Title: Targeted conservation efforts for four karst species: Foushee cavesnail (*Amicola cora*), Foushee cave springtail (*Typhlogastrura fousheensis*), gray bat (*Myotis griscesens*), and Indiana bat (*Myotis sodalis*).

Summary: This project will seek to develop conservation actions for protecting endemic species found in Foushee Cave from development and disturbance and provide additional protection for Foushee Cave as a hibernaculum for endangered bats. Following delineation of the recharge boundary for Foushee Cave, parcel based priorities will be set for habitat restoration and protection, including acquisitions, easements, and conservation enrollment. Where conservation opportunities occur, we will provide information on available programs and assist with landowner negotiations to secure protection of Foushee Cave and land within the recharge boundary.

Project Leader: Michael Slay, Ozark Karst Program Director

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SWG Funds Requested: $30,000

Amount and Source of Matching Funds: $30,000 (50%) from The Nature Conservancy, Ozark Underground Laboratory, and Tumbling Creek Cave Foundation.

Total Project Cost: $60,000

Date Submitted: January 16, 2009

Need:
Foushee Cave, located in Independence County, Arkansas, is one of the most biologically significant caves in Arkansas (Graening, 2003). The cave is home to great diversity of organisms including the Foushee cavesnail (*Amicola cora*), Foushee cave springtail (*Typhlogastrura fousheensis*), gray bat (*Myotis griscesens*), and Indiana bat (*Myotis sodalis*). Although the cave is gated, it is privately owned, has no special status (e.g., not a nature preserve, etc.), and the land within the drainage basin of the cave is not protected from development or other land use practices.

The Foushee cavesnail, *Amnicola cora*, was first described in 1979 (Hubricht, 1979). This snail occurs only in Foushee Cave, and recent surveys confirmed the snail still occuring in the cave (Graening, 2003; Slay and Taylor, 2007). While there is some ongoing monitoring being conducted by The Nature Conservancy, with funding provided by US Fish and Wildlife Service, there are currently no long-term efforts directed toward protecting this species.

The Foushee cave springtail was originally identified as *Schaefferia alabamensis* in Youngteadt and Youngsteadt (1978). However, these cave springtail specimens were ultimately determined to be a new species and described as *Typhlogstrura fousheensis* by Christiansen and Wang (2006), and the name should be changed to *Typhlogstrura fousheensis* in the Arkansas Wildlife Action Plan. Recent studies did not record this species from surrounding caves, and the species appears to be endemic to Foushee Cave (Graening et al, 2004; Graening et al, 2006).

In addition to these two endemic invertebrates, both the endangered gray bat and endangered Indiana bat are known to hibernate in the cave during the winter (D. Kampwerth, USFWS, personal communication). The presence of these endangered bats facilitated the installation of an entrance gate, which currently affords the only protection for Foushee Cave and its inhabitants.

Because Foushee Cave is one of the most biologically significant caves in Arkansas and harbors several single-site endemics and endangered species, there is a need to develop conservation actions to protect this site and the surrounding landscape.

Funding Priorities Addressed:
This project will lay the necessary groundwork to accomplish the funding priority related to Foushee Cavesnail, gray bat, Indiana bat, and Foushee cave springtail (to protect habitat and recharge zone from development and disturbance and to protect bat hibernacula) identified by the Karst Taxa and Habitat Team and included in the 2009 Request for Preproposals.

Location of Work:
Work will be conducted within portions of the Ozark Highlands ecoregion, within the Ozark Highlands - White River eco-basin, White River Hills sub-ecoregion. Specifically, the work will occur in Independence County.

Conservation Priorities Addressed:
This project will lay the groundwork for accomplishing the funding priority determined by the State Wildlife Action Plan Steering Committee for 2009, namely to protect habitat and recharge zone from development and disturbance and to protect bat hibernacula at Foushee Cave for the Foushee Cavesnail, gray bat, Indiana bat, and Foushee cave springtail.

In addition, the project will address the following issues that impede accomplishing this priority:

1. Conduct recharge delineation for Foushee Cave.

2. Identify all land parcels that occur within recharge area.
3. Identify all land owners within in recharge area.
4. Determine highest priority parcels for acquisition, easement, or conservation enrollment.

Goal:
The goal of this project is to establish a mechanism to target and implement conservation easements and land acquisitions to benefit the Foushee Cavesnail, gray bat, Indiana bat, and Foushee cave springtail. To accomplish this goal, we will delineate the recharge area for Foushee Cave, identify all land parcels and land owners within recharge area, and determine highest priority parcels for acquisition, easement, or conservation enrollment.

Methods and Monitoring:
Using standard dye tracing techniques (Aley 1999; see also Appendix 1), we will delineate the recharge boundary for Foushee Cave. Using digital topography maps, potential locations for dye introduction and dye retrieval (e.g. Foushee Cave and nearby springs) will be identified. Landowners of these possible introduction and retrieval sites will be indentified through the Independence County Assessor's Office, and they will be contacted to discuss the project and arrange permission for access to land. Similar techniques were used successfully to conduct recent recharge delineations for two populations of the endangered Benton cave crayfish (*Cambarus aculabrum*) in northwest Arkansas (Aley and Slay 2006; Aley and Slay 2007). Prior to introducing dye, Arkansas Department of Environmental Quality will be contacted to acquire the necessary clearance for conducting a dye trace study in Arkansas. Based on the recharge area, land parcel information from recent plat books will be georeferenced, using ArcGIS 9.3. These digitized land parcels will be used to identify all landowners with property within the recharge boundary for Foushee Cave. We will then facilitate contact with respective landowners to promote protection and restoration concepts. Where opportunities for land acquisition or conservation easement occur, we will provide information on available programs and assist with landowner negotiations to secure protection of Foushee Cave.

Measures of Success

Several performance measures will be used to measure success of this project, and these measures can be used to indicate results to resource managers, scientists, and non-scientists.

- Percent (%) of recharge boundary in protected conservation status.
- Percent (%) of landowners willing to implement conservation actions within the recharge boundary.

Expected Outcomes:
A delineated recharge boundary for Foushee Cave will provide the basis for all conservation efforts designed to protect species occurring in the cave. The recharge boundary will allow potential threats to be identified which could be addressed proactively. In addition, identifying land parcels for acquisition, easement, or conservation enrollment will allow agencies and non-governmental organizations such as Arkansas Natural Heritage Commission, Arkansas Game and Fish, and The Nature Conservancy to begin acquiring land.

Deliverables

1. Monitoring methodology entered into the Natural Resources Monitoring Partnership.
2. Recharge boundary delineated for Foushee Cave.
3. Priority list of landowners and conservation recommendations for land within recharge boundary of Foushee Cave.
4. Update the database associated with the Arkansas Comprehensive Wildlife Conservation Strategy.

5. Produce final report.
6. Present results of project to scientific community (probably during AGFC forum) in Fall 2012.
7. Present results of project as part of 2-3 public lectures.

Existing Resources Utilized:
This project takes advantage of past cooperation between The Nature Conservancy, Ozark Underground Laboratory, and Tumbling Creek Cave Foundation and builds on recent conservation efforts by the cooperators jointly and independently. All cooperators contribute expertise in dye tracing, recharge delineation, threats assessment, GIS based parcel prioritization, and land acquisition areas of this project.

Deliverables Calendar:

Deliverable	**Time Frame**
Monitoring methodology entered into the Natural Resources Monitoring Partnership.	Month 1 -2
Recharge boundary delineated for Foushee Cave.	Month 2-12
Priority list of landowners and conservation recommendations.	Month 12-22
Update the database associated with the Arkansas Comprehensive Wildlife Conservation Strategy.	Month 23-24
Produce final report.	Month 23-24
Present results of project to scientific community (probably during AGFC forum) in Fall 2012.	Month 21-24
Present results of project as part of 2-3 public lectures.	Month 18-24

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Michael Slay has been working in karst conservation for eight years in the five states that contain the caves and springs of the Ozark Highlands Ecoregion. Before joining The Nature Conservancy as the Ozark Karst Program Director, Mike coordinated karst research during positions held at the University of Arkansas, Buffalo National River NPS, Illinois Natural History Survey, and Missouri Department of Conservation. Since joining The Nature Conservancy, Mike has worked with multiple partners such as US Fish & Wildlife Service, US Forest Service, Arkansas Game & Fish Commission, Missouri Department of Conservation, Oklahoma Biological Survey, and Illinois Natural History Survey to conserve and protect karst species and habitats, including species found in spring habitats. Mike has coordinated the exploration, species monitoring, and habitat analysis in several hundred caves and springs, and he has assisted with the discovery of over 15 karst species new to science. Mike received his undergraduate degree and M.S. in Biology at the University of Arkansas. In addition to conducting karst research and implementing karst conservation actions, Mike has authored and co-authored 10 peer-reviewed journal articles related to the discovery and conservation of karst species.

Tom Aley has been conducting groundwater hydrology studies for over forty years and is president of Ozark Underground Laboratory in Protem, Missouri. He is licensed Geologist in Arkansas and has conducted a majority of the dye trace studies involving recharge areas for threatened and endangered karst species in the Ozarks. Tom has also done extensive groundwater studies in many other states and multiple countries concerning water quality degradation and impact on both aquatic species and humans. Tom serves on two ESA recovery teams for endangered aquatic species, and has established the Tumbling Creek Cave Foundation for the protection of the Tumbling Creek Cavesnail, and cave species known only from a single cave in Missouri. Tom has authored and co-authored many peer-reviewed journal articles related to the conservation, protection, and ecology of karst species and habitats.

Appendix 1.

Procedures and criteria analysis of flouroscein, eosine, rhodamine wt, sulforhodamine b, and pyranine dyes in water and charcoal samplers.

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PROCEDURES AND CRITERIA
ANALYSIS OF FLUORESCEIN, EOSINE, RHODAMINE WT, SULFORHODAMINE B, AND PYRANINE DYES IN WATER AND CHARCOAL SAMPLERS

March 21, 2005

Thomas Aley, PHG 179
President
Ozark Underground Laboratory, Inc.

PROCEDURES

Introduction

This document describes standard procedures and criteria currently in use at the Ozark Underground Laboratory 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 up-graded. The Ozark Underground Laboratory maintains a summary of changes in standard procedures and criteria.

Dye Nomenclature

Fluorescein is C.I. Acid yellow 73, Color Index Number 45350. Rhodamine WT is Acid Red 388; there is no assigned Color Index Number for this dye. Eosine (sometimes called eosin) is Acid Red 87, Color Index Number 45380. Sulforhodamine B is C.I. Acid Red 52, Color Index Number 45100. Pyranine is Solvent Green 7 (also called D&C Green 8), Color Index Number 59040.

Description of the Samplers

The charcoal samplers are packets of fiberglass screening partially filled with approximately 4.25 grams of activated coconut charcoal. The charcoal used by the Ozark Underground Laboratory is Barnebey and Sutcliffe coconut shell carbon, 6 to 12 mesh, catalog type AC.

The most commonly used samplers are about 4 inches long by two inches wide. A cigar-shaped sampler is made for use in very small diameter wells (such as 1 inch diameter wells); this is a special order item and should be specifically requested when it is needed. All of the samplers are closed by heat sealing.

Placement of Samplers

Samplers (also called charcoal packets) are placed so as to be exposed to as much water as possible. 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 being flat against it. Two or more separately anchored packets are typically used for sampling springs and streams. 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 placed in sample holders made of PVC pipe fittings. Brass hose fittings are installed 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 consists of 1.5 inch diameter pipe and pipe fittings.

Charcoal packets can also 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. Some sort of weight should be added near the charcoal packet to insure 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 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. 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 narrow 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 stainless steel weight may be added to the top of the disposable bailer. We have had good success with two to three ounce segments of stainless steel pipe which have an outside diameter of 1.315 inches and an inside diameter of 1.049 inches; such pipe weighs about 1.7 pounds per linear foot. The weight of the stainless steel is approximately 497 pounds per cubic foot. The pipe segments can be attached over the anchoring cord at the top of the bailer. All weights should be cleaned prior to use; the cleaning approach should comply with decontamination procedures in use at the project site.

Placement of samplers requires adjustment to field conditions. The above placement comments are intended as guidance, not firm requirements.

Rinsing of Charcoal Packets Prior to Sampling

Charcoal packets routinely contain some fine powder that washes off rapidly when they are placed in water. Since such material could remain in monitoring wells, charcoal packets to be placed in such wells are 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 from 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.

Where convenient, the collected samplers should be briefly rinsed in the water being sampled. This is typically 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 permanent type felt marker pen. 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 are not inserted inside the sample bags.

For most projects the Ozark Underground Laboratory supplies the Whirl-Pak bags. Prior to use, 1% of the new bags are randomly selected. Each bag is soaked in the standard eluting solution and then analyzed for the presence of any of the tracer dyes being used.

Collected samplers are kept in the dark to minimize algal growth on the charcoal prior to analysis work. We prefer (and in some studies require) that samples be placed on "blue ice" or ice upon collection and that they be shipped refrigerated with "blue ice" by overnight express. Do not ship samplers packed in 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.

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 are often collected. They should be collected in either glass or plastic; the Ozark Underground Laboratory routinely uses 50 ml research grade polypropylene copolymer Perfector Scientific vials (Catalog Number 2650) for such water samples. The vials should be placed in the dark and refrigerated immediately after collection. They should be refrigerated until shipment. For most projects the Ozark Underground Laboratory supplies the vials. Prior to use, 1% of the new vials are randomly selected. Each vial is soaked in the standard eluting solution and then analyzed for the presence of any of the tracer dyes being used.

When water or charcoal samplers are collected for shipment to the Ozark Underground Laboratory they should be shipped promptly. We receive good overnight and second day air service from both UPS and Fed Ex; Airborne Service is excessively slow, and the Postal Service does not provide next day service to us.

Each shipment of charcoal samplers or water samples must be accompanied by a sample tracking sheet. These sheets (which bear the title "Samples for Fluorescence Analysis") are provided by the Ozark Underground Laboratory and summarize placement and collection data. These sheets can be augmented by a client's chain of custody forms or any other relevant documentation. Figure 1 is one of our blank sample forms.

Receipt of Samplers

Samplers shipped to the Ozark Underground Laboratory are refrigerated upon receipt. Prior to cleaning and analysis, samplers are assigned a laboratory identification number. All samples are logged in upon receipt.

It sometimes occurs that there are discrepancies between the chain-of-custody sheets and the actual samples received. When this occurs, a "Discrepancy Sheet" form is completed and sent to the shipper of the sample for resolution. A copy of this form is enclosed as Figure 2. The purpose of the form is to help resolve discrepancies, even when they may be minor.

Cleaning of Samplers

Samplers are cleaned by spraying them with jets of clean water. At the Laboratory we use unchlorinated water for the cleansing to minimize dye deterioration. 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.

After washing, the packets are shaken to remove excess water. Next, the packets are cut open and the charcoal is emptied into a new disposable plastic beaker. The beaker has been pre-labeled with the laboratory identification number. The charcoal is now ready for elution. The emptied fiberglass screen packet is discarded. At stations where two or more charcoal packets are collected, one is selected for analysis and the other is frozen and retained until the end of the study. In some studies the analysis protocol stipulates that a fixed percentage (often 5%) of the samples should be duplicates; in these cases the second charcoal packet is separately analyzed. Note that these are duplicate samples, not replicate samples since each packet is, of necessity, placed in a somewhat different location and is therefore exposed to somewhat different conditions.

Cleaning of Glassware

Most of our work uses disposable plastic containers. A small amount of glassware is occasionally used for preparation of standards. It is dedicated to this use. In the event that any glassware does come in contact with tracer dyes it will be carefully cleaned before re-use. To do this cleaning, containers are rinsed several times in clean water. Glassware that may be contaminated with dyes is washed with detergent, and then again rinsed. Next, the glassware is soaked for one hour or more in a bleach and water solution. Upon removal from this soaking, the glassware is rinsed again and allowed to air dry.

Figure 1. Sample Collection Data Sheet

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SAMPLE COLLECTION DATA SHEET for FLUORESCENCE ANALYSIS

Project:_____**Week No:**_____ **Samples Collected By:**_____

Samples Shipped By:_____**Samples Received By:**_____

Date Samples Shipped:____/____/____ **Date Samples Received:**____/____/____ **Time Samples Received:**_____:_____ **Return Cooler?**

Yes_____**No**_____

Bill to:_____**Send Results to:** _____

Analyze for: Fluorescein_____**Eosine**_____**Rhodamine WT**_____**Other**_____**Ship cooler to:** _____

OUL use only		***Please indicate stations where dye was visible in the field*** *for field technician use - use black ink only*						*OUL use only*
# CHAR REC'D	**LAB NUMBER**	**STATION NUMBER 1-4 Numbers**	**STATION NAME**	**PLACED**		**COLLECTED**		**# WATER REC'D**
				DATE	**TIME**	**DATE**	**TIME**	

COMMENTS:_____

_____

This sheet filled out by OUL staff? Yes_____**No**_____**Charts for samples on this page proofed by OUL:**_____

Page _____ of _____

Figure 2. Discrepancy Sheet

OZARK UNDERGROUND LABORATORY, INC.					
DISCREPANCIES BETWEEN CHAIN-OF-CUSTODY SHEETS AND ACTUAL SAMPLES RECEIVED					**Page _____ of _____**
Company & Project Name:				**Date Rec'd by OUL:**	**Wk # _____**
Lab #	**Sta #**	**Station Name**	**Date Pulled**	**Problem**	**Solution**
Comments:					

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, some water, and a strong basic solution such as aqueous ammonia.

The standard elution solution now used at the Ozark Underground Laboratory is a mixture of 5% aqua ammonia and 95% isopropyl alcohol solution and sufficient potassium hydroxide flakes to saturate the solution. The isopropyl alcohol 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 we use will elute fluorescein, eosine, rhodamine WT, sulforhodamine B, and pyranine dyes. It is also suitable for separating fluorescein peaks from peaks of some naturally present materials found in some 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.

Analysis on the Shimadzu RF-5000U or RF-5301

The Laboratory uses two Shimadzu spectrofluorophotometers. One is a model RF-5000U, and the other is a model RF-5301. Both of these instruments are capable of synchronous scanning. The RF-5301 is the primary instrument used; the RF-5000U is primarily used as a back-up instrument except for tracing studies which were begun using this instrument. The OUL also owns a Shimadzu RF-540 spectrofluorometer which is occasionally used for special purposes.

A sample of the elutant is withdrawn from the sample container using a disposable polyethylene pipette. Approximately 3 ml of the elutant 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 spectral range of the cuvettes is 340 to 800 nm. The pipettes and cuvettes are discarded after one use.

The cuvette is then placed in the RF-5000U or the RF-5301. Both instruments are controlled by a programmable computer. Each instrument is capable of conducting substantial data analysis.

Our instruments are operated and maintained in accordance with the manufacturer's recommendations. On-site installation of the instruments and a training session on the use of spectrofluorophotometers was provided by Delta Instrument Company.

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 "very fast" on the RF-5000U; it is “fast” on the RF-5301. The typical sensitivity setting used on both instruments is "high."

Our typical analysis of an elutant sample where pyranine dye may be present includes a synchronous scanning of excitation and emission spectra with a 35 nm separation between excitation and emission wavelengths. For this dye, the excitation scan is from 360 to 600 nm; the emission scan is from 395 to 635 nm. The emission fluorescence from the scan is plotted on a graph. The typical scan speed setting is "very fast" on the RF-5000U; it is “fast” on the RF-5301. The typical sensitivity setting on both instruments is "high."

Excitation and emission slit width settings vary between the two instruments. The widths vary with the dyes for which we are sampling and for the matrix in which the dyes may be present. Excitation and emission slit width settings are summarized in Table 1.

Table 1. Excitation and emission slit width settings routinely used for dye analysis. Units are nanometers (nm)

Parameter	**RF5000U**	**RF5301**
Excitation slit for Eos, Fl, RWT, and SRB in elutant	5	3
Emission slit for Eos, Fl, RWT, and SRB in elutant	3	1.5
Excitation slit for Eos, Fl, RWT, and SRB in water	5	5
Emission slit for Eos, Fl, RWT, and SRB in water	10	3
Excitation slit for Pyranine in elutant	5	5
Emission slit for Pyranine in elutant	3	3
Excitation slit for Pyranine in pH adjusted water	5	5
Emission slit for Pyranine in pH adjusted water	3	3

Eos = 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; peaks are picked to the nearest 0.1 nm. All samples run on the RF-5000U and RF-5301 are stored on disk and printed on normal typing paper with a laser printer; sample information is printed on the chart.

All samples analyzed are recorded in a bound journal.

Quantification

We calculate the magnitude of fluorescence peaks for fluorescein, eosine, rhodamine WT, sulforhodamine B, and pyranine dyes. Dye quantities are expressed in microgram per liter (parts per billion; ppb). On the RF-5000U and RF-5301 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.

Where there are multiple fluorescence peaks it is sometimes necessary to calculate dye concentrations based upon the height of the fluorescence peak rather that the area. The heights of the peaks are also proportional to dye concentrations.

We run dye concentration standards each day the machine is used. Ten separate 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 pyranine in well water from the Jefferson City-Cotter Formation. A sample of the standard is placed for at least two hours in a high ammonia atmosphere to adjust the pH to a value of 9.5 or greater.
5) 10 ppb fluorescein and 100 ppb rhodamine WT in elutant.
6) 10 ppb eosine in elutant.
7) 100 ppb sulforhodamine B in elutant.
8) 10 ppb pyranine 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 insure 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, sulforhodamine B, and pyranine. The resulting solutions contain 100 mg/l dye (100 parts per million dye). 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 Finnpipette 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. Ozark Underground Laboratory 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 Finnpipette. (see description in Step 2). All other volume measurements are made with Rheinland Kohn Geprufte 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) A solution containing 1 ppm fluorescein dye and 10 ppm rhodamine WT dye.

2) A solution containing 1 ppm eosine.

3) A solution containing 10 ppm sulforhodamine B dye.

4) A solution containing 1 ppm pyranine.

Step 4. A series of eight short-term dye standards are made from solutions in Step 3. These standards were identified earlier in this section. In the experience of the Ozark Underground Laboratory these standards have a useful shelf life in excess of one week. However, in practice, they are kept under refrigeration and new standards are made weekly.

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 dilution is 100 fold. One part of the test sample is combined with 99 parts of water (if the test sample is water) or with 99 parts of the standard elutant (if the test sample is elutant). 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 Finnpipette. 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%

All other volume measurements are made with Rheinland Kohn Geprufte Sicherheit 50 ml. capacity pump dispenser which will pump within plus or minus 1% of the set value.

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.

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 typically includes approximately 1% of materials used.

Reports

Reports are provided in accordance with the needs of the client. At a minimum we provide copies of the analysis graphs and a listing of stations and samples where dye was detected. The reports indicate dye concentrations.

Work at the Ozark Underground Laboratory is directed by Mr. Thomas Aley. Mr. Aley has 40 years of professional experience in hydrology and hydrogeology. He is certified as a Professional Hydrogeologist (Certificate #179) by the American Institute of Hydrology. Mr. Aley has 35 years of professional experience in groundwater tracing with fluorescent tracing agents.

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 five dyes. 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 centrifuged or, if this is not effective, diluted prior to analysis.

Table 2 provides normal emission wavelength ranges and detection limits for the five dyes when analyzed on the OUL's RF-5000U spectrofluorophotometer. Table 3 provides similar data for the OUL's RF-5301. As indicated earlier in Table 1, the analytical protocols used on the two instruments are somewhat different, especially in regard to the widths of excitation and emission slit settings.

Table 2. RF-5000U Spectrofluorophotometer. Normal emission wavelength ranges and detection limits for fluorescein, eosine, rhodamine WT, sulforhodamine B, and pyranine dyes in water and elutant samples. Detection limits are based upon the as-sold weight of the dye mixtures normally used by the OUL.

Dye and Matrix	Normal Acceptable Emission Wavelength Range (nm)	Detection Limit (ppb)
Eosine in Elutant	533.0 to 539.6	0.035
Eosine in Water	529.6 to 538.4	0.008
Fluorescein in Elutant	510.7 to 515.0	0.010
Fluorescein in Water	505.6 to 510.5	0.0005
Pyranine in Elutant	500.4 to 504.6	0.055
Pyranine in Water*	501.2 to 505.2	0.030
Rhodamine WT in Elutant	561.7 to 568.9	0.275
Rhodamine WT in Water	569.4 to 574.8	0.050
Sulforhodamine B in Elutant	567.5 to 577.5	0.150
Sulforhodamine B in Water	576.2 to 579.7	0.040

* pH adjusted water with pH of 9.5 or greater.

Note: The protocols for the analysis of pyranine dye are substantially different than those for the other dyes. As a result, there is less potential interference between pyranine and fluorescein than might otherwise be indicated by the emission wavelength values shown in the table.

Table 3. RF-5301 Spectrofluorophotometer. Normal emission wavelength ranges and detection limits for fluorescein, eosine, rhodamine WT, sulforhodamine B, and pyranine dyes in water and elutant samples. Detection limits are based upon the as-sold weight of the dye mixtures normally used by the OUL.

Dye and Matrix	Normal Acceptable Emission Wavelength Range (nm)	Detection Limit (ppb)
Eosine in Elutant	538.1 to 543.9	0.050
Eosine in Water	533.4 to 537.9	0.015
Fluorescein in Elutant	514.0 to 518.1	0.025
Fluorescein in Water	508.0 to 511.7	0.002
Pyranine in Elutant	502.1 to 508.1	0.015
Pyranine in Water*	504.1 to 510.1	0.010
Rhodamine WT in Elutant	565.4 to 572.0	0.170
Rhodamine WT in Water	572.7 to 578.0	0.015
Sulforhodamine B in Elutant	572.8 to 579.6	0.080
Sulforhodamine B in Water	580.1 to 583.7	0.008

* pH adjusted water with pH of 9.5 or greater.

Note: The protocols for the analysis of pyranine dye are substantially different than those for the other dyes. As a result, there is less potential interference between pyranine and fluorescein than might otherwise be indicated by the emission wavelength values shown in the table.

Criteria for Determining Positive Dye Recoveries

The following sections identify normal criteria used by the OUL for determining positive dye recoveries. Beginning January 1, 2001, the primary analytical instrument in use at the OUL was the RF-5301; the RF-5000U was the principal backup instrument. Studies which were in progress prior to January 1, 2001 continued to have samples analyzed on the RF-5000U.

Except for pyranine dye, the analytical protocol used for the RF-5301 provides for the use of narrower excitation and/or emission slit settings than the RF-5000U protocol. This enhances our ability to discriminate between dyes and other fluorescent compounds. The protocol which is possible with the RF-5301 (as contrasted with the RF-5000U) also provides for a better balance in the sizes of the fluorescence peaks associated with an equal concentration of all of the dyes.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Eosine Dye Recoveries in Elutants from Charcoal Samplers.

There is generally little or no detectable fluorescence background in the general range of eosine dye encountered in most groundwater tracing studies. The following four criteria are used to identify fluorescence peaks which are deemed to be eosine dye.

Criterion 1. There must be at least one fluorescence peak at the station in question in the range of 538.1 to 543.9 nm for samples analyzed by the RF-5301. The range must be 533.0 to 539.6 nm for samples analyzed by the RF-5000U.

Criterion 2. The dye concentration associated with the fluorescence peak must be at least 3 times the detection limit. For the RF-5301, the eosine detection limit in elutant samples is 0.050 ppb, thus this dye concentration limit equals 0.150 ppb. For the RF-5000U the eosine detection limit in elutant samples is 0.035 ppb, thus this dye concentration limit equals 0.105 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 Ozark Underground Laboratory for Determining Positive Eosine Dye Recoveries in Water Samples.

There is generally little or no detectable fluorescence background in the general range of eosine dye encountered in most groundwater tracing studies. The following three criteria are used to identify fluorescence peaks which are deemed to be eosine dye.

Criterion 1. The associated charcoal samplers for the station should also contain eosine 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 eosine dye from our groundwater tracing work. For samples analyzed on the RF-5301, the fluorescence peak should generally be in the range of 533.4 to 537.9 nm. For samples analyzed on the RF-5000U, the fluorescence peak should generally be in the range of 529.6 to 538.4 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 analyzed on the RF-5301 is 0.015 ppb, thus this dye concentration limit equals 0.045 ppb. For samples analyzed on the 5000U the detection limit is 0.008 ppb, thus this dye concentration limit equals 0.024 ppb.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Fluorescein Dye Recoveries in Elutants from Charcoal Samplers.

There is often some fluorescence background in the range of fluorescein dye present at some of the stations used in groundwater tracing studies. We routinely conduct background sampling prior to the introduction of any tracer dyes to characterize this background fluorescence and to identify the existence of any tracer dyes which may be present in the area. The fact that a fluorescence peak is identified in our analytical results is not proof that it is fluorescein dye or that it is fluorescein dye from the trace of concern. The following 4 criteria are used to identify fluorescence peaks which are deemed to be fluorescein dye recoveries from our tracing work.

Criterion 1. There must be at least one fluorescence peak at the station in question in the range of 514.0 to 518.1 nm for samples analyzed by the RF-5301. The range must be 510.7 to 515.0 for samples analyzed by the RF-5000U.

Criterion 2. The dye concentration associated with the fluorescence peak must be at least 3 times the detection limit. For the RF-5301, the fluorescein detection limit in elutant samples is 0.025 ppb, thus this dye concentration limit equals 0.075 ppb. For the RF-5000U, the fluorescein detection limit in elutant samples is 0.010 ppb, thus this dye concentration limit equals 0.030 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 Ozark Underground Laboratory for Determining Positive Fluorescein Dye Recoveries in Water Samples.

There is commonly some fluorescence background in the general range of fluorescein dye at some sampling stations used in groundwater tracing studies. The following criteria are used to identify fluorescence peaks which are deemed to be fluorescein dye in water.

Criterion 1. The associated charcoal samplers for the station should also contain fluorescein 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 fluorescein dye from our groundwater tracing work. For samples analyzed on the RF-5301, the fluorescence peak should generally be in the range of 508.0 to 511.7 nm. For samples analyzed on the RF-5000U, the fluorescence peak should generally be in the range of 505.6 to 510.5 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 analyzed on the RF-5301 is 0.002 ppb, thus this dye concentration limit equals 0.006 ppb. For the RF-5000U the detection limit is 0.0005 ppb, thus this dye concentration limit equals 0.0015 ppb.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Rhodamine WT Dye Recoveries in Elutants from Charcoal Samplers.

There is generally little or no detectable fluorescence background in the general range of Rhodamine WT dye encountered in most groundwater tracing studies. The following four criteria are used to identify fluorescence peaks which are deemed to be Rhodamine WT.

Criterion 1. For samples analyzed on the RF-5301, there must be at least one fluorescence peak at the station in question in the range of 565.4 to 572.0 nm. For samples analyzed on the RF-5000U, there must be at least one fluorescence peak at the station in question in the range of 561.7 to 568.9 nm.

Criterion 2. The dye concentration associated with the Rhodamine WT peak must be at least 3 times the detection limit. For the RF-5301, the detection limit in elutant samples is 0.170 ppb, thus this dye concentration limit equals 0.510 ppb. For the RF-5000U, the detection limit in elutant samples is 0.275 ppb, thus this dye concentration limit equals 0.825 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 Ozark Underground Laboratory for Determining Positive Rhodamine WT Dye Recoveries in Water Samples.

The following criteria are used to identify fluorescence peaks which are deemed to be Rhodamine WT dye in water.

Criterion 1. 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. For samples analyzed with the RF-5301, the fluorescence peak should generally be in the range of 572.7 to 578.0 nm. For samples analyzed with the RF-5000U, the fluorescence peak should generally be in the range of 569.4 to 574.8 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 analyzed on the RF-5301 is 0.015 ppb, thus this dye concentration limit is 0.045 ppb. For samples analyzed on the RF-5000U the detection limit is 0.050 ppb, thus this dye concentration limit equals 0.150 ppb.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Sulforhodamine B Dye Recoveries in Elutants from Charcoal Samplers.

There is generally little or no detectable fluorescence background in the general range of sulforhodamine B dye encountered in most groundwater tracing studies. The following four criteria are used to identify fluorescence peaks which are deemed to be sulforhodamine B.

Criterion 1. For samples analyzed on the RF-5000U, there must be at least one fluorescence peak at the station in question in the range of 567.5 to 577.5 nm. The acceptable range for samples analyzed on the RF-5301 is 572.8 to 579.6 nm.

Criterion 2. The dye concentration associated with the sulforhodamine B peak must be at least 3 times the detection limit. For the RF-5000U, the detection limit in elutant samples is 0.150 ppb, thus this dye concentration limit equals 0.450 ppb. For the RF-5301, 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 Ozark Underground Laboratory for Determining Positive Sulforhodamine B dye Recoveries in Water Samples.

The following criteria are used to identify fluorescence peaks which are deemed to be sulforhodamine B dye in water.

Criterion 1. The associated charcoal samplers for the station should also contain sulforhodamine B dye in accordance with the criteria listed earlier. 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 sulforhodamine B dye from the tracing work under investigation. For samples analyzed with the RF-5000U, the fluorescence peak should generally be in the range of 576.2 to 579.7 nm. For samples analyzed with the RF-5301, 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. For samples analyzed on the RF-5301 the detection limit in water is 0.008 ppb, thus this dye concentration limit equals 0.024 ppb. For samples analyzed on the RF-5000U the detection limit in water samples is 0.040 ppb, thus this dye concentration limit equals 0.120 ppb.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Pyranine Dye Recoveries in Elutants from Charcoal Samplers.

It must be remembered that the analysis protocol for pyranine dye is different than the protocol for the other four dyes discussed in this document. If the other dyes are present in a sample analyzed for pyranine dye their emission fluorescence peaks (if any) will be appreciably different than the values presented above. Because of this, there is very little analytical interference between fluorescein and pyranine dyes when both are present in a sample.

There is often some detectable fluorescence background encountered in the general range of pyranine dye in groundwater tracing studies. The following four criteria are used to identify fluorescence peaks which are deemed to be pyranine.

Criterion 1. For samples analyzed on the RF-5000U, there must be at least one fluorescence peak at the station in question in the range of 500.4 to 504.6 nm. The acceptable range for samples analyzed on the RF-5301 is 502.1 to 508.1 nm.

Criterion 2. The dye concentration associated with the pyranine dye peak must be at least 3 times the detection limit. For the RF-5000U, the detection limit in elutant samples is 0.055 ppb, thus this dye concentration limit equals 0.165 ppb. For the RF-5301, the detection limit in elutant samples is 0.015 ppb, thus this dye concentration limit equals 0.045 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 pyranine dye. 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 Ozark Underground Laboratory for Determining Positive Pyranine Dye Recoveries in Water Samples.

It must be remembered that the analysis protocol for pyranine dye is different than the protocol for the other four dyes discussed in this document. If the other dyes are present in a sample analyzed for pyranine dye their emission fluorescence peaks (if any) will be appreciably different than the values presented above. Because of this, there is very little analytical interference between fluorescein and pyranine dyes when both are present in a sample.

The fluorescence of pyranine decreases below a pH of about 9.5. Prior to analysis water samples are placed in a high ammonia atmosphere for at least two hours. A pyranine dye in water standard is placed in the same atmosphere as the samples. Prior to analysis samples are tested to insure that their pH is 9.5 or greater. If pyranine dye concentrations in a sample are so great as to require dilution for quantification of the dye concentration the diluting water used is OUL reagent water which has been pH adjusted in a high ammonia atmosphere.

The following criteria are used to identify fluorescence peaks which are deemed to be pyranine dye in water.

Criterion 1. The associated charcoal samplers for the station should also contain pyranine dye in accordance with the criteria listed earlier. 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 pyranine dye from the tracing work under investigation. For samples analyzed with the RF-5000U, the fluorescence peak should generally be in the range of 501.2 to 505.2 nm. For samples analyzed with the RF-5301, the fluorescence peak should generally be in the range of 504.1 to 510.1 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. For samples analyzed on the RF-5301 the detection limit in water is 0.010 ppb, thus this dye concentration limit equals 0.030 ppb. For samples analyzed on the RF-5000U the detection limit in water samples is 0.030 ppb, thus this dye concentration limit equals 0.090 ppb.

STATE WILDLIFE GRANT PROGRAM
SUBGRANT PROJECT BUDGET

1. Budget summary
Complete the project budget summary form below.

Budget Category	State Wildlife Grant Funds (Federal)	Cash Match (Non-Federal)	In-Kind Match (Non-Federal)	Total Project Cost
Salaries	3,300.00	17,200.00	5,000.00	25,500.00
Contract Services	20,000.00		-	20,000.00
Supplies and Materials	490.00	1,725.00		2,215.00
Travel	600.00	1,400.00		2,000.00
Equipment	-	-	-	-
Indirect Costs	5,610.00	4,675.00		10,285.00
TOTAL	30,000.00	25,000.00	5,000.00	60,000.00

2. Non-Federal Match (cash and/or in-kind)
Matching funds included in the grant budget are subject to the same requirements and conditions that apply to federal funds. These requirements include the certifications and assurances submitted with the grant application and any conditions attached to the grant award.

Additional details about match can be found here:
http://wsfrprograms.fws.gov/subpages/toolkitfiles/43cfr12.pdf

3. Budget Narrative
In addition to completing the subgrant project budget summary above, a detailed, itemized budget justification must also be completed on a separate sheet. It must contain the reason for each requested budget item and provide the basis and rationale for its cost. All requested (federal and non-federal) items must be thoroughly justified and clearly tied to project tasks, schedule and deliverables.

4. Indirect Costs
Indirect costs will only be approved if the applicant has an existing, approved rate from a cognizant federal agency. A copy of the current federal approval must be submitted with the grant proposal. Indirect cost rates greater than 10 percent must be must be justified in the budget narrative.

5. Grant period
Project costs and cash and/or in-kind matching can only be incurred after a formal grant award is made by the U.S. Fish and Wildlife Service and a grant agreement is executed by and between the Arkansas Game and

Budget and match questions may be addressed to
Matthew Warriner
Federal Aid Coordinator

Detailed Budget Narrative

Salaries SWG $3,300 TNC/OUL/TCCF Match $22,200 Total $25,500

Mike Slay, Ozark Karst Program Director, The Nature Conservancy, will provide approximately 100 days (0.39 FTE) for overall project management, supervision, implementation, monitoring, and reporting. Additional staff, including Conservation GIS Specialist and Karst/Rivers Technician, will provide technical support as needed. Salaries include fringe benefits and some overtime may be required. OUL is providing personnel match of $1,000 and TCCF is providing personnel match of $4,000.

Supplies SWG $350 TNC/OUL/TCCF Match $1,725 Total $2,075

Supplies include batteries, monitoring equipment, shipping containers for dye packet transport, and water and food supplies during field work.

Contract SWG $20,000 TNC/OUL/TCCF Match $0 Total $20,000

Contract to OUL to conduct dye trace study.

Travel SWG $600 TNC/OUL/TCCF Match $1,400 Total $2,000

Travel expenses include mileage reimbursement at 0.55 cents per mile for travel to and from the field site. It also includes costs for travel to meetings and presentation expenses.

Other SWG $140 TNC/OUL/TCCF Match $0 Total $140

Other expenses includes communication services such as mailings and telecommunications and miscellaneous expenses.

Indirect Costs SWG $5,610 TNC Match $4,675 Total $10,285

The Nature Conservancy has a current negotiated indirect cost rate of 23% that is accepted by USFWS.

Appendix 2.

Indirect Cost Negotiated Agreement with the US Fish and Wildlife Service

Nonprofit Organization
Indirect Cost Negotiation Agreement

EIN #: 53-0242652

Organization:

The Nature Conservancy
4245 North Fairfax Drive, Suite 100
Arlington, Virginia 22203-1606

Date: August 13, 2008

Report No(s).: 08-A-682(07F)
08-A-683(09P)

Filing Ref.:
Last Negotiation Agreement
dated July 24, 2007

The indirect cost rates contained herein are for use on grants, contracts, and other agreements with the Federal Government to which 2 CFR 230 (OMB Circular A-122) applies, subject to the limitations in Section II.A. of this agreement. The rates are negotiated by the U.S. Department of the Interior, National Business Center, and the subject organization in accordance with the authority contained in 2 CFR 230.

Section I: Rates

Type	Effective Period From	To	Rate	Locations	Applicable To
Final	07/01/06	06/30/07	23.28% 1/	All	All Programs
Fixed Carryforward	07/01/08	06/30/09	23.28% 1/	All	All Programs
		Fringe Benefit Rates			
Final	07/01/06	06/30/07	40.00% 2/	All	Regular Fringes
Final	07/01/06	06/30/07	12.00% 3/	All	Short-Term Fringes
Final	07/01/06	06/30/07	12.00% 4/	All	Foreign Fringes
Provisional	07/01/08	06/30/09	41.00% 2/	All	Regular Fringes
Provisional	07/01/08	06/30/09	12.00% 3/	All	Short-Term Fringes
Provisional	07/01/08	06/30/09	13.00% 4/	All	Foreign Fringes

1/ **Base:** Total direct costs, less external transfers, the value of land sold or donated to government agencies and other conservation organizations. Equipment costs valued between $5,000 and $50,000 are included in the base limited to the first year of capitalization. **All subawards, regardless of dollar amounts, are included in the base.**

Note: TNC has agreed to make all reasonable efforts to implement the exclusion of the portion of subawards in excess of $25,000 subject to a new system implementation in the FY 2011 rate negotiation.

2/ **Base:** Total salaries and wages for regular employees.

3/ **Base:** Total salaries and wages for short-term employees.

4/ **Base:** Total salaries and wages for foreign employees.

Note: The foreign fringes rate is applicable to benefits that are paid centrally by TNC's headquarters. Additional benefits are paid locally by TNC's foreign locations which are charged directly to government awards.

Treatment of fringe benefits: Fringe benefits applicable to direct salaries and wages are treated as direct costs; fringe benefits applicable to indirect salaries and wages are treated as indirect costs.

Treatment of paid absences: (a) For employees paid on TNC's U.S. payroll, the costs of vacation, holiday and sick leave pay are included in the organization's fringe benefit rate and are not included in the direct cost of salaries and wages. Claims for direct salaries and wages must exclude those amounts paid or accrued to employees for periods when they are on vacation, holiday or sick leave. Other paid absences are billed directly. (b) For employees paid on local payrolls in other country programs, paid absences are billed directly.

Section II: General

A. **Limitations:** Use of the rates contained in this agreement is subject to any applicable statutory limitations. Acceptance of the rates agreed to herein is predicated upon these conditions: (1) no costs other than those incurred by the subject organization were included in its indirect cost rate proposal, (2) all such costs are the legal obligations of the grantee/contractor, (3) similar types of costs have been accorded consistent treatment, and (4) the same costs that have been treated as indirect costs have not been claimed as direct costs (for example, supplies can be charged directly to a program or activity as long as these costs are not part of the supply costs included in the indirect cost pool for central administration).

B. **Audit:** All costs (direct and indirect, federal and non-federal) are subject to audit. Adjustments to amounts resulting from audit of the cost allocation plan or indirect cost rate proposal upon which the negotiation of this agreement was based will be compensated for in a subsequent negotiation.

C. **Changes:** The rates contained in this agreement are based on the organizational structure and the accounting system in effect at the time the proposal was submitted. Changes in organizational structure, or changes in the method of accounting for costs which affect the amount of reimbursement resulting from use of the rates in this agreement, require the prior approval of the responsible negotiation agency. Failure to obtain such approval may result in subsequent audit disallowance.

D. **Fixed Carryforward Rate:** The fixed carryforward rate is based on an estimate of the costs that will be incurred during the period for which the rate applies. When the actual costs for such periods have been determined, an adjustment will be made to the rate for future periods, if necessary, to compensate for the difference between the costs used to establish the fixed rate and the actual costs.

E. **Agency Notification:** Copies of this document may be provided to other federal offices as a means of notifying them of the agreement contained herein.

F. **Record Keeping:** Organizations must maintain accounting records that demonstrate that each type of cost has been treated consistently either as a direct cost or an indirect cost. Records pertaining to the costs of program administration, such as salaries, travel, and related costs, should be kept on an annual basis.

G. **Reimbursement Ceilings:** Grantee/contractor program agreements providing for ceilings on indirect cost rates or reimbursement amounts are subject to the ceilings stipulated in the contract or grant agreements. If the ceiling rate is higher than the negotiated rate in Section I of this agreement, the negotiated rate will be used to determine the maximum allowable indirect cost.

H. **Use of Other Rates:** If any federal programs are reimbursing indirect costs to this grantee/contractor by a measure other than the approved rates in this agreement, the grantee/contractor should credit such costs to the affected programs, and the approved rate should be used to identify the maximum amount of indirect cost allocable to these programs.

I. **Central Service Costs:** Where central service costs are estimated for the calculation of indirect cost rates, adjustments will be made to reflect the difference between provisional and final amounts.

J. **Other:**

1. The purpose of an indirect cost rate is to facilitate the allocation and billing of indirect costs. Approval of the indirect cost rate does not mean that an organization can recover more than the actual costs of a particular program or activity.

2. Programs received or initiated by the organization subsequent to the negotiation of this agreement are subject to the approved indirect cost rate if the programs receive administrative support from the indirect cost pool. It should be noted that this could result in an adjustment to a future rate.

3. New indirect cost proposals are necessary to obtain approved indirect cost rates for future fiscal or calendar years. The proposals are due in our office 6 months prior to the beginning of the year to which the proposed rates will apply.

Section III: Acceptance

Listed below are the signatures of acceptance for this agreement:

By the Nonprofit Organization:

/s/
Signature

Stephen C. Howell
Name (Type or Print)

Chief Financial & Administrative Officer
Title

8/11/08
Date

By the Cognizant Federal Government Agency:

/s/
Signature

Deborah A. Moberly
Name

Indirect Cost Coordinator
Indirect Cost Services
Title

U.S. Department of the Interior
National Business Center
Agency

Date **August 13, 2008**

Negotiated by Elena Chan
Telephone (916) 566-7111