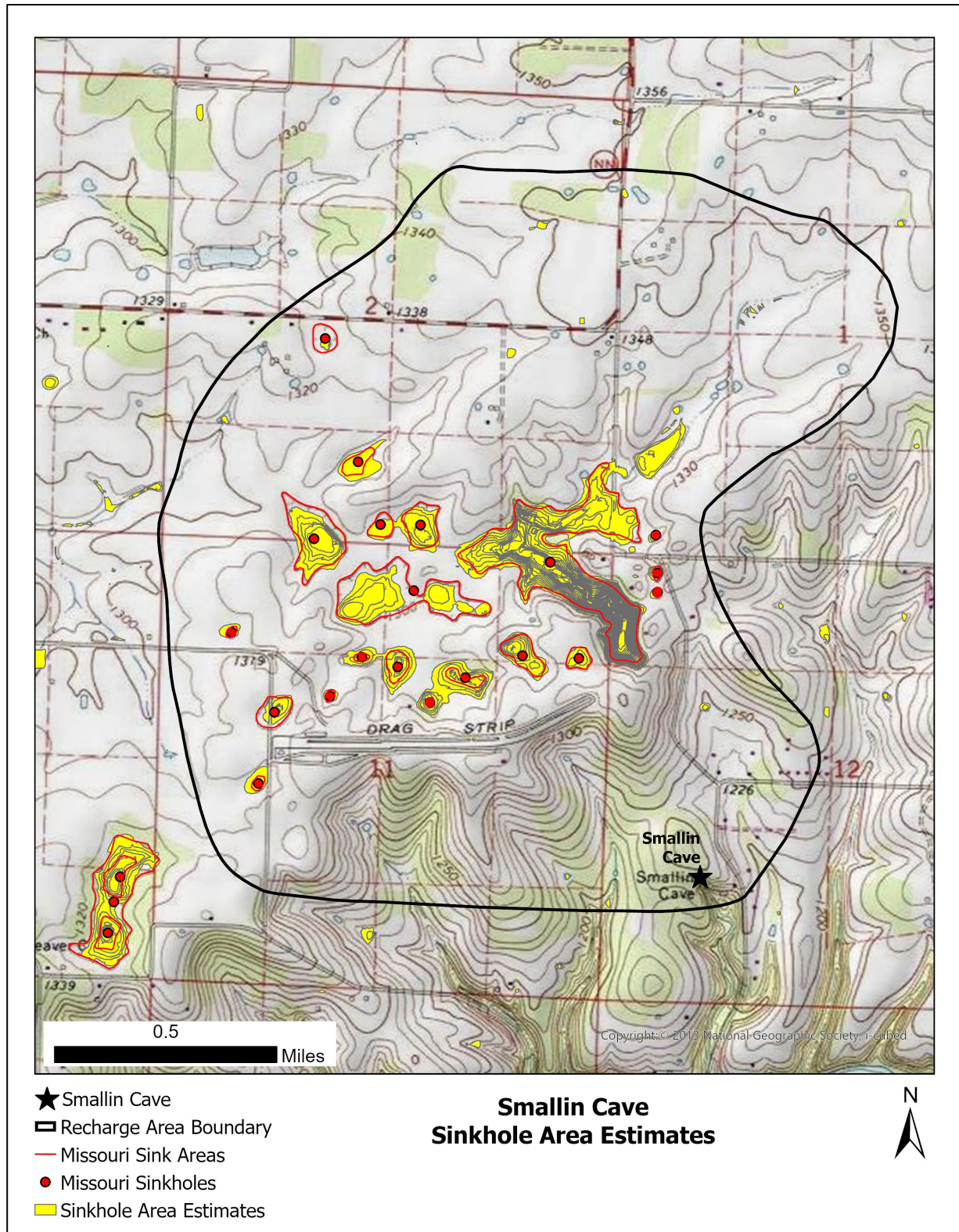


3.9 Sinkhole Area Estimates

The estimated sink areas generated using the methodology of Doctor and Young (2013) are shown in Figure 25. The estimated sink areas correlate well with the known sinkholes and sink areas as identified by the Missouri Department of Natural Resources. Notably, the process did not identify known sinkholes that did not have a corresponding sink area (e.g., the sinkhole used for Trace 23-02).

There were several sink areas identified that were not in the datasets from the Missouri Department of Natural Resources. These sink areas include several unplowed areas ($n=2$) in farm fields (i.e., likely natural sinkholes as identified by the landowner), as well as stormwater management structures such as detention basins ($n=8$) throughout the recharge area. Other sink areas were confirmed in satellite imagery to represent ponds, which may or may not also be natural sinkholes ($n=2$). The 2017 LiDAR data pre-dates the Northtown Park development.

Figure 25. Sinkhole areas estimated using ArcGIS Pro, shown alongside known sinkholes and sink areas. The grey lines represent inferred topographic lines within the sinks.



4.0 HAZARD AREA AND VULNERABILITY MAPPING

Vulnerability mapping is based on the concept that not all lands pose equal risks of introducing contaminants into karst groundwater systems. Various factors affect the relative level of risk. Key among these factors is the type of groundwater recharge that is related to various land areas. Discrete recharge is localized and concentrated. In contrast, diffuse recharge is dispersed.

Discrete recharge zones within the delineated recharge area include sinkholes, losing stream segments, and stormwater management structures. In the delineated recharge area, there are 20 sinkholes and 14 corresponding sink areas identified by the Missouri Department of Natural Resources. There were several additional sinkholes identified during OUL fieldwork. There are also four losing stream segments within the recharge area identified by the Missouri Department of Natural Resources. These losing streams are shown on the Ozark 7.5-minute quadrangle map as intermittent streams but cease flowing within a few hours of the end of a precipitation event. The rapid increase in flow rates of springs and cave streams to precipitation events is primarily due to discrete recharge that rapidly enters and is transported through the karst groundwater system. Vulnerability mapping in the study area focuses substantial attention on areas likely to contain discrete recharge zones. These include surface stream channels, sinkholes, likely sinkholes, and stormwater management structures.

Diffuse recharge is water that slowly infiltrates through soils before reaching the epikarstic zone. The epikarst is the weathered upper portion of the bedrock. The thickness of the epikarst is variable. The epikarstic zone is drained by localized solutionally widened fractures and other karst features that ultimately convey recharging waters into the conduits feeding cave streams and springs. Substantial quantities of water are routinely detained within the epikarstic zone. The slow discharge of this detained water from the epikarst zone is largely responsible for sustaining the flow of springs in the area during drier periods of the year. Waters entering karst groundwater systems through diffuse recharge receive more effective natural cleansing than is the case for discrete recharge waters. As contrasted with discrete recharge waters, diffuse recharge water has more contact with soil particles. This increases the effectiveness of filtration and the removal of contaminants by adsorption onto soil particles.

4.1 Vulnerability Classifications

Vulnerability mapping for recharge area delineation studies has routinely depicted risks posed to groundwater quality by various portions of the recharge areas. The OUL has routinely depicted four categories in such assessments, as described in Section 2.8.

Low Vulnerability lands. No Low Vulnerability lands were identified in this study since it is likely that most or all of the study area could be developed for suburban or urban uses in the foreseeable future and these uses are inconsistent with Low Vulnerability lands.

Moderate Vulnerability lands. There are 598 acres of moderate vulnerability lands in the recharge area. These lands are located in the uplands that do not contribute to sinkholes in the recharge area, as well as the dissected lands side gradient to the drainage ways that contribute drainage to the topographic basin of Smallin Cave. The predominant soil series in this group is the Captina-Needley complex, which is characterized by silt loams with 1 to 3 percent slopes.

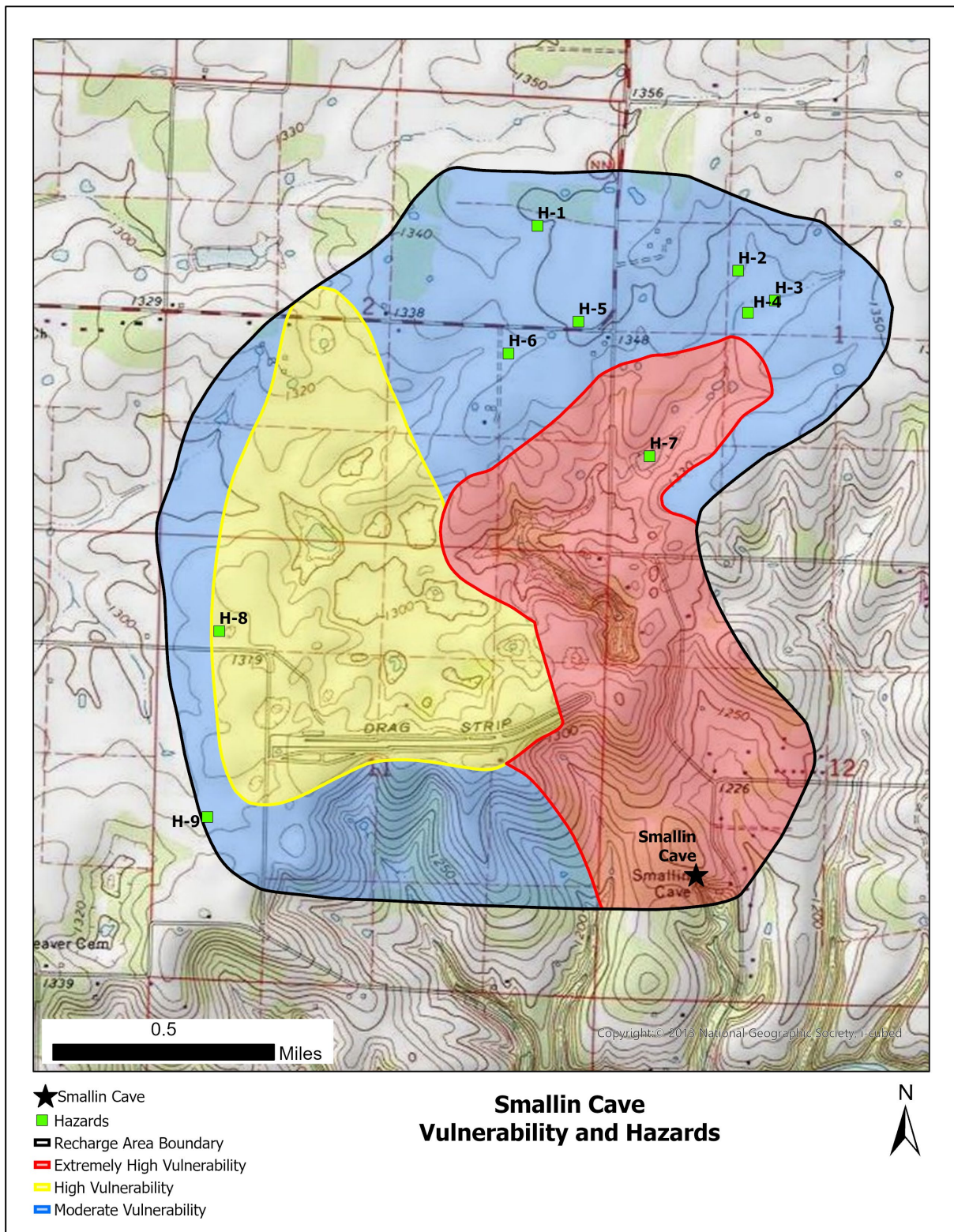
High Vulnerability Lands. There are 352.5 acres of high vulnerability lands in the recharge area. These lands have a high density of sinkholes and sink areas as mapped by The Missouri Department of Natural Resources' (MODNR) Geological Survey. High vulnerability lands also include those that contribute drainage to sinkholes. The predominant soil series in this category are the Goss cherty silt loam with 2 to 9 and 9 to 14 percent slopes with karst features including sinkholes.

Extremely High Vulnerability Lands. There are 394 acres of extremely high vulnerability lands that were identified in the recharge area. These are lands within 1,000 feet of known cave passages as well as lands that contribute drainage to the Jeff Cave sinkhole area. Major soil series within this group include Goss cherty silt loam with 2 to 9 and 9 to 14 percent slopes with karst features including sinkholes, as well as the Goss-Gasconade complex with 2 to 50% slopes.

Table 13. Land in each vulnerability class within the Smallin Cave recharge area.

Vulnerability Class	Total Acreage	Total Square Miles	Percent of Recharge Area.
Moderate Vulnerability Land	598	0.93	44.5%
High Vulnerability Land	353	0.55	26.3%
Extremely High Vulnerability Land	393	0.62	29.2%
Total Recharge Area	1,344	2.1	100.0

Figure 26. Vulnerability mapping and hazard areas in the Smallin Cave recharge area.



4.2 Hazard Areas

Stormwater runoff from urban and suburban areas, and particularly from paved surfaces, is a well-recognized source of surface and groundwater contamination. Meister and Kefer (1981) compared the quality of urban stormwater runoff with similar values from an area of forest and meadows. Biochemical Oxygen Demand (BOD) values indicate the amount of oxygen required to break down organic matter, and thus are a good indicator of the amount of organic matter contained in water. Meister and Kefer (1981) found that the BOD values of urban stormwater were about 10 times greater than those for forest and meadow runoff.

Nutrients, such as nitrates, aid in the breakdown of organic matter. Meister and Kefer (1981) found that urban runoff contained an average of about 20 times more nitrates than did runoff from forests and meadows. Urban stormwater runoff also contains unnaturally large concentrations of other materials that can adversely impact caves and groundwater.

As should be expected, the concentration of contaminants in stormwater runoff is much greater in the first flush of runoff water. Jenkins (1988) notes that the first half-inch of runoff water from storms often contains 80 to 95% of the total annual pollutant load.

Portions of the study area have experienced suburban development. These developments include a number of stormwater detention basins. While these ponds decrease the magnitude of downstream surface flows, they routinely adversely impact groundwater quality in karst areas for the following reasons:

1. The stormwater detention basins are typically constructed in small water courses and sinkholes and these areas are routinely characterized by more rapid water infiltration into the subsurface than is the case for adjacent lands.
2. The detention basins concentrate stormwater runoff from larger areas than under natural conditions. The design of the detention ponds in the study area is dependent on rapid infiltration of stormwater runoff into the subsurface, thereby increasing total groundwater recharge.
3. The waters detained in the basins are routinely the first flush of stormwater runoff, and this water is routinely the most highly contaminated runoff water.
4. Stormwater detention basins, as currently constructed in the study area, provide little if any water quality improvement for the detained waters.
5. During construction, upper horizons of the soil are often removed from the floor of the detention basins and are used as dike material. These upper horizons are routinely the best suited natural earth material for detaining or removing groundwater contaminants.

Identified Hazard Areas within the Smallin Cave recharge area are limited to stormwater management structures:

H-1. Stormwater management structure. Concrete-lined channel within low-lying area which functions as stormwater detention basin.

H-2. Stormwater management structure. Unlined channel that feeds into adjacent stormwater detention basin (H-4) constructed in a known losing stream segment.

H-3. Stormwater management structure. Unlined channel located in known losing stream segment, feeds adjacent detention basin (H-4).

H-4. Stormwater detention basin. Unlined basin constructed in known losing stream segment with no apparent outlet.

H-5. Stormwater detention basin. Unlined basin constructed with no apparent outlet.

H-6. Stormwater detention basin. Concrete-lined channels within stormwater detention basin. Basin has concrete outlet that appears to discharge water into adjacent farm field.

H-7. Stormwater detention basin. Unlined basin constructed in known losing stream segment with no apparent outlet. See Section 3.1 for information on the dye introduction into this stormwater detention basin as well as Figure 27.

H-8. Stormwater detention basin. Unlined basin constructed in known sink area. There is no apparent outlet.

H-9. Stormwater detention basin. Concrete-lined channel within stormwater detention basin. Basin has concrete outlet that appears to discharge water into adjacent farm field.

5.0 SPRING FLOW, TEMPERATURE, AND WATER LEVELS

5.1 Spring Flow Measurements

In the spring of 2023, the OUL made flow rate measurements on two dates at Smallin Cave (SM01) and the spring on Parched Corn Hollow (SM06). Flow rates were measured with a Pygmy Price current meter and a top-setting wading rod and US Geological Survey standard methodologies were used to calculate stream flow rates. Table 14 includes flow rate measurements made during the study.

On March 1, 2023, comparable flow rates were observed at Smallin Cave and the spring on Parched Corn Hollow. According to National Weather Service data for February 2023, 0.95" of precipitation was recorded in the Ozark area in the week prior to the March 1st flow rate measurements. The total precipitation amount for the month of February was 3.44".

Flow rates were measured again on March 10th at Smallin Cave and the spring on Parched Corn Hollow. In the week prior to these measurements, approximately 4" of precipitation was recorded in the Ozark area. Flow rates measured at Smallin Cave on March 10th (4331 gpm) were over five times greater than those recorded on March 1 (863 gpm). Flow rates measured at the spring on Parched Corn Hollow were nearly 3.5 times higher on March 10th (2892 gpm) than those measured on March 1st (836 gpm).

Table 14. Flow rate measurements at two springs in the study area. Flow rates are reported in gallons per minute (gpm)

Location	3/1/2023	3/10/2023
Smallin Cave	863	4331
Parched Corn Spring	836	2892

5.2 Temperature and Water Level Monitoring

Temperature and water level data can help characterize aquifers. The rate at which temperature and flow in springs and cave streams respond to storm flows can be affected by the interconnectedness between the surface and sub-surface in karst terrains. Abrupt temperature changes and rapid increases in water level in springs and cave streams are indicative of systems where groundwater recharges quickly through openings in the limestone.

Onset HOBO MX 2001 water level loggers were installed in the stream in Smallin Cave (SM01) and in the spring on Parched Corn Hollow (SM06) to monitor temperature and water levels at the two groundwater discharge points. Placement of the water level loggers allowed for continuous monitoring of temperature and water level at the two locations from the period of March 1st to May 19th, 2023, when the loggers were removed. Temperature and water level values were recorded every five minutes and logged data were downloaded to the HOBO Connect smartphone application during each sampling event via a Bluetooth transmitter.

Figures 27 and 28 display temperature and water level data for Smallin Cave and the spring on Parched Corn Hollow, respectively. Water temperatures recorded in Smallin Cave consistently stayed around 58 degrees Fahrenheit throughout the study period, except during periods of heavy precipitation, when water temperature would change sharply depending on surface temperatures. On one occasion during the overnight hours of March 23rd and 24th, 4.5 inches of rain fell in the Ozark area according to National Weather Service observational data. Surface low temperature was recorded at 46 degrees Fahrenheit, producing a cold rain relative to groundwater temperatures. The effects of this precipitation event can clearly be observed in Figure 25 where water temperatures in Smallin Cave decreased by four degrees Fahrenheit in a matter of hours.

Similar water temperature trends can be observed in the spring on Parched Corn Hollow; however, the average water temperature in the Parched Corn spring was lower than that of Smallin Cave early in the study. Groundwater temperatures approach the mean annual surface temperature for a given location. In Ozark, MO that is approximately 58 degrees Fahrenheit. The average water temperature recorded in the spring on Parched Corn Hollow was lower than that of Smallin Cave, likely due to the location of the datalogger in the spring discharge. The Parched Corn datalogger was placed in the spring outlet, which is exposed to ambient air temperatures. The colder surface temperatures likely affected the water temperatures recorded at the Parched Corn spring, whereas the Smallin Cave datalogger was located inside the cave and was not exposed to outside air temperatures.

Water level increases corresponded with rapid temperature changes caused by storm flows at both monitoring points. Base flow recorded in Smallin Cave during the study period averaged around 0.5 feet, but

relatively rapid increases in water level coincided with precipitation events. The maximum water level recorded at Smallin Cave during the monitoring period was 1.03 feet. Due to the nature of the spring outlet and the placement of the water level logger, base flow water levels recorded at the spring on Parched Corn were shallower than at Smallin Cave, averaging between 0.1 and 0.2 feet in depth. The highest water level recorded at the Parched Corn spring was 0.35 feet.

Figure 27. Temperature and water level data recorded in Smallin Cave.

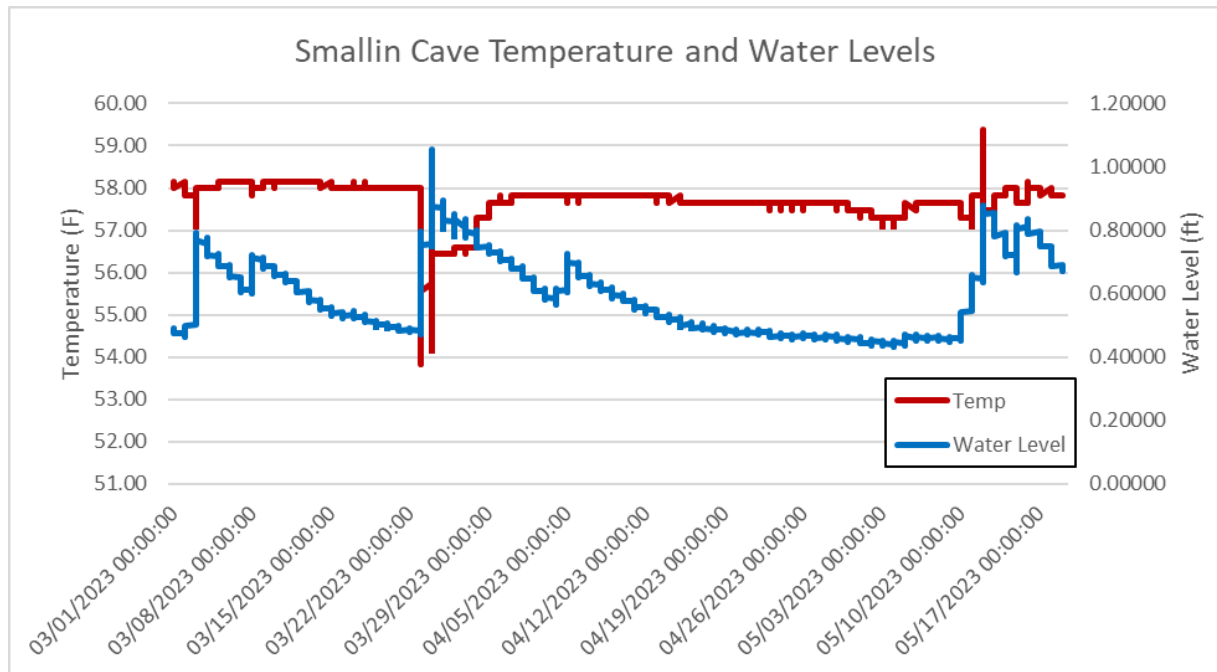
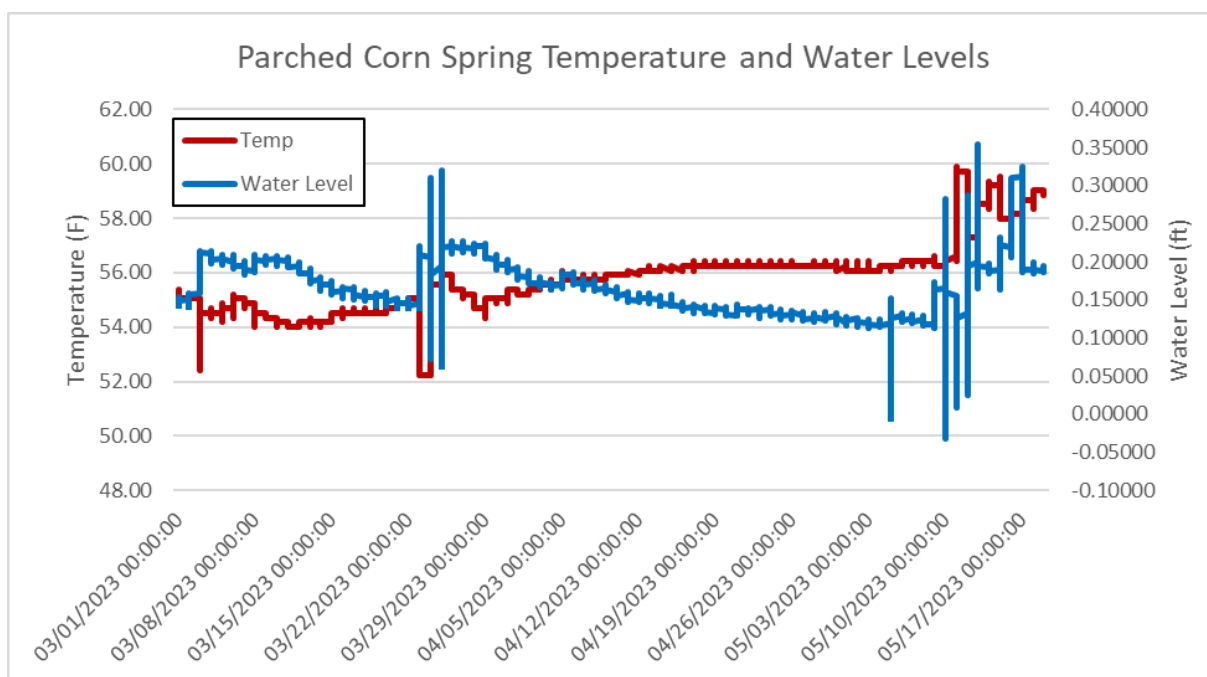


Figure 28. Temperature and water level data recorded in the spring on Parched Corn Hollow.



6.0 Discussion and Key Findings

6.1 Discussion

Nine dye traces were conducted to delineate the recharge area for Smallin Cave, seven of which resulted in detections of dye at sampling stations. Four of the nine traces resulted in detections at Smallin Cave and one trace resulted in a detection at Fielden Cave, an associated cave within 150 feet of Smallin Cave. All traces detected at Smallin Cave were from sinkhole features, indicating the cave system's primary source of water is the sinkhole plain to the north and west of the cave. The trace detected at Fielden Cave was from a losing stream 1,400 feet east of the cave. Six of the nine traces were detected at one or more of three major springs along the Finley River: Smallin Cave, the spring on Parched Corn Hollow, and Cave Spring.

The time of first dye arrival at a particular sampling station can help characterize groundwater flow paths in dye tracing studies. Short travel times between dye introduction sites and sampling stations are indicative of discreet recharge zones where well-developed connections between the surface and subsurface waters exist. Discrete groundwater recharge zones have little ability to filter out sediment or contaminants carried by surface runoff and can be areas of high vulnerability to groundwater systems.

In most instances, travel times and groundwater flow rates are determined using time of first detection of the dye in activated carbon samplers or grab samples of water. Generally, samples were collected on a weekly basis in this study. In addition, many of the dye introductions were made using dry sets, which require precipitation to mobilize the dye. Consequently, it was often impossible to determine travel times of less than a week in this study unless sampling occurred on a shorter interval, or the dye was visually observed in the

field. Additionally, when using dry sets, it is often difficult to know when the dye was mobilized; however, in this case since OUL staff live near the study area, we were able to make fairly accurate approximations of when dye from dry sets was mobilized during storm events.

The shortest travel times observed in this study occurred during Trace 23-01, when RWT dye introduced into the stormwater detention basin in the Northtown Park development was detected in samples collected at the spring on Parched Corn Hollow 48 hours later. With a straight-line distance of 7,975 feet between the dye introduction point and the spring, groundwater flow rates were estimated to be at least 3,988 feet per day during this trace. Based on dye mixture detections in carbon samplers at the site, travel time for this same trace to reach Smallin Cave was between 14 and 21 days under the hydrological conditions at the time.

The shortest travel times observed to Smallin Cave occurred during Trace 23-08, when RWT dye introduced into a sinkhole on Indian Valley Road was visually observed 5,490 feet away at Smallin Cave approximately 58 hours later. This produced an estimated groundwater flow rate of 2,288 feet per day during this trace. These rapid travel times to both Smallin Cave and the spring on Parched Corn Hollow are indicative of an open conduit system with little filtration of sediment and contaminants between the recharge zones and the aquifer.

In addition to groundwater travel times, an examination of dye concentrations detected at sampling stations can help characterize groundwater flow paths. In most cases the OUL attempts to introduce just enough dye to make detections with our analytical methodologies while minimizing its visibility to the public. OUL analytical methods can detect dye in the parts per trillion which is invisible to the naked eye. Very high concentrations of dye measured in activated carbon samplers or water samples, as well as visible dye present at groundwater resurgences, often indicates direct conduit flow between the dye introduction point and the spring or cave. This scenario occurred during Trace 23-08, where 4 pounds of RWT was introduced into the Indian Valley Road sinkhole over a mile away from Smallin Cave. RWT was clearly visible in the cave stream 58 hours after introduction and dye concentrations of 13,000 parts per billion were measured in elutant from the activated carbon samplers. This shows that the recharge area of Smallin Cave is characterized by well-developed connections between the surface and sub-surface and multiple discrete recharge zones. Discrete groundwater recharge zones have little ability to filter out sediment or contaminants carried by surface runoff and can be areas of high vulnerability to groundwater systems. The Burlington-Keokuk limestones in which Smallin Cave has developed are well known for their large cave passages and open groundwater conduits. Jeep powered trams take tourists through these conduits in the limestone passages of Fantastic Caverns north of Springfield, Missouri.

Using information from the dye traces conducted in this study, a recharge area of approximately 3.3 square miles was delineated for Smallin Cave. The recharge area encompasses most of the sinkhole plain located north and west of the cave, including at least 20 documented sinkholes and several that have not been formally identified by the MODNR (Figure 25). The southern portion of the recharge area lies in a dissected landscape of high ridges and deep hollows. Management considerations for the protection of caves and groundwater resources will differ in these two portions of the recharge area.

Vulnerability classes were assigned according to land use and proximity to sinkholes and cave passages. Approximately 598 acres were identified as having moderate vulnerability, 353 acres were classified

as high vulnerability and 393 acres were classified as extremely high vulnerability. There are no low vulnerability lands within the recharge area.

The impetus for this study was concern over the effects of suburban development and other stressors related to the growth of the surrounding community on cave and groundwater resources in the Ozark area. Sediment laden storm flows have caused considerable siltation issues in Smallin and Fielden Caves. This can be detrimental to the cave system by destroying aquatic habitat for cave species, impairing groundwater quality, and ruining the aesthetic character that makes Smallin Cave a popular tourist attraction. The famous rimstone dams in Smallin Cave slow the water before it flows over their tops, causing the sediment carried in the water to deposit upstream of the natural dams. Flow velocities necessary to flush the sediment out of the pools are higher than the velocities that allowed for the deposition of the sediment, making it difficult for natural processes to move the sediment out of the pools once it's been deposited. The sediment deposition destroys Bristly Cave Crayfish habitat by filling interstitial spaces in the stream bed used by the crayfish and the aquatic organisms they eat.

Over the course of the study, examples of insufficient erosion control measures at construction sites, poorly sited stormwater control structures, and agricultural inputs directly into sinkholes were observed throughout the recharge area (Figures 29-31). Dye traces conducted in this study demonstrated the existence of direct connections between these areas of residential development and the Smallin Cave system. Considering the open nature of the recharge area and the rate at which surface water reaches the groundwater system, the unnatural amounts of sediment observed in Smallin Cave are the result of land use practices on the surface.

Land use issues not only affect Smallin Cave, but also adversely impact groundwater quality throughout the area and water quality in the Finley River. This study demonstrated multiple connections between major springs along the Finley River. Consequently, the water quality issues that are affecting Smallin Cave are also affecting other caves and springs in the area. These systems ultimately drain to the Finley River. The OUL estimates that greater than 75% of the water entering the Finley River in the study area has passed through the groundwater system. The Finley River is a defining feature of the city of Ozark. Efforts to protect water quality in the Finley River must focus on the protection of water quality in the caves and springs contributing water to the river, as these aquatic systems are inextricably connected.

While these issues are common in karst settings, the threats urban and suburban development place on cave and groundwater resources are not insurmountable. In 2015 the OUL completed a large study for the Northwest Arkansas Regional Planning Commission to delineate the Cave Springs recharge area. Cave Springs provides habitat for the largest known population of the Federally Threatened Ozark Cavefish (*Amblyopsis rosae*). Individual environmental review of development projects by the U.S. Fish and Wildlife Service (FWS) often caused project delays for the nearby towns of Rogers, Springdale, Lowell, and Cave Springs, Arkansas. The recharge area delineation of Cave Springs by the OUL enabled the Planning Commission to develop the Cave Springs Karst Resource Conservation Regulations, in cooperation with FWS, to facilitate development in the surrounding communities while also maintaining the ecological integrity of the Cave Springs system. This model could be used by Christian County and the City of Ozark to ensure these communities can grow responsibly and protect caves, springs, and other aquatic resources that draw people to the area.

Figure 29. Structural failure of a stormwater detention basin full of sediment laden water. Note the breach in the dam just to the right of the concrete structure. Trace 23-01 was conducted at this location before the failure. Discharge from this structure contributes water directly to Smallin Cave.



Figure 30. Excessive sedimentation in a losing stream that flows under Hemlock Road. The sediment is the result of inadequate erosion control measures from nearby residential development and road work. This losing stream contributes water to Fielden Cave, less than 1,400 feet away.



Figure 31. Sediment in the Fielden Cave stream after storm flows. At this flow rate the cave stream typically runs clear.



6.2 Key Findings

1. Smallin Cave is habitat for one of the largest populations of Bristly Cave Crayfish (*Cambarus setosus*) observed in the state, which is a Missouri Species of Conservation Concern. Smallin Cave is also a major tourist attraction and cultural resource for the Ozark area.
2. The recharge area for Smallin Cave was delineated by conducting nine dye traces. The surface area that contributes water to Smallin Cave is approximately 3.3 square miles, encompassing much of the sinkhole plain to the north and west of the cave.
3. Dye tracing, spring flow measurements, and water temperature data show that the Smallin Cave system is closely connected to the surface, largely through discrete recharge zones where surface water can enter the cave system rapidly with ineffective removal of sediment and contaminants.
4. Smallin Cave and the spring on Parched Corn Hollow have a shared recharge area and are part of a larger groundwater drainage system contributing to the Finely River.
5. The majority of Smallin Cave's recharge area is vulnerable to stressors associated with the growth of the City of Ozark and Christian County.

6. Inadequate erosion control measures, suburban development, and agricultural practices are the primary stressors threatening the water quality of Smallin Cave. These issues should be addressed through the establishment of appropriate best management practices and ordinances to protect the most vulnerable groundwater recharge zones such as sinkholes and losing stream reaches in the recharge area.

7.0 REFERENCES

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Appendix A: Dye Tracing Results



A Recharge Area Delineation for Smallin Cave
Ozark, Missouri
July 2023

Ozark Underground Laboratory, Inc.

Smallin Cave Recharge Area Delineation

City of Ozark

Table 1. Results for charcoal samplers analyzed for the presence of fluorescein, eosine, rhodamine WT (RWT) and sulforhodamine B (SRB) dyes.

Peak wavelengths are reported in nanometers (nm); dye concentrations are reported in parts per billion (ppb).

OUL Number	Station Number	Station Name	Date/Time Placed	Date/Time Collected	Fluorescein		Eosine		RWT		SRB	
					Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)
G2133	SM01	Smallin Cave	12/14/22 0950	12/21/22 1155	ND		ND		ND		ND	
G2134	SM01	Smallin Cave	12/21/22 1155	1/4/23 1400	ND		ND		ND		ND	
G2549	SM01	Smallin Cave	1/4/23 1400	1/20/23 1003	ND		ND		ND		ND	
G2654	SM01	Smallin Cave	1/20/23 1003	2/3/23 1010	ND		ND		ND		ND	
G2813	SM01	Smallin Cave	2/3/23 1010	2/10/23 1025	ND		ND		566.4	10.4	ND	
G2907	SM01	Smallin Cave	2/10/23 1025	2/17/23 0950	ND		ND		567.1	82.5	ND	
G2992	SM01	Smallin Cave	2/17/23 0950	2/24/23 0945	ND		ND		ND		574.8 **	60.8
G3279	SM01	Smallin Cave	2/24/23 0945	3/10/23 0920	ND		ND		568.9	17.0	ND	
G3726	SM01	Smallin Cave	3/10/23 0920	3/23/23 1035	ND		ND		568.3	23.4	ND	
G4090	SM01	Smallin Cave	3/23/23 1035	3/30/23 0950	ND		ND		573.8 **	2.14	ND	
G4511	SM01	Smallin Cave	3/30/23 0950	4/6/23 0905	ND		ND		ND		ND	
G5212	SM01	Smallin Cave	4/6/23 0905	5/4/23 1425	ND		ND		565.9	4.34	ND	
G5316	SM01	Smallin Cave	5/4/23 1425	5/11/23 0843	ND		542.6	1,660	566.8	13,000	ND	
G5425	SM01	Smallin Cave	5/11/23 0843	5/19/23 0920	ND		541.6	678	567.4 **	171	ND	
G2135	SM02	Fielden Cave	12/14/22 1000	12/21/22 1150	ND		ND		ND		ND	
G2136	SM02	Fielden Cave	12/21/22 1150	1/4/23 1405	ND		ND		ND		ND	
G2550	SM02	Fielden Cave	1/4/23 1405	1/20/23 0958	ND		ND		ND		ND	
G2655	SM02	Fielden Cave	1/20/23 0958	2/3/23 1006	ND		ND		ND		ND	
G2814	SM02	Fielden Cave	2/3/23 1006	2/10/23 1020	ND		ND		ND		ND	
G2908	SM02	Fielden Cave	2/10/23 1020	2/17/23 0940	ND		ND		ND		ND	
G2993	SM02	Fielden Cave	2/17/23 0940	2/24/23 0935	ND		ND		ND		ND	
G3281	SM02	Fielden Cave	2/24/23 0935	3/10/23 0940	ND		ND		ND		ND	
G3727	SM02	Fielden Cave	3/10/23 0940	3/23/23 1030	ND		ND		ND		ND	
G4091	SM02	Fielden Cave	3/23/23 1030	3/30/23 0940	ND		ND		ND		ND	
G4512	SM02	Fielden Cave	3/30/23 0940	4/6/23 0900	ND		ND		ND		ND	
G5213	SM02	Fielden Cave	4/6/23 0900	5/4/23 1410	ND		ND		ND		ND	
G5317	SM02	Fielden Cave	5/4/23 1410	5/11/23 0838	ND		ND		ND		ND	
G5426	SM02	Fielden Cave	5/11/23 0838	5/19/23 1912	516.3	3,490	ND		ND		ND	
G2137	SM03	1st Drainage East of Smallin	12/21/22 0900	1/4/23 1237	ND		ND		ND		ND	
G2551	SM03	1st Drainage East of Smallin	1/4/23 1237	1/20/23 0820	ND		ND		ND		ND	
G2656	SM03	1st Drainage East of Smallin	1/20/23 0820	2/3/23 0925	ND		ND		ND		ND	
G2815	SM03	1st Drainage East of Smallin	2/3/23 0925	2/10/23 0915	ND		ND		ND		ND	
G2909	SM03	1st Drainage East of Smallin	2/10/23 0915	2/17/23 0840	ND		ND		ND		ND	
G2994	SM03	1st Drainage East of Smallin	2/17/23 0840	2/24/23 0855	ND		ND		ND		ND	



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Smallin Cave Recharge Area Delineation

City of Ozark

OUL Number	Station Number	Station Name	Date/Time Placed	Date/Time Collected	Fluorescein		Eosine		RWT		SRB	
					Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)
G3282	SM03	1st Drainage East of Smallin	2/24/23 0855	3/10/23 0825	ND		ND		ND		ND	
G3728	SM03	1st Drainage East of Smallin	3/10/23 0825	3/23/23 0905	ND		ND		ND		ND	
G4092	SM03	1st Drainage East of Smallin	3/23/23 0905	3/30/23 0751	ND		ND		ND		ND	
G4513	SM03	1st Drainage East of Smallin	3/30/23 0751	4/6/23 0740	ND		ND		ND		ND	
G5214	SM03	1st Drainage East of Smallin	4/6/23 0740	5/4/23 1355	ND		ND		ND		ND	
G5318	SM03	1st Drainage East of Smallin	5/4/23 1355	5/11/23 0742	ND		ND		ND		ND	
G5427	SM03	1st Drainage East of Smallin	5/11/23 0742	5/19/23 0739	ND		ND		ND		ND	
G2138	SM04	2nd Drainage East of Smallin	12/21/22 0915	1/4/23 1230	ND		ND		ND		ND	
G2552	SM04	2nd Drainage East of Smallin	1/4/23 1230	1/20/23 0835	ND		ND		ND		ND	
G2657	SM04	2nd Drainage East of Smallin	1/20/23 0835	2/3/23 0935	ND		ND		ND		ND	
G2816	SM04	2nd Drainage East of Smallin	2/3/23 0935	2/10/23 0922	ND		ND		ND		ND	
G2910	SM04	2nd Drainage East of Smallin	2/10/23 0922	2/17/23 0855	ND		ND		ND		ND	
G2995	SM04	2nd Drainage East of Smallin	2/17/23 0855	2/24/23 0900	ND		ND		ND		ND	
G3283	SM04	2nd Drainage East of Smallin	2/24/23 0900	3/10/23 0832	ND		ND		ND		ND	
G3729	SM04	2nd Drainage East of Smallin	3/10/23 0832	3/23/23 0915	ND		ND		ND		ND	
G4093	SM04	2nd Drainage East of Smallin	3/23/23 0915	3/30/23 0805	ND		ND		ND		ND	
G4514	SM04	2nd Drainage East of Smallin	3/30/23 0805	4/6/23 0749	ND		ND		ND		ND	
G5215	SM04	2nd Drainage East of Smallin	4/6/23 0749	5/4/23 1350	ND		ND		ND		ND	
G5319	SM04	2nd Drainage East of Smallin	5/4/23 1350	5/11/23 0753	ND		ND		ND		ND	
G5428	SM04	2nd Drainage East of Smallin	5/11/23 0753	5/19/23 0748	ND		ND		ND		ND	
G2139	SM05	3rd Drainage East of Smallin	12/21/22 0925	1/4/23 1210	ND		ND		ND		ND	
G2553	SM05	3rd Drainage East of Smallin	1/4/23 1210	1/20/23 0852	ND		ND		ND		ND	
G2658	SM05	3rd Drainage East of Smallin	1/20/23 0852	2/3/23 0945	ND		ND		ND		ND	
G2817	SM05	3rd Drainage East of Smallin	2/3/23 0945	2/10/23 0933	ND		ND		ND		ND	
G2911	SM05	3rd Drainage East of Smallin	2/10/23 0933	2/17/23 0905	ND		ND		ND		ND	
G2996	SM05	3rd Drainage East of Smallin	2/17/23 0905	2/24/23 0908	ND		ND		ND		ND	
G3284	SM05	3rd Drainage East of Smallin	2/24/23 0908	3/10/23 0845	ND		ND		ND		ND	
G3730	SM05	3rd Drainage East of Smallin	3/10/23 0845	3/23/23 0924	ND		ND		ND		ND	
	SM05	3rd Drainage East of Smallin	3/23/23 0924	3/30/23 0815	waso							
G4515	SM05	3rd Drainage East of Smallin	3/30/23 0815	4/6/23 0730	ND		ND		ND		ND	
G5216	SM05	3rd Drainage East of Smallin	4/6/23 0730	5/4/23 1335	ND		ND		ND		ND	
G5321	SM05	3rd Drainage East of Smallin	5/4/23 1335	5/11/23 0725	ND		ND		ND		ND	
G5429	SM05	3rd Drainage East of Smallin	5/11/23 0725	5/19/23 0725	ND		ND		ND		ND	
G2141	SM06	Spring on Parched Corn Hollow	12/21/22 0939	1/4/23 1200	ND		ND		ND		ND	
G2554	SM06	Spring on Parched Corn Hollow	1/4/23 1200	1/20/23 0903	ND		ND		567.0	42.7	ND	
G2659	SM06	Spring on Parched Corn Hollow	1/20/23 0903	2/3/23 0835	515.5	31.1	ND		567.7	618	ND	



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OUL Number	Station Number	Station Name	Date/Time Placed	Date/Time Collected	Fluorescein		Eosine		RWT		SRB	
					Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)
G2818	SM06	Spring on Parched Corn Hollow	2/3/23 0835	2/10/23 0820	515.5	14.8	ND		565.8	33.2	ND	
G2912	SM06	Spring on Parched Corn Hollow	2/10/23 0820	2/17/23 0825	515.3	11.9	ND		564.7	7.98	ND	
G2997	SM06	Spring on Parched Corn Hollow	2/17/23 0825	2/24/23 0810	515.2	1.63	ND		566.0	8.73	ND	
G3285	SM06	Spring on Parched Corn Hollow	2/24/23 0810	3/10/23 0735	514.4	0.809	ND		564.8	7.52	ND	
G3731	SM06	Spring on Parched Corn Hollow	3/10/23 0735	3/23/23 0932	514.4 **	0.305	ND		ND		ND	
G4094	SM06	Spring on Parched Corn Hollow	3/23/23 0932	3/30/23 0825	ND		ND		ND		ND	
G4516	SM06	Spring on Parched Corn Hollow	3/30/23 0825	4/6/23 0721	ND		ND		ND		ND	
G5217	SM06	Spring on Parched Corn Hollow	4/6/23 0721	5/4/23 1327	ND		ND		ND		ND	
G5322	SM06	Spring on Parched Corn Hollow	5/4/23 1327	5/11/23 0706	ND		541.4	255	567.4	614	ND	
G5430	SM06	Spring on Parched Corn Hollow	5/11/23 0706	5/19/23 0715	ND		541.2	54.9	568.4 **	10.9	ND	
G2142	SM07	Finley River Upstream of Parched Corn	12/21/22 1000	1/4/23 1150	ND		ND		ND		ND	
G2555	SM07	Finley River Upstream of Parched Corn	1/4/23 1150	1/20/23 0910	ND		ND		ND		ND	
G2661	SM07	Finley River Upstream of Parched Corn	1/20/23 0910	2/3/23 0820	ND		ND		ND		ND	
G3007	SM07	Finley River Upstream of Parched Corn	2/3/23 0820	2/24/23 0805	ND		ND		ND		ND	
G3738	SM07	Finley River Upstream of Parched Corn	2/24/23 0805	3/23/23 0941	ND		ND		ND		ND	
G4095	SM07	Finley River Upstream of Parched Corn	3/23/23 0941	3/30/23 0845	ND		ND		ND		ND	
G4517	SM07	Finley River Upstream of Parched Corn	3/30/23 0845	4/6/23 0715	ND		ND		ND		ND	
G5218	SM07	Finley River Upstream of Parched Corn	4/6/23 0715	5/4/23 1318	ND		ND		ND		ND	
G5431	SM07	Finley River Upstream of Parched Corn	5/4/23 1318	5/19/23 0705	ND		539.7	0.437	ND		ND	
G2143	SM08	Parched Corn Hollow	12/21/22 1005	1/4/23 1145	ND		ND		ND		ND	
G2556	SM08	Parched Corn Hollow	1/4/23 1145	1/20/23 0915	ND		ND		ND		ND	
G2662	SM08	Parched Corn Hollow	1/20/23 0915	2/3/23 0825	513.6 **	0.553	ND		566.2	14.0	ND	
G2819	SM08	Parched Corn Hollow	2/3/23 0825	2/10/23 0810	514.4	1.06	ND		566.0	4.72	ND	
G2913	SM08	Parched Corn Hollow	2/10/23 0810	2/17/23 0815	514.4	1.91	ND		ND		ND	
G2998	SM08	Parched Corn Hollow	2/17/23 0815	2/24/23 0755	ND		ND		ND		ND	
G3743	SM08	Parched Corn Hollow	2/24/23 0755	3/23/23 0938	ND		ND		ND		ND	
	SM08	Parched Corn Hollow	3/23/23 0938	3/30/23 0830	was							
G4518	SM08	Parched Corn Hollow	3/30/23 0830	4/6/23 0710	ND		ND		ND		ND	
G5219	SM08	Parched Corn Hollow	4/6/23 0710	5/4/23 1315	ND		ND		ND		ND	
G5432	SM08	Parched Corn Hollow	5/4/23 1315	5/19/23 0658	ND		541.3	4.52	566.2	7.07	ND	
G2144	SM09	Smallin Branch	12/21/22 1015	1/4/23 1250	ND		ND		ND		ND	
G2557	SM09	Smallin Branch	1/4/23 1250	1/20/23 0755	ND		ND		ND		ND	
G2663	SM09	Smallin Branch	1/20/23 0755	2/3/23 0907	ND		ND		ND		ND	
G2821	SM09	Smallin Branch	2/3/23 0907	2/10/23 0855	ND		ND		565.4	5.04	ND	
G2914	SM09	Smallin Branch	2/10/23 0855	2/17/23 1012	ND		ND		567.1	39.5	ND	
G2999	SM09	Smallin Branch	2/17/23 1012	2/24/23 0835	ND		ND		ND		573.8 **	21.4



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OUL Number	Station Number	Station Name	Date/Time Placed	Date/Time Collected	Fluorescein		Eosine		RWT		SRB	
					Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)
G3286	SM09	Smallin Branch	2/24/23 0835	3/10/23 0810	ND		ND		567.9	15.1	ND	
G3732	SM09	Smallin Branch	3/10/23 0810	3/23/23 1015	ND		ND		565.0	10.5	ND	
	SM09	Smallin Branch	3/23/23 1015	3/30/23 0738	wso							
G4519	SM09	Smallin Branch	3/30/23 0738	4/6/23 0838	ND		ND		ND		ND	
G5221	SM09	Smallin Branch	4/6/23 0838	5/4/23 1450	ND		ND		ND		ND	
G5324	SM09	Smallin Branch	5/4/23 1450	5/11/23 0822	ND		542.6	194	567.8	2,220	ND	
	SM09	Smallin Branch	5/11/23 0822	5/19/23 0837	wso							
G2145	SM10	McClellan Spring	12/21/22 1030	1/4/23 1335	ND		ND		ND		ND	
G2558	SM10	McClellan Spring	1/4/23 1335	1/20/23 1015	ND		ND		ND		ND	
G2664	SM10	McClellan Spring	1/20/23 1015	2/3/23 1023	ND		ND		ND		ND	
G2822	SM10	McClellan Spring	2/3/23 1023	2/10/23 1040	ND		ND		ND		ND	
G2915	SM10	McClellan Spring	2/10/23 1040	2/17/23 0958	ND		ND		ND		ND	
G3001	SM10	McClellan Spring	2/17/23 0958	2/24/23 1005	ND		ND		ND		ND	
G3287	SM10	McClellan Spring	2/24/23 1005	3/10/23 0955	ND		ND		ND		ND	
G3733	SM10	McClellan Spring	3/10/23 0955	3/23/23 1115	ND		ND		ND		ND	
G4096	SM10	McClellan Spring	3/23/23 1115	3/30/23 1010	ND		ND		ND		ND	
G4521	SM10	McClellan Spring	3/30/23 1010	4/6/23 0912	ND		ND		ND		ND	
G5222	SM10	McClellan Spring	4/6/23 0912	5/4/23 1437	ND		ND		ND		ND	
G5325	SM10	McClellan Spring	5/4/23 1437	5/11/23 0935	ND		ND		ND		ND	
G2146	SM11	Seep Downstream Smallin Cave	12/21/22 1050	1/4/23 1350	ND		ND		ND		ND	
G2559	SM11	Seep Downstream Smallin Cave	1/4/23 1350	1/20/23 1025	ND		ND		ND		ND	
G2665	SM11	Seep Downstream Smallin Cave	1/20/23 1025	2/3/23 1034	ND		ND		ND		ND	
G2823	SM11	Seep Downstream Smallin Cave	2/3/23 1034	2/10/23 1055	ND		ND		ND		ND	
G2916	SM11	Seep Downstream Smallin Cave	2/10/23 1055	2/17/23 1006	ND		ND		566.4	8.41	ND	
G3002	SM11	Seep Downstream Smallin Cave	2/17/23 1006	2/24/23 0957	ND		ND		ND		572.5 **	14.6
G3288	SM11	Seep Downstream Smallin Cave	2/24/23 0957	3/10/23 1010	ND		ND		567.5	14.0	ND	
G3734	SM11	Seep Downstream Smallin Cave	3/10/23 1010	3/23/23 1130	ND		ND		566.5	3.25	ND	
G4097	SM11	Seep Downstream Smallin Cave	3/23/23 1130	3/30/23 1020	ND		ND		ND		ND	
G4522	SM11	Seep Downstream Smallin Cave	3/30/23 1020	4/6/23 0940	ND		ND		ND		ND	
G5223	SM11	Seep Downstream Smallin Cave	4/6/23 0940	5/4/23 1425	ND		ND		566.8 *	2.43	ND	
G5326	SM11	Seep Downstream Smallin Cave	5/4/23 1425	5/11/23 0911	ND		542.2	90.7	567.6	1,040	ND	
G5433	SM11	Seep Downstream Smallin Cave	5/11/23 0911	5/19/23 0850	515.6	70.5	541.2	279	567.8	1,250	ND	
G2147	SM12	Cave Spring	12/21/22 1110	1/4/23 1305	ND		ND		ND		ND	
G2561	SM12	Cave Spring	1/4/23 1305	1/20/23 0750	ND		ND		ND		ND	
G2666	SM12	Cave Spring	1/20/23 0750	2/3/23 0859	ND		ND		ND		ND	
G2824	SM12	Cave Spring	2/3/23 0859	2/10/23 0847	ND		ND		ND		ND	



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OUL Number	Station Number	Station Name	Date/Time Placed	Date/Time Collected	Fluorescein		Eosine		RWT		SRB	
					Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)
G2917	SM12	Cave Spring	2/10/23 0847	2/17/23 1022	ND		ND		ND		ND	
G3003	SM12	Cave Spring	2/17/23 1022	2/24/23 0830	ND		ND		ND		ND	
G3289	SM12	Cave Spring	2/24/23 0830	3/10/23 0805	ND		ND		ND		ND	
G3735	SM12	Cave Spring	3/10/23 0805	3/23/23 1012	ND		ND		ND		ND	
G4098	SM12	Cave Spring	3/23/23 1012	3/30/23 0715	ND		ND		568.4	1,800	ND	
G4523	SM12	Cave Spring	3/30/23 0715	4/6/23 0821	ND		ND		567.7	179	ND	
G5224	SM12	Cave Spring	4/6/23 0821	5/4/23 1505	ND		ND		567.0	166	ND	
G5327	SM12	Cave Spring	5/4/23 1505	5/11/23 1011	ND		ND		567.1	54.8	ND	
G5434	SM12	Cave Spring	5/11/23 1011	5/19/23 0819	ND		ND		565.7	12.8	ND	
G2148	SM13	Jeff Cave	12/21/22 1130	1/4/23 1320	ND		ND		ND		ND	
G2562	SM13	Jeff Cave	1/4/23 1320	1/20/23 0950	ND		ND		ND		ND	
G2667	SM13	Jeff Cave	1/20/23 0950	2/3/23 1000	ND		ND		ND		ND	
G2825	SM13	Jeff Cave	2/3/23 1000	2/10/23 0952	ND		ND		ND		ND	
G2918	SM13	Jeff Cave	2/10/23 0952	2/17/23 0920	ND		ND		ND		ND	
G3004	SM13	Jeff Cave	2/17/23 0920	2/24/23 0922	ND		ND		ND		ND	
G3290	SM13	Jeff Cave	2/24/23 0922	3/10/23 0901	ND		ND		ND		ND	
G3736	SM13	Jeff Cave	3/10/23 0901	3/23/23 0850	ND		ND		ND		ND	
	SM13	Jeff Cave	3/23/23 0850	3/30/23 0930	ns							
G4526	SM13	Jeff Cave	3/30/23 0930	4/6/23 0805	ND		ND		ND		ND	
G5323	SM13	Jeff Cave	4/6/23 0805	5/11/23 0950	ND		ND		ND		ND	
G5437	SM13	Jeff Cave	5/11/23 0950	5/19/23 0803	ND		ND		ND		ND	
G2149	SM14	2nd Drainage West of Smallin	12/21/22 1215	1/4/23 1325	ND		ND		ND		ND	
G2563	SM14	2nd Drainage West of Smallin	1/4/23 1325	1/20/23 0745	ND		ND		ND		ND	
G2668	SM14	2nd Drainage West of Smallin	1/20/23 0745	2/3/23 0853	ND		ND		ND		ND	
G2826	SM14	2nd Drainage West of Smallin	2/3/23 0853	2/10/23 0840	ND		ND		ND		ND	
G2919	SM14	2nd Drainage West of Smallin	2/10/23 0840	2/17/23 1018	ND		ND		ND		ND	
G3005	SM14	2nd Drainage West of Smallin	2/17/23 1018	2/24/23 0825	ND		ND		ND		ND	
G3291	SM14	2nd Drainage West of Smallin	2/24/23 0825	3/10/23 0756	ND		ND		ND		ND	
G3737	SM14	2nd Drainage West of Smallin	3/10/23 0756	3/23/23 1006	ND		ND		ND		ND	
	SM14	2nd Drainage West of Smallin	3/23/23 1006	3/30/23 0732	was							
G4524	SM14	2nd Drainage West of Smallin	3/30/23 0732	4/6/23 0815	ND		ND		ND		ND	
G5225	SM14	2nd Drainage West of Smallin	4/6/23 0815	5/4/23 1500	ND		ND		ND		ND	
G5328	SM14	2nd Drainage West of Smallin	5/4/23 1500	5/11/23 0815	ND		ND		ND		ND	
G5435	SM14	2nd Drainage West of Smallin	5/11/23 0815	5/19/23 0825	ND		ND		ND		ND	
G2150	SM15	Finley River Downstream of Riverside Park	12/21/22 1230	1/4/23 1442	ND		ND		ND		ND	
G2564	SM15	Finley River Downstream of Riverside Park	1/4/23 1442	1/20/23 0740	ND		ND		ND		ND	



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OUL Number	Station Number	Station Name	Date/Time Placed	Date/Time Collected	Fluorescein		Eosine		RWT		SRB	
					Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)
G2669	SM15	Finley River Downstream of Riverside Park	1/20/23 0740	2/3/23 0848	ND		ND		ND		ND	
G3006	SM15	Finley River Downstream of Riverside Park	2/3/23 0848	2/24/23 0820	ND		ND		ND		ND	
G3739	SM15	Finley River Downstream of Riverside Park	2/24/23 0820	3/23/23 1002	ND		ND		ND		ND	
G4099	SM15	Finley River Downstream of Riverside Park	3/23/23 1002	3/30/23 0725	ND		ND		ND		ND	
G4525	SM15	Finley River Downstream of Riverside Park	3/30/23 0725	4/6/23 0833	ND		ND		ND		ND	
G5226	SM15	Finley River Downstream of Riverside Park	4/6/23 0833	5/4/23 1455	ND		ND		ND		ND	
G5436	SM15	Finley River Downstream of Riverside Park	5/4/23 1455	5/19/23 0832	515.0	0.629	540.8	0.962	ND		ND	
G2565	SM16	Camp Cora Spring	12/31/22 1215	1/11/23 1400	ND		ND		ND		ND	
G3741	SM16	Camp Cora Spring	1/11/23 1400	3/23/23 0823	ND		ND		ND		ND	
G2566	SM17	Winoka Spring	12/31/22 1245	1/11/23 1425	ND		ND		ND		ND	
G3742	SM17	Winoka Spring	1/11/23 1425	3/23/23 0755	ND		ND		ND		ND	

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Smallin Cave Recharge Area Delineation

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Table 2. Results for water samples analyzed for the presence of fluorescein, eosine, rhodamine WT (RWT) and sulforhodamine B (SRB) dyes.
Peak wavelengths are reported in nanometers (nm); dye concentrations are reported in parts per billion (ppb).

OUL Number	Station Number	Station Name	Date/Time Collected	Fluorescein		Eosine		RWT		SRB	
				Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)
G2209	SM01	Smallin Cave	12/14/22 0950	ND		ND		ND		ND	
G2828	SM01	Smallin Cave	2/10/23 1025	ND		ND		ND		ND	
G2957	SM01	Smallin Cave	2/17/23 0950	ND		ND		ND		ND	
G3015	SM01	Smallin Cave	2/24/23 0945	ND		ND		ND		ND	
G3429	SM01	Smallin Cave	3/10/23 0920	ND		ND		ND		ND	
G3772	SM01	Smallin Cave	3/23/23 1035	ND		ND		ND		ND	
G4129	SM01	Smallin Cave	3/30/23 0950	ND		ND		ND		ND	
G5248	SM01	Smallin Cave	5/4/23 1425	ND		ND		ND		ND	
G5329	SM01	Smallin Cave	5/11/23 0843	ND		534.3	38.1	572.4	3.25	ND	
G5438	SM01	Smallin Cave	5/19/23 0920	ND		535.0	0.383	575.4 (1)	0.089	ND	
G2210	SM02	Fielden Cave	12/14/22 1000	ND		ND		ND		ND	
G5439	SM02	Fielden Cave	5/19/23 0912	507.5	0.195	ND		ND		ND	
G2206	SM04	2nd Drainage East of Smallin	1/4/23 1230	ND		ND		ND		ND	
G4130	SM05	3rd Drainage East of Smallin	3/30/23 0815	ND		ND		ND		ND	
G5249	SM05	3rd Drainage East of Smallin	5/4/23 1335	ND		ND		ND		ND	
G2567	SM06	Spring on Parched Corn Hollow	1/20/23 0903	ND		ND		573.7	1.82	ND	
G2709	SM06	Spring on Parched Corn Hollow	2/3/23 0835	506.2 (1)	0.011	ND		ND		ND	
G2829	SM06	Spring on Parched Corn Hollow	2/10/23 0820	507.6	0.035	ND		ND		ND	
G2958	SM06	Spring on Parched Corn Hollow	2/17/23 0825	ND		ND		ND		ND	
G3016	SM06	Spring on Parched Corn Hollow	2/24/23 0810	ND		ND		ND		ND	
G3430	SM06	Spring on Parched Corn Hollow	3/10/23 0735	ND		ND		ND		ND	
G5330	SM06	Spring on Parched Corn Hollow	5/11/23 0706	ND		534.3	5.00	ND		ND	
G5441	SM06	Spring on Parched Corn Hollow	5/19/23 0715	ND		536.0 (1)	0.062	ND		ND	
G5442	SM07	Finley River Upstream of Parched Corn	5/19/23 0705	ND		ND		ND		ND	
G2710	SM08	Parched Corn Hollow	2/3/23 0825	ND		ND		ND		ND	
G2830	SM08	Parched Corn Hollow	2/10/23 0810	ND		ND		ND		ND	
G2959	SM08	Parched Corn Hollow	2/17/23 0815	ND		ND		ND		ND	
G4131	SM08	Parched Corn Hollow	3/30/23 0830	ND		ND		ND		ND	
G5443	SM08	Parched Corn Hollow	5/19/23 0658	ND		ND		ND		ND	
G2831	SM09	Smallin Branch	2/10/23 0855	ND		ND		ND		ND	
G2961	SM09	Smallin Branch	2/17/23 1012	ND		ND		ND		ND	
G3017	SM09	Smallin Branch	2/24/23 0835	ND		ND		ND		ND	
G3431	SM09	Smallin Branch	3/10/23 0810	ND		ND		ND		ND	
G3773	SM09	Smallin Branch	3/23/23 1015	ND		ND		ND		ND	



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OUL Number	Station Number	Station Name	Date/Time Collected	Fluorescein		Eosine		RWT		SRB	
				Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)	Peak (nm)	Conc (ppb)
G4132	SM09	Smallin Branch	3/30/23 0738	ND		ND		ND		ND	
G5331	SM09	Smallin Branch	5/11/23 0822	ND		534.1	32.0	573.2	2.79	ND	
G5444	SM09	Smallin Branch	5/19/23 0837	ND		536.6	0.536	570.4 **	0.605	ND	
G2962	SM11	Seep Downstream Smallin Cave	2/17/23 1006	ND		ND		ND		ND	
G3018	SM11	Seep Downstream Smallin Cave	2/24/23 0957	ND		ND		ND		ND	
G3432	SM11	Seep Downstream Smallin Cave	3/10/23 1010	ND		ND		ND		ND	
G3774	SM11	Seep Downstream Smallin Cave	3/23/23 1130	ND		ND		ND		ND	
G5332	SM11	Seep Downstream Smallin Cave	5/11/23 0911	ND		534.2	3.18	573.8	5.37	ND	
G5445	SM11	Seep Downstream Smallin Cave	5/19/23 0850	ND		535.7	0.387	572.9	1.09	ND	
G4133	SM12	Cave Spring	3/30/23 0715	ND		ND		574.9	0.097	ND	
G4527	SM12	Cave Spring	4/6/23 0821	ND		ND		571.0 (1)	0.116	ND	
G5250	SM12	Cave Spring	5/4/23 1505	ND		ND		ND		ND	
G5333	SM12	Cave Spring	5/11/23 1011	ND		ND		ND		ND	
G5446	SM12	Cave Spring	5/19/23 0819	ND		ND		ND		ND	
G4134	SM14	2nd Drainage West of Smallin	3/30/23 0732	ND		ND		ND		ND	
G5447	SM15	Finley River Downstream of Riverside Park	5/19/23 0832	ND		ND		ND		ND	
G2207	SM16	Camp Cora Spring	12/31/22 1215	ND		ND		ND		ND	
G2208	SM17	Winoka Spring	12/31/22 1245	ND		ND		ND		ND	



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Footnotes:

ND = No dye detected

NT = No time given

NDT = No date or time given

* = A fluorescence peak is present that does not meet all the criteria for a positive dye result. However, it has been calculated as though it was the tracer dye.

** = A fluorescence peak is present that does not meet all the criteria for this dye. However, it has been calculated as a positive dye result.

(1) = A fluorescence peak is present that does not meet all the criteria for this dye. However, it has been calculated as a positive dye result because dye is present in the corresponding charcoal sampler.

wso = A water sample only was collected for this time period.

ns = No samples were collected for this time period.

Appendix B:

Ozark Underground Laboratory Procedures and Criteria Document

**PROCEDURES AND CRITERIA
ANALYSIS OF FLUORESCENT
DYES
IN WATER AND CHARCOAL SAMPLERS:**

**FLUORESCEIN, EOSINE, RHODAMINE
WT, AND SULFORHODAMINE B DYES**

Revision Dates:

March 3, 2015

Pages A-14 to A-18 corrected to match Table 4 on **December 27, 2018**

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TRACER DYES AND SAMPLE TYPES

Dye Nomenclature

Dye manufacturers and retailers use a myriad of names for the dyes. This causes confusion among dye users and report readers. The primary dyes used at the OUL and described in this document are included in Table 1 below.

Table 1. Primary OUL Dye Nomenclature.

OUL Common Name	Color Index Number	Color Index Name	Other Names
Fluorescein	45350	Acid Yellow 73	uranine, uranine C, sodium fluorescein, fluorescein LT and fluorescent yellow/green
Eosine	45380	Acid Red 87	eosin, eosine OJ, and D&C Red 22
Rhodamine WT	None assigned	Acid Red 388	fluorescent red (but not the same as rhodamine B)
Sulforhodamine B	45100	Acid Red 52	pontacyl brilliant pink B, lissamine red 4B, and fluoro brilliant pink

The OUL routinely provides dye for tracing projects. Dyes purchased for groundwater tracing are always mixtures that contain both dye and an associated diluent. Diluents enable the manufacturer to standardize the dye mixture so that there are minimal differences among batches. Additionally, diluents are often designed to make it easier to dissolve the dye mixture in water, or to produce a product which meets a particular market need (groundwater tracing is only a tiny fraction of the dye market). The percent of dye in “as-sold” dye mixtures often varies dramatically among manufacturers and retailers, and retailers are sometimes incorrect about the percent of dye in their products. The OUL subjects all of its dyes to strict quality control (QC) testing. Table 2 summarizes the as-sold dye mixtures used by the OUL.

Table 2. As-Sold Dye Mixtures at the OUL.

OUL Common Name	Form	Dye Equivalent
Fluorescein	Powder	75% dye equivalent, 25% diluent
Eosine	Powder	75% dye equivalent, 25% diluent
Rhodamine WT	Liquid	20% dye equivalent, 80% diluent
Sulforhodamine B	Powder	75% dye equivalent, 25% diluent

Analytical results are based on the as-sold weights of the dyes provided by the OUL. The use of dyes from other sources is discouraged due to the wide variability of dye equivalents within the market. However, if alternate source dyes are used, a sample should be provided to the OUL for quality control and to determine if a correction factor is necessary for the analytical results.

Types of Samples

Typical samples that are collected for fluorescent tracer dye analysis include charcoal samplers (also called activated carbon or charcoal packets) and water samples.

The charcoal samplers are packets of fiberglass screening partially filled with 4.25 grams of activated coconut charcoal. The charcoal used by the OUL is Calgon 207C coconut shell carbon, 6 to 12 mesh, or equivalent. The most commonly used charcoal samplers are about 4 inches long by 2 inches wide. A cigar-shaped sampler is made for use in very small diameter wells (such as 1-inch diameter piezometers); this is a special order item and should be specifically requested in advance when needed. All of the samplers are closed by heat sealing.

In specialized projects, soil samples have been collected from soil cores and analyzed for fluorescent tracer dyes. Project-specific procedures have been developed for projects such as these. For additional information, please contact the OUL.

INTRODUCTION

This document describes standard procedures and criteria currently in use at the Ozark Underground Laboratory (OUL) as of the date shown on the title page. Some samples may be subjected to different procedures and criteria because of unique conditions; such non-standard procedures and criteria are identified in reports for those samples. Standard procedures and criteria change as knowledge and experience increases and as equipment is improved or upgraded. The OUL maintains a summary of changes in standard procedures and criteria.

FIELD PROCEDURES

Field procedures included in this section are intended as guidance, and not firm requirements. Placement of samplers and other field procedures require adjustment to field conditions. Personnel at the OUL are available to provide additional assistance for implementation of field procedures specific to specialized field conditions.

Placement of Samplers

Charcoal samplers are placed so as to be exposed to as much water as possible. Water should flow through the packet. In springs and streams they are typically attached to a rock or other anchor in a riffle area. Attachment of the packets often uses plastic tie wires. In swifter water galvanized wire (such as electric fence wire) is often used. Other types of anchoring wire can be used. Electrical wire with plastic insulation is also good. Packets are attached so that they extend outward from the anchor rather than laying flat against it. Two or more separately anchored packets are typically used for sampling springs and streams. The placement of multiple packets is recommended in order to minimize the chance of loss during the sampling period. The use of fewer packets is discouraged except when the spring or stream is so small that there is not appropriate space for placing multiple packets.

When pumping wells are being sampled, the samplers are typically placed in sample holders made of plastic pipe fittings. Brass hose fittings can be at the end of the sample holders so that the sample holders can be installed on outside hose bibs and water which has run through the samplers can be directed to waste through a connected garden hose. The samplers can be unscrewed in the middle so that charcoal packets can be changed. The middle portions of the samplers consist of 1.5 inch diameter pipe and pipe fitting.

Charcoal packets can be lowered into monitoring wells for sampling purposes. In general, if the well is screened, samplers should be placed approximately in the middle of the screened interval. Due to the typically lower volume of water that flows through a well, only one charcoal sampler should be used per well. However, multiple packets can be placed in a single well at depths to test different depth horizons when desirable. A weight should be added near the charcoal packet to ensure that it will not float. The weight should be of such a nature that it will not affect water quality. One common approach is to anchor the packets with a white or uncolored plastic cable tie to the top of a dedicated weighted disposable bailer. We typically run nylon cord from the top of the well to the charcoal packet and its weight. ***Do not use colored cord*** since some of them are colored with fluorescent dyes. Nylon fishing line should not be used since it can be readily cut by a sharp projection in the well.

In some cases, especially with small diameter wells and appreciable well depths, the weighted disposable bailers sink very slowly or may even fail to sink because of friction and floating of the anchoring cord. In such cases a weight may be added to the top of the disposable bailer. Stainless steel weights are ideal, but are not needed in all cases. All weights should be cleaned prior to use; the cleaning approach should comply with decontamination procedures in use at the project site.

Optional Preparation of Charcoal Samplers

Charcoal packets routinely contain some fine powder that washes off rapidly when they are placed in water. While not usually necessary, the following optional preparation step is suggested if the fine charcoal powder is problematic.

Charcoal packets can be triple rinsed with distilled, demineralized, or reagent water known to be free of tracer dyes. This rinsing is typically done by soaking. With this approach,

approximately 25 packets are placed in one gallon of water and soaked for at least 10 minutes. The packets are then removed from the water and excess water is shaken off the packets. The packets are then placed in a second gallon of water and again soaked for at least 10 minutes. After this soaking they are removed from the water and excess water is shaken off the packets. The packets are then placed in a third gallon of water and the procedure is again repeated. Rinsed packets are placed in plastic bags and are placed at sampling stations within three days. Packets can also be rinsed in jets of water for about one minute; this requires more water and is typically difficult to do in the field with water known to be free of tracer dyes.

Collection and Replacement of Samplers

Samplers are routinely collected and replaced at each of the sampling stations. The frequency of sampler collection and replacement is determined by the nature of the study. Collections at one week intervals are common, but shorter or longer collection frequencies are acceptable and sometimes more appropriate. Shorter sampling frequencies are often used in the early phases of a study to better characterize time of travel. As an illustration, we often collect and change charcoal packets 1, 2, 4, and 7 days after dye injection. Subsequent sampling is then weekly.

The sampling interval in wells at hazardous wastes sites should generally be no longer than about a week. Contaminants in the water can sometimes use up sorption sites on the charcoal that would otherwise adsorb the dye. This is especially important if the dye might pass in a relatively short duration pulse.

Where convenient, the collected samplers should be briefly rinsed in the water being sampled to remove dirt and accumulated organic material. This is not necessary with well samples. The packets are shaken to remove excess water. Next, the packet (or packets) are placed in a plastic bag (Whirl-Pak® bags are ideal). The bag is labeled on the outside with a black permanent type felt marker pen, such as a Sharpie®. ***Use only pens that have black ink;*** colored inks may contain fluorescent dyes. The notations include station name or number and the date and time of collection. Labels must not be inserted inside the sample bags.

Collected samplers are kept in the dark to minimize algal growth on the charcoal prior to analysis work. New charcoal samplers are routinely placed when used charcoal packets are collected. The last set of samplers placed at a stream or spring is commonly not collected.

Water Samples

Water samples are often collected. They should be collected in either glass or plastic; the OUL routinely uses 50 milliliter (mL) research-grade polypropylene copolymer Perfector Scientific vials (Catalog Number 2650) for such water samples. No more than 30 mL of water is required for analysis. The sides of the vials should be labeled with the project name, sample ID, sample date and time with a black permanent felt tip pen. ***Do not label the lid only.*** The vials should be placed in the dark and refrigerated immediately after collection, and maintained under refrigeration until shipment. The OUL supplies vials for the collection of water samples.

Sample Shipment

When water or charcoal samplers are collected for shipment to the OUL they should be shipped promptly. We prefer (and in some studies require) that samples be refrigerated with frozen re-usable ice packs upon collection and that they be shipped refrigerated with frozen ice packs by overnight express. ***Do not ship samplers packed in wet ice*** since this can create a potential for cross contamination when the ice melts. Our experience indicates that it is not essential for samplers to be maintained under refrigeration; yet maintaining them under refrigeration clearly minimizes some potential problems. A product known as "green ice" should not be used for maintaining the samples in a refrigerated condition since this product contains a dye which could contaminate samples if the "green ice" container were to break or leak.

We receive good overnight and second day air service from both UPS and FedEx. The U.S. Postal Service does not typically provide next day service to us. DHL does not provide overnight service to us. FedEx is recommended for international shipments. The OUL does not receive Saturday delivery.

Each shipment of charcoal samplers or water samples ***must be accompanied by a sample custody document***. The OUL provides a sheet (which bears the title "Samples for Fluorescence Analysis") that can be used if desired. These sheets can be augmented by a client's chain-of-custody forms or any other relevant documentation. OUL's custody document works well for charcoal samplers because it allows for both the placement date and time as well as the collection date and time. Many other standard chain-of-custody documents do not allow for these types of samples. Attachment 1 includes a copy of OUL's Sample Collection Data Sheet.

Please write legibly on the custody documents and ***use black ink***. Check the accuracy of the sample sheet against the samples prior to shipment to identify and correct errors that may delay the analysis of your samples following receipt at the laboratory.

Supplies Provided by the OUL

The OUL provides supplies for the collection of fluorescent tracer dyes. Supplies provided upon request are charcoal packets, Whirl-Pak® bags (to contain the charcoal packets after collection for shipment to the laboratory), and water vials. These supplies are subjected to strict QA/QC procedures to ensure the materials are free of any potential tracer dye contaminants. The charge for these materials is included in the cost of sample analysis. Upon request, coolers and re-freezable ice packs are also provided for return shipment of samples.

The OUL also has tracer dyes available for purchase. These dyes are subject to strict QA/QC testing. All analytical work is based upon the OUL as-sold weight of the dyes.

LABORATORY PROCEDURES

The following procedures are followed upon receipt of samples at the laboratory.

Receipt of Samples

Samplers shipped to the OUL are logged in and refrigerated upon receipt. Prior to cleaning and analysis, samplers are assigned a laboratory identification number.

It sometimes occurs that there are discrepancies between the sample collection data sheet and the actual samples received. When this occurs, a "Discrepancy Sheet" form is completed and sent to the shipper of the sample for resolution. The purpose of the form is to help resolve discrepancies, even when they may be minor. Many discrepancies arise from illegible custody documents. ***Please write legibly*** on the custody documents and ***use black ink***. Check the accuracy of the sample sheet against the samples prior to shipment to identify and correct errors that may delay the analysis of your samples following receipt at the laboratory.

Cleaning of Charcoal Samplers

Samplers are cleaned by spraying them with jets of clean water from a laboratory well in a carbonate aquifer. OUL uses non-chlorinated water for the cleansing to minimize dye deterioration. We do not wash samplers in public water supplies. Effective cleansing cannot generally be accomplished simply by washing in a conventional laboratory sink even if the sink is equipped with a spray unit.

The duration of packet washing depends upon the condition of the sampler. Very clean samplers may require less than a minute of washing; dirtier samplers may require several minutes of washing.

Elution of the Charcoal

There are various eluting solutions that can be used for the recovery of tracer dyes. The solutions typically include an alcohol, water, and a strong basic solution such as aqueous ammonia and /or potassium hydroxide.

The standard elution solution used at the OUL is a mixture of 5% aqua ammonia and 95% isopropyl alcohol solution and sufficient potassium hydroxide pellets to saturate the solution.

The isopropyl alcohol solution is 70% alcohol and 30% water. The aqua ammonia solution is 29% ammonia. The potassium hydroxide is added until a super-saturated layer is visible in the bottom of the container. This super-saturated layer is not used for elution. Preparation of eluting solutions uses dedicated glassware which is never used in contact with dyes or dye solutions.

The eluting solution will elute fluorescein, eosine, rhodamine WT, and sulforhodamine B dyes. It is also suitable for separating fluorescein peaks from peaks of some naturally present materials found in samplers.

Fifteen mL of the eluting solution is poured over the washed charcoal in a disposable sample beaker. The sample beaker is capped. The sample is allowed to stand for 60 minutes. After this time, the liquid is carefully poured off the charcoal into a new disposable beaker which has been appropriately labeled with the laboratory identification number. A few grains of charcoal may inadvertently pass into the second beaker; no attempt is made to remove these from the second sample beaker. After the pouring, a small amount of the elutant will remain in the initial sample beaker. After the transfer of the elutant to the second sample beaker, the contents of the first sample beaker (the eluted charcoal) are discarded. Samples are kept refrigerated until analyzed.

pH Adjustment of Water Samples

The fluorescence intensity of several of the commonly used fluorescent tracer dyes is pH dependent. The pH of samples analyzed for fluorescein, eosine, and pyranine dyes are adjust to a target pH of greater than 9.5 in order to obtain maximum fluorescence intensities.

Adjustment of pH is achieved by placing samples in a high ammonia atmosphere for at least two hours in order to increase the pH of the sample. Reagent water standards are placed in the same atmosphere as the samples. If dye concentrations in a sample are off-scale and require dilution for quantification of the dye concentration, the diluting water used is OUL reagent water that has been pH adjusted in a high ammonia atmosphere. Samples that are only analyzed for rhodamine WT or sulforhodamine B are not required to be pH adjusted.

Analysis on the Shimadzu RF-5301

The OUL uses a Shimadzu spectrofluorophotometer model RF-5301. This instrument is capable of synchronous scanning. The OUL also owns a Shimadzu RF-540 spectrofluorometers that is occasionally used for special purposes.

A sample of the elutant or water is withdrawn from the sample container using a disposable polyethylene pipette. Approximately 3 mL of the sample is then placed in disposable rectangular polystyrene cuvette. The cuvette has a maximum capacity of 3.5 mL. The cuvette is designed for fluorometric analysis; all four sides and the bottom are clear. The acceptable spectral range of these cuvettes is 340 to 800 nm. The pipettes and cuvettes are discarded after one use.

The cuvette is then placed in the RF-5301. This instrument is controlled by a programmable computer and operated by proprietary software developed for dye tracing applications.

Our instruments are operated and maintained in accordance with the manufacturer's recommendations. On-site installation of our first instrument and a training session on its use was provided by the instrument supplier. Repairs are made by a Shimadzu-authorized repairman.

Our typical analysis of an elutant sample where fluorescein, eosine, rhodamine WT, or

sulforhodamine B dyes may be present includes synchronous scanning of excitation and emission spectra with a 17 nm separation between excitation and emission wavelengths. For these dyes, the excitation scan is from 443 to 613 nm; the emission scan is from 460 to 630 nm. The emission fluorescence from the scan is plotted on a graph. The typical scan speed setting is "fast" on the RF-5301. The typical sensitivity setting used is "high."

Table 3. Excitation and emission slit width settings routinely used for dye analysis.

Parameter	Excitation Slit (nm)	Emission Slit (nm)
ES, FL, RWT, and SRB in elutant	3	1.5
ES, FL, RWT, and SRB in water	5	3

Note: ES = Eosine. FL = Fluorescein. RWT = Rhodamine WT. SRB = Sulforhodamine B.

The instrument produces a plot of the synchronous scan for each sample; the plot shows emission fluorescence only. The synchronous scans are subjected to computer peak picks using proprietary software; peaks are picked to the nearest 0.1 nm. Instrument operators have the ability to manually adjust peaks as necessary based upon computer-picked peaks and experience. All samples run on the RF-5301 are stored electronically with sample information. All samples analyzed are recorded in a bound journal.

Quantification

We calculate the magnitude of fluorescence peaks for fluorescein, eosine, rhodamine WT, and sulforhodamine B dyes in both elutant and water samples. Dye quantities are expressed in microgram per liter (parts per billion; ppb). The dye concentrations are calculated by separating fluorescence peaks due to dyes from background fluorescence on the charts, and then calculating the area within the fluorescence peak. This area is proportional to areas obtained from standard solutions.

We run dye concentration standards each day the RF-5301 is used. Six standards are used; the standard or standards appropriate for the analysis work being conducted are selected. All standards are based upon the as-sold weights of the dyes. The standards are as follows:

- 1) 10 ppb fluorescein and 100 ppb rhodamine WT in well water from the Jefferson City-Cotter Formation
- 2) 10 ppb eosine in well water from the Jefferson City-Cotter Formation
- 3) 100 ppb sulforhodamine B in well water from the Jefferson City-Cotter Formation.
- 4) 10 ppb fluorescein and 100 ppb rhodamine WT in elutant.
- 5) 10 ppb eosine in elutant.
- 6) 100 ppb sulforhodamine B in elutant.

Preparation of Standards

Dye standards are prepared as follows:

Step 1. A small sample of the as-sold dye is placed in a pre-weighed sample vial and the vial is again weighed to determine the weight of the dye. We attempt to use a sample weighing between 1 and 5 grams. This sample is then diluted with well water to make a 1% dye solution by weight (based upon the as-sold weight of the dye). The resulting dye solution is allowed to sit for at least four hours to ensure that all dye is fully dissolved.

Step 2. One part of each dye solution from Step 1 is placed in a mixing container with 99 parts of well water. Separate mixtures are made for fluorescein, rhodamine WT, eosine, and sulforhodamine B. The resulting solutions contain 100 mg/L dye (100 parts per million dye mixture). The typical prepared volume of this mixture is appropriate for the sample bottles being used; we commonly prepare about 50 mL of the Step 2 solutions. The dye solution from Step 1 that is used in making the Step 2 solution is withdrawn with a digital Finnpiquette which is capable of measuring volumes between 0.200 and 1.000 mL at intervals of 0.005 mL. The calibration certificate with this instrument indicates that the accuracy (in percent) is as follows:

At 0.200 mL, 0.90%

At 0.300 mL, 0.28%

At 1.000 mL, 0.30%

The Step 2 solution is called the long term standard. OUL experience indicates that Step 2 solutions, if kept refrigerated, will not deteriorate appreciably over periods of less than a year. Furthermore, these Step 2 solutions may last substantially longer than one year.

Step 3. A series of intermediate-term dye solutions are made. Approximately 45 mL of each intermediate-term dye solution is made. All volume measurements of less than 5 mL are made with a digital Finnpiquette. (see description in Step 2). All other volume measurements are made with Rheinland Kohn Geprüfte Sicherheit 50 mL capacity pump dispenser which will pump within plus or minus 1% of the set value. The following solutions are made; all concentrations are based on the as-sold weight of the dyes:

- 1) 1 ppm fluorescein dye and 10 ppm rhodamine WT dye.
- 2) 1 ppm eosine.
- 3) 10 ppm sulforhodamine B dye.

Step 4. A series of six short-term dye standards are made from solutions in Step 3. These standards were identified earlier in this section. In the experience of the OUL these standards have a useful shelf life in excess of one week. However, in practice, Step 4 elutant standards are made weekly, and Step 4 water standards are made daily.

Dilution of Samples

Samples with peaks that have arbitrary fluorescence unit values of 500 or more are diluted a

hundred fold to ensure accurate quantification.

Some water samples have high turbidity or color which interferes with accurate detection and measurement of dye concentrations. It is often possible to dilute these samples and then measure the dye concentration in the diluted sample.

The typical dilutions are either 10 fold (1:10) or 100 fold (1:100). A 1:10 dilution involves combining one part of the test sample with 9 parts of water (if the sample is water) or elutant (if the sample is elutant). A 1:100 dilution involves combining one part of the test sample is combined with 99 parts of water or elutant, based upon the sample media. Typically, 0.300 mL of the test solution is combined with 29.700 mL of water (or elutant as appropriate) to yield a new test solution.

All volume measurements of less than 5 mL are made with a digital Finnpiquette. All other volume measurements are made with Rheinland Kohn Geprüfte Sicherheit 50 mL capacity pump dispenser which will pump within plus or minus 1% of the set value.

The water used for dilution is from a carbonate aquifer. All dilution water is pH adjusted to greater than pH 9.5 by holding it in open containers in a high ammonia concentration chamber. This adjustment takes a minimum of two hours.

Quality Control

Laboratory blanks are run for every sample where the last two digits of the laboratory numbers are 00, 20, 40, 60, or 80. A charcoal packet is placed in a pumping well sampler and at least 25 gallons of unchlorinated water is passed through the sampler at a rate of about 2.5 gallons per minute. The sampler is then subjected to the same analytical protocol as all other samplers.

System functioning tests of the analytical instruments are conducted in accordance with the manufacturer's recommendations. Spiked samples are also analyzed when appropriate for quality control purposes.

All materials used in sampling and analysis work are routinely analyzed for the presence of any compounds that might create fluorescence peaks in or near the acceptable wavelength ranges for any of the tracer dyes. This testing includes approximately 1% of materials used.

Project specific QA/QC samples may include sample replicates and sample duplicates. A replicate sample is when a single sample is analyzed twice. A sample duplicate is where two samples are collected in a single location and both are analyzed. Sample replicates and duplicates are run for QA/QC purposes upon request of the client. These results are reported in the Certificate of Analysis.

Reports

Sample analysis results are typically reported in a Certificate of Analysis. However, specialized reports are provided in accordance with the needs of the client. Certificates of Analysis typically provide a listing of station number, sample ID, and dye concentrations if detected. Standard data format includes deliverables in MS Excel and Adobe Acrobat (.pdf)

format. Hard copy of the data package, and copies of the analytical charts are available upon request.

Work at the OUL is directed by Mr. Thomas Aley. Mr. Aley has 45 years of professional experience in hydrology and hydrogeology. He is certified as a Professional Hydrogeologist (Certificate #179) by the American Institute of Hydrology and licenced as a Professional Geologist in Missouri, Arkansas, Kentucky, and Alabama. Additional details regarding laboratory qualifications are available upon request.

Waste Disposal

All laboratory wastes are disposed of according to applicable state and federal regulations. Waste elutant and water samples are collected in 15 gallon poly drums and disposed with a certified waste disposal facility as non-hazardous waste.

In special cases, wastes for a particular project may be segregated and returned to the client upon completion of the project. These projects may have samples that contain contaminants that the client must account for all materials generated and disposed. These situations are managed on a case-by-case basis.

CRITERIA FOR DETERMINATION OF POSITIVE DYE RECOVERIES

Normal Emission Ranges and Detection Limits

The OUL has established normal emission fluorescence wavelength ranges for each of the four dyes described in this document. The normal acceptable range equals mean values plus and minus two standard deviations. These values are derived from actual groundwater tracing studies conducted by the OUL.

The detection limits are based upon concentrations of dye necessary to produce emission fluorescence peaks where the signal to noise ratio is 3. The detection limits are realistic for most field studies since they are based upon results from actual field samples rather than being based upon values from spiked samples in a matrix of reagent water or the elutants from unused activated carbon samplers. In some cases detection limits may be smaller than reported if the water being sampled has very little fluorescent material in it. In some cases detection limits may be greater than reported; this most commonly occurs if the sample is turbid due to suspended material or a coloring agent such as tannic compounds. Turbid samples are typically allowed to settle, centrifuged, or, if these steps are not effective, diluted prior to analysis.

Table 4 provides normal emission wavelength ranges and detection limits for the four dyes when analyzed on the OUL's RF-5301 for samples analyzed as of March 3, 2015.

Table 4. RF-5301 Spectrofluorophotometer. Normal emission wavelength ranges and detection limits for fluorescein, eosine, rhodamine WT, and sulforhodamine B dyes in water and elutant samples.

Fluorescent Dye	Normal Acceptable Emission Wavelength Range (nm)		Detection Limit (ppb)	
	Elutant	Water	Elutant	Water
Eosine	539.3 to 545.1	532.5 to 537.0	0.050	0.015
Fluorescein	514.1 to 519.2	505.9 to 509.7	0.025	0.002
Rhodamine WT	564.6 to 571.2	571.9 to 577.2	0.170	0.015
Sulforhodamine B	575.2 to 582.0	580.1 to 583.7	0.080	0.008

Note: Detection limits are based upon the as-sold weight of the dye mixtures normally used by the OUL.

Fluorescein and eosine detection limits in water are based on samples pH adjusted to greater than 9.5.

It is important to note that the normal acceptable emission wavelength ranges are subject to change based on instrument maintenance, a change in instrumentation, or slight changes in dye formulation. Significant changes in normal acceptable emission wavelength ranges will be updated in this document as they occur.

Fluorescence Background

Due to the nature of fluorescence analysis, it is important to identify and characterize any potential background fluorescence at dye introduction and monitoring locations prior to the introduction of any tracer dyes.

There is generally little or no detectable fluorescence background in or near the general range of eosine, rhodamine WT, and sulforhodamine B dyes encountered in most groundwater tracing studies. There is often some fluorescence background in or near the range of fluorescein dye present at some of the stations used in groundwater tracing studies.

Criteria for Determining Dye Recoveries

The following sections identify normal criteria used by the OUL for determining dye recoveries. The primary instrument in use is a Shimadzu RF-5301.

EOSINE

Normal Criteria Used by the OUL for Determining Eosine Dye Recoveries in Elutants from Charcoal Samplers

Criterion 1. There must be at least one fluorescence peak in the range of 539.3 to 545.1 nm in the sample.

Criterion 2. The dye concentration associated with the fluorescence peak must be at least 3 times the detection limit. The eosine detection limit in elutant samples is 0.050 ppb, thus this dye concentration limit equals 0.150 ppb.

Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Criterion 4. The shape of the fluorescence peak must be typical of eosine. Much background fluorescence yields low, broad, and asymmetrical fluorescence peaks rather than the more narrow and symmetrical fluorescence peaks typical of eosine. In addition, there must be no other factors which suggest that the fluorescence peak may not be eosine dye from our groundwater tracing work.

Normal Criteria Used by the OUL for Determining Eosine Dye Recoveries in Water Samples

Criterion 1. In most cases, the associated charcoal samplers for the station should also contain eosine dye in accordance with the criteria listed above. This criterion may be waived if no charcoal sampler exists.

Criterion 2. There must be no factors which suggest that the fluorescence peak may not be eosine dye from our groundwater tracing work. The fluorescence peak should generally be in the range of 532.5 to 537.0 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. Our eosine detection limit in water samples is 0.015 ppb, thus this dye concentration limit equals 0.045 ppb.

Criterion 4. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

FLUORESCCEIN

Normal Criteria Used by the OUL for Determining Fluorescein Dye Recoveries in Elutants from Charcoal Samplers

Criterion 1. There must be at least one fluorescence peak in the range of 514.1 to 519.2 nm in the sample.

Criterion 2. The dye concentration associated with the fluorescence peak must be at least 3 times the detection limit. The fluorescein detection limit in elutant samples is 0.025 ppb, thus this dye concentration limit equals 0.075 ppb.

Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Criterion 4. The shape of the fluorescence peak must be typical of fluorescein. Much background fluorescence yields low, broad, and asymmetrical fluorescence peaks rather than the more narrow and symmetrical fluorescence peaks typical of fluorescein. In addition, there must be no other factors which suggest that the fluorescence peak may not be fluorescein dye from our groundwater tracing work.

Normal Criteria Used by the OUL for Determining Fluorescein Dye Recoveries in Water Samples

Criterion 1. In most cases, the associated charcoal samplers for the station should also contain fluorescein dye in accordance with the criteria listed above. This criterion may be waived if no charcoal sampler exists.

Criterion 2. There must be no factors which suggest that the fluorescence peak may not be fluorescein dye from our groundwater tracing work. The fluorescence peak should generally be in the range of 505.9 to 509.7 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. Our fluorescein detection limit in water samples is 0.002 ppb, thus this dye concentration limit equals 0.006 ppb.

Criterion 4. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

RHODAMINE WT

Normal Criteria Used by the OUL for Determining Rhodamine WT Dye Recoveries in Elutants from Charcoal Samplers

Criterion 1. There must be at least one fluorescence peak in the sample in the range of 564.6 to 571.2 nm.

Criterion 2. The dye concentration associated with the rhodamine WT peak must be at least 3 times the detection limit. The detection limit in elutant samples is 0.170 ppb, thus this dye concentration limit equals 0.510 ppb.

Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Criterion 4. The shape of the fluorescence peak must be typical of rhodamine WT. In addition, there must be no other factors which suggest that the fluorescence peak may not be dye from the groundwater tracing work under investigation.

Normal Criteria Used by the OUL for Determining Rhodamine WT Dye Recoveries in Water Samples

Criterion 1. In most cases, the associated charcoal samplers for the station should also contain rhodamine WT dye in accordance with the criteria listed above. These criteria may be waived if no charcoal sampler exists.

Criterion 2. There must be no factors which suggest that the fluorescence peak may not be rhodamine WT dye from the tracing work under investigation. The fluorescence peak should generally be in the range of 571.9 to 577.2 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. Our rhodamine WT detection limit in water samples is 0.015 ppb, thus this dye concentration limit is 0.045 ppb.

Criterion 4. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

SULFORHODAMINE B

Normal Criteria Used by the OUL for Determining Sulforhodamine B Dye Recoveries in Elutants from Charcoal Samplers

Criterion 1. There must be at least one fluorescence peak in the sample in the range of 575.2 to 582.0 nm.

Criterion 2. The dye concentration associated with the sulforhodamine B peak must be at least 3 times the detection limit. The detection limit in elutant samples is 0.080 ppb, thus this dye concentration limit equals 0.240 ppb.

Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Criterion 4. The shape of the fluorescence peak must be typical of sulforhodamine B. In addition, there must be no other factors which suggest that the fluorescence peak may not be dye from the groundwater tracing work under investigation.

Normal Criteria Used by the OUL for Determining Sulforhodamine B dye Recoveries in Water Samples

Criterion 1. In most cases, the associated charcoal samplers for the station should also contain sulforhodamine B dye in accordance with the criteria listed earlier. This criterion may be waived if no charcoal sampler exists.

Criterion 2. There must be no factors which suggest that the fluorescence peak may not be sulforhodamine B dye from the tracing work under investigation. The fluorescence peak should generally be in the range of 580.1 to 583.7 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. The detection limit in water is 0.008 ppb, thus this dye concentration limit equals 0.024 ppb.

Criterion 4. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question

Standard Footnotes

Sometimes not all the criteria are met for a straight forward determination of tracer dye in a sample. For these reasons, the emission graph is scrutinized carefully by the analytical technician and again during the QA/QC process. Sometimes the emission graphs require interpretation as to whether or not a fluorescence peak represents the tracer dye or not. Background samples from each of the sampling stations aid in the interpretation of the emission fluorescence graphs. When the results do not meet all the criteria for a positive dye detection, often the fluorescence peak is quantified and flagged with a footnote to the result as not meeting all the criteria for a positive dye detection. Standard footnotes are as follows:

Single asterisk (*): A fluorescence peak is present that does not meet all the criteria for a positive dye recovery. However, it has been calculated as though it were the tracer dye.

Double asterisk (**): A fluorescence peak is present that does not meet all the criteria for this dye. However, it has been calculated as a positive dye recovery.

Other footnotes specific to the fluorescence signature are sometimes also used. These footnotes are often developed for a specific project.

The quantification of fluorescence peaks that do not meet all the criteria for a positive dye detection can be important for interpretation of the dataset as a whole.

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