Standard Operating Procedure
Sediment Sampling in Streams and Small Impoundments

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1.0 Scope and Application

Instructions presented in this Standard Operating Procedure (SOP) are for collecting representative sediment samples from surface water bodies. Sediment can be considered as solid material that is submerged/saturated (at least temporarily) or suspended in any surface water body. This includes sludges, lake bottom sediments, perennial and intermittent stream sediments, and marine sediments. This SOP describes in detail the sampling proposed for the Illinois River Watershed Sampling.

The Illinois River watershed in eastern Oklahoma and western Arkansas has been receiving inputs from agricultural runoff, including from fields where poultry waste has been applied. These wastes, along with the chemical constituents that make up the poultry waste, have apparently impacted the water quality and sediments in the streams and lakes in the Illinois River Watershed. The purposes of the work being performed are to evaluate and document 1) the linkage and relationship, if any, between the disposal of poultry wastes and environmental (primarily aquatic system) contamination within the Illinois River Watershed, and; 2) the resulting harm/injury to natural resources that may have resulted from the disposal of poultry wastes within the Illinois River Watershed.

2.0 Sampling Methods

Presented below are sampling instructions for the most common tube sampling technique for collecting sediment samples. For additional information, see Plumb, 1981 (00-0231), Spigolon, 1993 (00-0232), and Shelton (94-458). Prior to sample collection, water body characteristics (size, depth, flow) should be recorded in the field logbook. Sampling should proceed from downstream locations to upstream locations so that disturbance from sampling does not affect sampling quality. In collecting sediment samples from any source, care must be taken to minimize disturbance and sample washing as it is retrieved through the liquid column. Sediment fines may be carried out of the sample during collection if the liquid above is flowing or deep. This may result in collection of a non-representative sample due to the loss of contaminants associated with these fines.

While a sediment sample is usually expected to be a solid matrix, the sampler should not place the sample in the bottle and then decant the excess liquid. If the sample is collected properly, any liquid in the bottle is representative of sediment conditions. If the liquid above the sediment collection point is either flowing or greater than 6 inches in depth, a corer or other device that eliminates sample washing may be used to collect the sample to minimize washing the sediment as it is retrieved. It may be necessary to decant standing water from the top of the core. This should be done carefully and prior to transfer to the sample bottle.

2.1 Sediment Sampling Locations

In-stream sediment core samples will be collected from over fifty locations in streams, rivers, and lakes within the Illinois River Watershed.

2.2 Sample Types

Sediment samples can either be discrete or composite samples. A discrete sample is defined as a single aliquot from of a specific location at a given point in time. Composites are samples composed of two or more specific aliquots (discrete samples) collected from one or several sampling locations and/or different points in time. This type of sample represents an average value and can, in certain instances, be used as an alternative to analyzing a number of individual discrete samples and calculating an average value. It should be noted, however, that compositing can mask the presence of contaminants by diluting isolated concentrations of analytes that may be present in the environmental matrix.

2.3 Sampler Selection

The choice of samplers is dictated by sampling objectives and site constraints based on water depth. Each sampling technique presents various advantages and disadvantages for its application. For example, sample disturbance, sample volume, chemical and physical reactivity between potential contaminants and sampling tool materials, and ease of decontamination vary from technique to technique.

Discrete sediment samples from shallow to moderately deep water can be collected efficiently using polycarbonate (or Lexane) tube. Polycarbonate tube samplers are easy to use, portable, and are a direct method for obtaining sediment samples. The tube is forced into the sediment

and then withdrawn using a vacuum/suction technique. Additional sample methods may be utilized if the tube sampling method is not effective in collecting fine grained sediments. Alternative methods that may be applied are the use of a ponar dredge according to procedures provided by the Oklahoma Water Resources Board's Beneficial Use Monitoring Program (BUMP) or stainless steel scoop. These methods will be used as contingencies.

2.4 Data to be Collected

At each sediment sampling location, a suite of physical variables should be recorded. These variables are intended to locate each probing point and to quantify factors likely to be associated with deposition and accumulation of soft sediments. Variables may include:

- Water depth.
- Qualitative rating surface sediment type—silt, sand, gravel, cobble.
- Qualitative assessment of presence or absence of cohesive soft sediment.
- Sample location river segment type—straight, inside bend, outside bend, depositional zones, etc.
- Presence or absence of benthic organisms (i.e. observation of larval forms present or encrusted on river bottom cobble and rock).

2.5 Sample Nomenclature

Samples should be labeled according to the identification scheme: AA-BBB-CC-DD

AA is defined by:

- SD = Sediment
- SL = Soil
- FW = Fish
- SW = Surface Water

- BN = Benthics
- ZP = Zooplankton/Phytoplankton

BBB is defined by a three digit station identification number.

CC is defined by a two digit number which identifies the sample location along a transect. If the transect is identifier is not applicable, 00 should be used.

DD is defined by a two digit number which identifies the type of sample; i.e.; the original sample (01), field blank (02), or a duplicate sample (03).

3.0 Sediment Sampling Procedures

It is anticipated that the sampling locations are composed of sand/gravel/cobble mix and may require the use a sieves to separate out the fine sediment. Three Hubbard plastic sieves, with stainless steel US mesh sizes (5, 10, 35), are to be used. The sieves should be shaken aggressively and the small amounts of sediment should be worked through the sieves (while wearing medical nitrile gloves). The sediment that is retained on and passes through the no. 35 sieve will be kept for the sample. The sediment on the no. 5 and 10 sieves will be discarded.

3.1 Tube Sampling Procedure

The following is a procedure to collect sediment samples using polycarbonate tubes.

1. Locate the proposed sample location with a GPS unit. If there are no fine grained sediments at the location, the sample crew should go upstream and/or downstream of the planned coordinate until fine grained sediments are encountered.
2. Once at the location of fine grained sediment, record the water depth from the top of surface water to the top of sediment.
3. Lower the polycarbonate tube until the tube make contacts with the top of the sediment.
4. Gradually force tube into sediment (use a hammer or slambar if necessary to obtain the desired sediment depth of 6 inches).

5. Fill the remainder of the tube with water and cap to create a vacuum between the top of the sediment and the tubing. Tape cap to tube using either electrical or duct tape to ensure an airtight seal.

6. Pull the tube from the sediment using proper lifting techniques. If the tube becomes stuck in the sediment, two people may be needed to pull the tube. If the desired sediment recovery (6 inches) is not achieved, remove the sediment within the tube and flush using river water. Move the location over a foot and repeat procedure.

7. When desired sediment retrieval is achieved and tube is removed from the sediment, place the cap on the bottom of the tubing and secure with tape. Note that more than one core may be needed in order to obtain at 500 mL of fine sediment for the parameters. In that case, one or more samples will be collected in the same general area as the original core.

8. With tube held vertically, remove the top cap and cut a hole in the tube with a hack saw just above the top of the sediment to drain off water.

9. Cut off the tube just above the sediment surface and recap the upper end.

10. Label the top cap with the sample location ID, date, time, and "top" description using a permanent marker. Record the date, time, location of the sample, and other salient observations in the field book. Tubes must be kept vertical at an angel greater than 45 degrees.

11. Photograph each tube at the sampling location with a sign that contains the sample information detailed above.

12. Re-GPS the location if substantially different than planned coordinate or mark for survey.

13. Transport cores to the site work area, keeping them on ice.

14. The sediment core will be processed at the site work area in order to describe its structure and create samples for chemical analysis.

15. Place the polycarbonate tube on a decontaminated worktable and secure. Aluminum foil or plastic sheeting can be used to cover the worktable. For large recoveries, cut the core liner (filled with sediment) lengthwise along opposite sides. (Note: cut through the liner wall without cutting significantly into the sediment core itself. Disturbed

sediment adjacent to the liner wall should not be sampled. Also, it is important not to contaminate the undisturbed interior of the core with plastic chips or other debris from the cutting process. The best hand tool available for cutting hard plastic liners is a jig saw being careful to eliminate any of the sample with which the blade has come into contact. For shorter recoveries i.e. less than one foot of sediment, either push the sediment out or allow the sediment to fall out of the tube while holding vertically.

16. Extend a tape measure along beside the sediment, starting at the original top end of the core.

17. Photograph the core with a digital camera. Photograph the core section in overlapping frames using a small label with core field ID number so that it appears in each frame. Advance the tape measure appropriately for any additional sections of the same core.

18. While the core section is still intact, record a description of the core structure, noting zones of different color, classification, layering, sorting, and sediment type (silt, sand, clay, gravel, etc.).

19. Cores will be sectioned into one sample from the 0-6 inch interval.

20. Using disposable scoops or by hand with medical gloves, place soil on aluminum foil or stainless steel bowl and homogenize. (Note that if more than one sample was collected for a location, the sediment from all samples will be combined and homogenized.) Some locations may be more gravelly than others and may require the use a sieves as mentioned in Section 3.0. After sieving, put sediment into the appropriate sample containers.

21. Label each container with a unique sample identification number as outlined in Section 2.5. Be sure to complete the label carefully and clearly, addressing all the categories or parameters.

22. Store the sample bottles on ice or in a refrigerator until transfer shipment to the analytical laboratories.

23. Complete all chain-of-custody documents and field sheets and record in the field logbook.

24. Decontaminate sampling equipment after use and between sample locations using phosphate-free soap and deionized water. Sample equipment may include hacksaw or jigsaw blades. All other equipment i.e. polycarbonate tubing, medical gloves, etc. are

disposable. Sample material not sent to the lab for analysis will be disposed in a municipal landfill.

25. Repeat the procedure for each sample location.

3.2 Stainless Steel Scoop Sampling

The following is a procedure to collect sediment samples using a stainless steel scoop or shovel.

1. Locate the proposed sample location with a GPS unit. If there are no fine grained sediments at the location, the sample crew should go upstream and/or downstream of the planned coordinate until fine grained sediments are encountered.

2. Once at location of fine grained sediment, record the water depth from the top of surface water to the top of sediment.

3. Lower the scoop into the sediment being careful not to stirrup any sediment. Slowly bring the scoop and sample to the surface, again being careful not the let the fine sediment be washed downstream.

4. Place sediment on aluminum foil or in a stainless steel bowl and homogenize. (Note that if more than one sample was collected for a location, the sediment from all samples will be combined and homogenized.) Some locations may be more gravelly than others and may require the use a sieves as mentioned in Section 3.0. After sieving, put sediment into the appropriate sample containers.

5. Label each container with a unique sample identification number as outlined in Section 2.5. Be sure to complete the label carefully and clearly, addressing all the categories or parameters.

6. Store the sample bottles on ice or in a refrigerator until transfer shipment to the analytical laboratories.

7. Complete all chain-of-custody documents and field sheets and record in the field logbook.

8. Decontaminate sampling equipment after use and between sample locations using phosphate-free soap and de-ionized water. Sample equipment may include stainless

steel scoop, sieves, bowl etc. All other equipment i.e. polycarbonate tubing, medical gloves, etc. are disposable.

9. Repeat the procedure for each sample location.

3.3 Ponar Dredge Sampling

The following is a procedure to collect sediment samples using a ponar dredge.

1. Locate the proposed sample location with a GPS unit. If there are no fine grained sediments at the location, the sample crew should go upstream and/or downstream of the planned coordinate until fine grained sediments are encountered.

2. Once at location of fine grained sediment, record the water depth from the top of surface water to the top of sediment.

3. Insert the tension spring and slowly lower the dredge until it reaches the sediment bed. Slowly raise the dredge about two feet and let it drop into the sediment. The force of the dredge in contact with the sediment will release the tension spring and close the dredge on the top 4 to 6 inches of sediment. Pull the dredge out of the water.

4. Open the dredge and place the sediment on aluminum foil or in a stainless steel bowl and homogenize. (Note that if more than one sample was collected for a location, the sediment from all samples will be combined and homogenized.) Some locations may be more gravelly than others and may require the use a sieves as mentioned in Section 3.0. After sieving, if needed, put sediment into the appropriate sample containers.

5. Label each container with a unique sample identification number as outlined in Section 2.5. Be sure to complete the label carefully and clearly, addressing all the categories or parameters.

6. Store the sample bottles on ice or in a refrigerator until transfer shipment to the analytical laboratories.

7. Complete all chain-of-custody documents and field sheets and record in the field logbook.

8. Decontaminate sampling equipment after use and between sample locations using phosphate-free soap and de-ionized water. Sample equipment may include stainless

steel scoop, sieves, bowl etc. All other equipment i.e. polycarbonate tubing, medical gloves, etc are disposable.

9. Repeat the procedure for each sample location.

4.0 Sample Containers, Preservation Techniques, Quality Control

For sediment samples, there is no need for sample preservation beyond storing and shipping the samples on ice. Periodically throughout the sampling, samples will be packed and shipped in coolers to the appropriate laboratories. Shipping addresses and contact information are listed below:

A&L Analytical Laboratories, Inc.
2790 Whitten Rd.
Memphis, TN 38133
Contact: Jimmy Ferguson or Scott McKee, (800) 264-4522
E-mail: smckee@allabs.com

General Engineering Laboratories, LLC (GEL)
701 Pine Ridge Rd, Unit 5
Golden, CO 80403
Contact: Paul Winkler, (720) 253-3093
E-mail: Paul.winkler@gel.com

Great Lakes Environmental Center (GLEC)
739 Hastings Street
Traverse City, MI 49686
Contact: Mailee W. Garton, (231) 941-2230
E-mail: mgarton@glec-tc.com

Environmental Microbiology Laboratory
1150 Bayhill Drive, Suite 100
San Bruno, CA 94066
Contact: Meagan S. Tatreau 858-268-2762
E-mail: mtatreau@emlab.com

Field control samples will be collected by the sampling team to determine whether data are of suitable quality. Control samples may include trip blanks, duplicates, decontamination (rinsate) blanks, or split sample. Duplicates collected as either co-located or split samples should be collected one of every twenty samples.

5.0 Documentation

Bound field logbooks should be used for the maintenance of field records. All aspects of sample collection and handling as well as visual observations shall be documented in the field logbooks. All entries in field logbooks should be legibly recorded and contain accurate and inclusive documentation of an individual's project activities.

6.0 Additional Information

Other contact information:

Bert Fisher, PhD
Lithochimeia, Inc.
222 South Kenosha Ave.
Tulsa, OK 74120
Telephone: 918-382-9784

Ronald French
CDM
100 North Tucker Blvd.
Suite 550
Saint Louis, MO 63101
314-241-8510

7.0 References

Plumb, R.H., Jr. 1981. Procedures for Handling and Chemical Analysis of Sediment and Water Samples. USACE WES/EPA.

Spigolon, S.J. 1993. Geotechnical Factors in the Dredgeability of Sediments: Report 2, Geotechnical Site Investigation Strategy for Dredging Projects. Contract Report DRP-93-3. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

American Society for Testing and Materials. 1995. ASTM Standard D4823-95, "Guide for Core-Sampling Submerged, Unconsolidated Sediments," pp 282-295. In: American Society for Testing and Materials (ASTM). 1997. ASTM Standards on Environmental Sampling. Second Edition. ASTM Publication: 03-418097-38.

American Society for Testing and Materials. 1992 (1996). ASTM Standard D3676-92, "Practice for Preparation of Sediment Samples for Chemical Analysis," pp 301-303. In: American Society for Testing and Materials (ASTM). 1997. ASTM Standards on Environmental Sampling. Second Edition. ASTM Publication: 03-418097-38.

U.S. Environmental Protection Agency (EPA) and U.S. Army Corps of Engineers (USACE). 1998. Great Lakes Dredged Material Testing and Evaluation Manual. Final Draft. Prepared by U.S. Environmental Protection Agency Regions 2, 3, 5 and Great Lakes National Program Office and U.S. Army Corps of Engineers Great Lakes & Ohio Division.

Shelton, Larry R. and Capel, Paul D. Guidelines for Collecting and Processing Samples of Stream Bed Sediment for Analysis of Trace Elements and Organic Contaminants for the National Water-Quality Assessment Program. United States Geological Survey (USGS). 1994. Open-File Report 94-458.

9.0 Revised Dates*

The following are other revision dates applicable to this SOP.

Revision 2 – January 18, 2006

Revision 1 – July 22, 2005

Standard Operating Procedure
Litter and Soil Sampling

SOP: 5-1
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Prepared: Roger Olsen Review: Darren Brown

Approved: _____ Date Approved: _____

1.0 Overview and Application

This standard operating procedure (SOP) describes field procedures used for collection of soil and litter/manure samples during the Illinois River watershed project.

2.0 Selection of Soil and Litter/Manure Sampling Locations

Sample locations will be selected from either contract growers' farms or company-owned facilities. At each of these farms/facilities, litter from the poultry houses will be collected. Fields where documentation of litter application from a specific farm and Integrator is available from the Oklahoma Department of Agriculture, Food and Forestry will also be selected for sample collection. Field locations selected will be within the Illinois River Watershed. Considerations for sample collection from farms/facilities and fields include:

1. Poultry litter/manure has been consistently generated,
2. Poultry litter/manure is currently being generated,
3. Poultry litter/manure has been consistently (every year for the at least the past 3 years) applied to land (Litter Application Locations, "LALs") associated with the Farm/Facility,
4. Availability of land upon which poultry litter/manure or other fertilizers have not been applied (Control Locations, "CLs").

To the extent possible, the following information should be collected for each associated Farm/Facility:

1. Name of Farm/Facility owner and Farm/Facility contact person,
2. Physical address and location (section-township-range) of Facility,
3. Contact address of Farm/Facility owner or Farm/Facility contact person,
4. Contact phone number of Farm/Facility owner or Farm/Facility contact person,
5. Whether or not one or more LALs can be accessed at the Farm/Facility,

6. The physical location of each LAL.
7. Whether or not one or more CLs can be accessed at the Farm/Facility.
8. The physical location of each CL.
9. Whether or not a litter/manure and/or nutrient management plan has been prepared for LALs at the Farm/Facility,
10. Estimates of the amounts, rates and dates of prior litter/manure applications to each LAL at the farm/facility,
11. Estimates of litter treatment or amendments added to each LAL (e.g., alum, etc), if any, and information as to amount, rate and dates of application
12. Number, type, dimensions, and capacity of poultry grower houses (or other poultry/egg production facilities, as appropriate) operated at Farm/Facility ("Poultry Houses").

Most of the above information may not be available to the field crews. These data may be acquired through the attorneys during deposition.

3.0 Sampling Documentation

3.1 Sampling Log Book and Sampling Forms

1. Sampling Log Books and/or Sampling Forms will be maintained by the field crews.
2. Pages in the Sampling Log Book will reference specific Sampling Forms by use of the Facility Identification.
3. The Sampling Log Book shall be bound and will be constructed of waterproof paper.
4. Entries in the Sampling Log Book or on the Sampling Form will be made in permanent ink, preferably black ink.
5. Each page of the Sampling Log Book will be dated.
6. The preparer will initial each page of the Sampling Log book.
7. If available, and to the extent possible, for each Farm/Facility sampled, the following information will be recorded in the Sampling Log Book or on the Sampling Forms:
    a. Name, address and phone number of the Farm/Facility owner,

b. Identification of the Farm/Facility, FAC1 – FAC8,

c. Name, address and phone number of the Farm/Facility operator,

d. Name, address and phone number of the Integrator responsible for the Farm/Facility,

e. Names, addresses and phone numbers of persons who have spread litter/manure on LALs associated with the Farm/Facility,

f. The amounts, rates and dates of prior litter/manure applications to specific LALs at the Farm/Facility (confirm State Reports),

g. The existence of prior soil sampling data for LALs or CLs at the facility (yes or no),

h. The water supply for the Farm/Facility,

i. The legal description (qtr-qtr-qtr-sec-twp-rng) of the property related to the Farm/Facility,

j. The legal description (qtr-qtr-qtr-sec-twp-rng) of the CLs at the Farm/Facility,

k. Type of animals generating litter (broilers, layers, pullets, turkeys, etc.),

l. Number of flocks of birds that have used the litter that is sampled,

m. The number of birds in each flock that have used the litter that is sampled,

n. The time since birds last used the litter,

o. Litter treatment (e.g. alum amendment), if any, and information as to amount, rate and date or dates of treatment,

p. Information as to any other fertilizers, chemicals or soil amendments added during the last five years,

q. Use of each LAL by cattle (yes or no) and typical number of cattle,

r. Specific information listed within this protocol,

s. Sketch map of each LAL with approximate dimensions; indicate local features on the sketch (vegetation, water bodies, adjacent fields, location of poultry houses, roads, old fence rows, livestock feeding areas, livestock grazing areas, etc); dimensions and features can also be placed on the aerial photographs,

t. Additional information such as identified springs, wells, seeps, or sinkholes should be indicated on the sketch map or aerial photograph

u. Land slope of each LAL (or LAL sub-area),

v. Distance to nearest water body,

w. Notes on weather (temperature, wind, last precipitation event, etc),

x. Type of vegetation currently on the LAL, if any, and any known vegetation grown in past 5 years,

y. Use of adjacent fields, and;

z. Other information as appropriate or relevant.

3.2 Photographic Record

A photographic record shall be made and maintained for all sampling activities on the LAL. Pictures of the LALs, CLs and the outsides of the poultry house will be taken. No pictures of sampling activities inside the poultry houses will be taken. A video recording will be made, to the extent possible, from a vantage point immediately outside the poultry house.

All photographs made shall be time and date stamped.

3.3 Chain-of-Custody

A Chain-of-Custody will be prepared for each set of samples transferred to the soil and litter processing lab (CDM Support Laboratory in Denver, Colorado). A second chain-of-custody will be prepared at the processing lab for the analytical laboratory.

The Chain-of-Custody to the soil processing lab shall, at a minimum, contain the following information:

1. The project name, ***Illinois River Watershed Soil and Litter/Manure Sampling,***
2. Name of person or entity relinquishing the sample and was part of the field crew,

3. Signature blocks with dates and times for all persons having custody (sampler, shipper, processing laboratory, etc),
4. For each sample related to a Chain-of-Custody:
    a. The unique numeric identifier on the submitted sample container/bag,
    b. The date and time interval the samples were collected,
    c. The sample "matrix" (i.e. SOIL or LITTER or WATER).

4.0 Soil Sampling

4.1 Litter Application Locations (LALs) and Control Locations (CLs)

4.1.1 Permissible Soil and Weather Conditions

1. Soils are not to be sampled if water saturated.
2. Soils are not to be sampled during precipitation events.

4.1.2 Division into Sampling Areas

A Sampling Area is an area within a LAL or CL that is reasonably homogenous with respect to soil types, soil properties, topography, landscapes, management history (to the extent known), and other relevant factors, as appropriate.

1. For each LAL or CL sampled, the LAL or CL shall be divided into a maximum of four Sampling Areas, identified as A, B, C and D.
2. Sampling Areas identified within the LAL or CL shall be a minimum of approximately one acre and shall not exceed approximately 10 acres.
3. In making determinations concerning the division of the LAL or CL into Sampling Areas, the person or persons making those determinations shall consult the USGS topographic map, aerial photograph, and/or other data including relevant USDA/NRCS soil survey. The data consulted shall be identified by reference in the Sampling Log Book.
4. The person or persons who make the determinations concerning the division of the LAL or CL into Sampling Areas shall prepare a sketch map of the LAL or CL and its constituent Sampling Areas. This sketch map shall show the approximate boundaries of each Sampling Area and the estimated area of each Sampling Area. This information can also be recorded on the aerial photographs.

4.2 Identification of Sub-Sampling Locations

A Sub-Sampling Location is a one to ten acre area within a Sampling Area at which individual soil samples will be collected. A total of 20 sub-sampling locations shall be sampled for each Sampling Area. The selection of Sub-Sampling Locations shall avoid:

1. Old fence rows,
2. Livestock feeding areas,
3. Livestock loafing areas, and;
4. Localized conditions atypical of the Sampling Area.

The geographic coordinates (Latitude and Longitude) of the first Sub-Sampling Location in each Sampling Area or a corner of the Sampling Area shall be determined using a Global Positioning System (GPS) receiver accurate to at least five (5) meters. These geographic coordinates will be recorded in the Sampling Log Book.

Representative Sub-Sampling locations will be documented with a time and date stamped photograph.

The following procedure will be used, when possible, to establish a grid system for each Sampling Area (Subareas A, B, C, or D).

1. On the map/aerial photo, select the general area to establish a grid pattern of twenty sampling locations. If the field configuration permits, the Subarea should be either a square or rectangle in shape.
2. The grid setup in either a square or rectangle shape will have 4 evenly spaced sample points within a width and 5 evenly spaced sample points within a length. In other words, the grid system will typically be a 4 by 5 grid, with sample points at the nodes.
3. If the selected grid location is near a fence line or tree line, the corner should be established by insetting a distance of Width (W) of Subarea grid divided by 8 (W/8).
4. Once the corner is established, determine the spacing of the remaining width (W) grid points by dividing the remaining width (RW) by 3. RW equals W – W/4. In summary, width grid points will be established at W/8 and then at distances of (W-W/4)/3.
5. For the length of the Subarea grid, the spacing of the length (L) grid points will be the remaining length (RL) divided by 4. Keeping in mind that the inset distance was W/8, RL equals L – W/4. In summary, length grid points will be established at W/8 and then at distances of (L-W/4)/4.

6. If the Subarea grid is more than 50 feet from a fence or tree line within a field, the grid spacing is simply W/3 by L/4.
7. If the field configuration does not permit the use of rectangular or square Subarea field configurations, try to establish a grid that provides for relatively uniform spacing within the field shape.
8. Record the grid spacing in the field book and, if possible, on the aerial photograph or map.
9. Once the grid spacing has been determined on the aerial photograph or map, the field crew shall use the maps to establish grid layout in the field. Conditions permitting, the grid points will be marked with pin flags, which will be removed after the grid point is sampled.

4.3 Soil Samples to be collected at each Sub-Sampling Location

For purposes of this Protocol, a Sub-Sampling Location shall be an area defined by a triangle with three-foot sides with the middle placed on the Sub-Sampling Location. When possible, one point of the triangle will be oriented in the north direction.

At each Sub-Sampling Location, core samples with a length of at least six inches will be collected at the corners of the triangle. The samples will be divided into three separate soil samples as follows:

1. Four (4) to Six (6) Inch Sample depth. This two inch sample will be collected by measuring the length of the core from the top of the sample. The two inch section of core will be placed in a plastic bag with the appropriate identification.
2. Two (2) to Four (4) Inch Sample depth. This two inch sample will be collected by measuring the length of the core from the top of the sample. The two inch section of core will be placed in a plastic bag with the appropriate identification.
3. Zero (0) to Two (2) Inch Sample depth. This two inch sample will be collected by measuring the length of the core from the top of the sample. The two inch section of core will be placed in a plastic bag with the appropriate identification.
4. One core sample will be collected at each corner of the triangle until enough sample is collected (approximately 100 to 200 grams, depending upon QA/QC needs). The first core will be at the triangle corner oriented to

the north. Additional cores, if necessary, will be collected at the remaining triangle corners.

5. The soil samples collected at each sub-sampling location will be collected with soil probe coring devices either marked for 6-inch, 4-inch and 2-inch depths, or with a vertical slot so that the core measurements can be made with a ruler. The diameters of all soil corers used should be the same, and should be of a diameter consistent with general practice for agricultural soil sampling.
6. Whenever a soil sample is to be collected, thatch and other plant residue shall be moved aside or lightly scuffed aside without removing the surface soil prior to pushing the soil probe core into the soil.
7. Coring devices will be manually driven to at least six inches in depth if possible. If coring devices are being driven with a post hole driver and the coring device shows no or very limited advancement after ten consecutive blows, the coring device will be considered to have reached refusal. The corer shall be extracted and the available core collected. Attempts to collect soil samples from the missing core depths can be made at the remaining triangle corners.
8. Core recovery will be noted for each 2-inch interval. Recovery will be qualified as good, poor, or no recovery. Poor recovery will note that an incomplete two-inch sample was recovered.
9. In the event that soil conditions do not permit the use of a soil probe coring device, samples may be collected with a shovel.
10. Thatch and other plant residue shall be removed prior to collecting a sample with a shovel.
11. When a shovel is used for collection the following procedure shall be followed:
    a. At each sub-sampling location, dig a hole at least 6 inches deep.
    b. During excavation, material from zero to 2 inches should be placed in a bag appropriately labeled for the depth. Then the material from 2 to 4 inches should be placed in a separate bag. And finally the material from 4 to 6 inches should be placed in a separate bag.
    c. Material from each depth interval may be placed on a plastic sheet to facility sample collection.

When possible, representative soil samples collected from the field will be documented with a time and date stamped photograph.

4.4 Handling of Samples

All individual samples from each sub-location will be placed in individual plastic bags. The sample number will be placed on the outside of the sample bag. Each sampling area (up to four sampling areas per LAL) will have 60 individual samples (20 sub-sample locations x 3 sample depths = 60 samples). For each subarea, the sample bags from each depth will be segregated and placed in a larger resealable reinforced plastic bag (typically one-gallon freezer bags). For example, all zero to 2-inch samples within sub-location "A" will be placed in the same one gallon resealable plastic bag. Soil samples from one LAL or CL will then be placed within one large plastic bag which will be sealed before it is placed in an insulated container (cooler). All samples will be shipped to the soil/litter processing laboratory for compositing.

Compositing of samples will be performed at the soil processing laboratory.

4.5 Field QA/QC Samples (Soils)

1. Field Duplicate Samples may be created at the soil processing lab.
2. Blind Standard: A blind standard of a certified reference soil may be sent to the analytical lab for every 50 samples send to the analytical laboratory. The blind standard will be sent by the soil processing lab.
3. Decontamination Blank: a sample of the final decontamination rinsate may be collected and forwarded to the soil processing laboratory for analysis at a frequency of one decon rinsate collected after sampling is completed at a facility or at a rate of one per 20 decontamination events . The decon blank will be generated in the field using laboratory grade distilled water.

4.6 Decontamination Procedures

Full decontamination will occur between every LAL property, or upon exit of a growers' field onto a public right-of-way. A decontamination station will be established and maintained at the boundary of the grower's property and the public right-of-way, unless a location has otherwise been designated by the grower or integrator.

Full decontamination steps will be as follows:

1. Bagged samples will be placed into a receiving bag held by the members of the decon team.
2. All electronic equipment will be transferred from the resealable plastic bags carrying the electronic equipment into the field into a plastic bag held by a member of the decon team.
3. All reusable tools will be decontaminated by removing all soil or other material by brushing/scraping the equipment. The equipment will then be washed with a phosphate free soap solution. This will be followed by a rinse a 6 or 10 percent bleach solution and then with distilled water.
4. All disposable PPE equipment such as gloves, coveralls, boot covers, etc. will be removed and disposed into a plastic trash bag held by the decon team. The trash bag will be placed into a second trash bag and tied shut.
5. The rubber boots worn by the field crews will then be decontaminated using the same procedures used to decontaminate the reusable tools. Upon decontamination of the rubber footwear, the field crew members may leave the field.
6. Any vehicles driven onto the LAL fields will be driven through the decon line with the front tires brushed to remove soil and other material, sprayed and brushed with a phosphate free detergent solution, and then sprayed with a bleach solution. Once the front tires and wheel wells have been decontaminated, the rear tires will be addressed using the same procedure before the vehicle enters the public right-of-way.

Decontamination between subareas within an LAL and not requiring Full Decontamination procedures will consist of removing soil material from the corer barrel and the knife or implement used to cut the soil samples prior to collection of the first soil sample from the next LAL subarea.

After discussion with Oklahoma Department of Agriculture, Food, and Forestry personnel, it was determined that the decontamination water and solutions will be considered *de minimus* material and will be disposed of on the ground on the right-of-way leading into the facility.

5.0 Litter/Manure Sampling

5.1 General Conditions

1. All litter/manure samples will be collected with litter/manure in place within Poultry Houses.

2. Litter/manure may be sampled at any time regardless of weather conditions.
3. More than one Poultry House may be sampled at a Farm/Facility. The litter/manure from each house will be maintained as a separate sample.
4. Bio-Security Protocol dictated by the Oklahoma Department of Agriculture, Food, and Forestry, and as supplemented by individual integrators and/or growers will be followed at all times.
5. The sampling team will consist of three individuals. One individual will enter the Poultry House and collect the samples. A second individual will accompany the first individual onto the property but will only video tape the first individual from a vantage point generally outside of the Poultry House. The third individual will maintain their position at a decontamination station anticipated to be at the public right-of-way entrance to the grower's property.
6. The individual responsible for the video taping will relay house entry times, house exit times, start of compositing times, and completion of sample compositing times to a third individual located at the public right of way entry to the grower's property via radio communications. The third individual will enter those times into the field book.
7. Prior to entry onto the grower's property, a decontamination/ sample handling station will be established on the public right-of-way adjacent to the grower's property, or on the grower's property if an adequate location is identified by the grower.

5.2 Location and Distribution of Poultry House Sub-Sample Collection Points

1. Broiler or Pullet Houses
    a. Sub-samples are collected from approximately 1/3 house-width zones.
    b. Approximately six samples are collected from each zone.
    c. Sub-samples should be located so as to obtain two samples from around the waters, feeders and walls on each side of the house.
    d. Depending upon the size of the poultry house, sub-samples are estimated to be spaced at 20 to 25 pace intervals within each 1/3 zone.

e. Sub-sampling locations alternate between the "sides" of each zone (i.e. a "zig-zag" pattern is traversed between sampling locations within a zone).

f. Sub-samples collected from adjacent zones should not be immediately adjacent.

2. Breeder Houses (partially slatted)

a. Sub-samples will be collected from both slatted and litter areas.

b. Twenty (20) sub-samples will be collected

c. Sub-samples will proportionally represent the relative aerial proportion of slatted and litter areas; for example if 2/3 of the house is under slats, and 1/3 is litter area, 14 litter/manure samples should be collected from under the slats and 7 litter/manure samples should be collected from the litter area.

d. Sub-samples taken beneath slats will be as fully penetrating of the manure as possible and will be distributed so as to obtain a representative sample of the entire slatted area.

e. Sub-samples from litter areas will be collected in the same manner (i.e. "zig-zag" pattern) as used for broiler or pullet houses.

3. Other Circumstances

a. Sampling of litter/manure within a Poultry House for circumstances and conditions other than those described for Broiler, Pullet or Breeder Houses will be conducted so as to obtain a representative sample of the litter/manure within that Poultry House.

b. The circumstances or conditions requiring a variation from the sampling protocol described for Broiler, Pullet or Breeder Houses will be documented in the Sampling Log Book.

c. A description of the method(s) and procedures used to collect a representative sample of the litter/manure within a Poultry House in which the sampling protocol for Broiler, Pullet or Breeder Houses cannot be followed will be documented in the Sampling Log Book.

d. The method(s) and procedures used to collect a representative sample of the litter/manure within a Poultry House in which the sampling protocol for Broiler, Pullet or Breeder Houses cannot be

followed will follow the principles embodied in the reference materials.

e. All sub-samples will be collected with an appropriate solid manure sampling device.

f. All samples from litter areas will be collected through the full thickness (surface to base) of the litter/manure.

g. All samples from slatted areas will, to the extent possible, be collected through the full thickness (surface to base) of the litter/manure.

h. Immediately after collection, all sub-samples will be placed in a plastic bag contained inside a 5-gallon plastic bucket.

i. For partially slatted houses, sub-samples from slatted and litter areas will be composited together.

4. Container

a. During sample collection, all samples will be placed into a 5-gallon bucket double-lined with plastic bags.

b. After sample collection, the material within the 5-gallon bucket will be manually mixed using either a clean hand trowel and/or the shovel used to collect the samples inside the poultry house.

c. The rough mixing/compositing will be accomplished by breaking the cake material and turning over, to the extent possible, the entire contents of the bucket without damaging the plastic bag liners.

d. The rough mixing/compositing will be conducted immediately outside the poultry house and immediately after sample collection.

e. After mixing, a small subsample (500 ml in volume) will be removed via hand trowel and placed into a sterile plastic bottle or whirl pack, which will be immediately sealed and labeled with the Sample ID.

f. The remaining material within the plastic bags lining the 5-gallon bucket will be tied shut. At the decon station, the plastic bags will be placed into another appropriately sized plastic bag which will be tied shut, sealed with duct tape, and the sample ID written on the duct tape with an indelible marker.

g. The small subsample container will also be placed into a resealable plastic container at the decon station. This second container will also be labeled with the sample ID and date using an indelible ink marker.

h. The samples will then be placed in an appropriately-sized cooler with ice that will also be double bagged in plastic bags and sealed shut.

i. The appropriate chain-of-custody will be placed inside each cooler and the cooler sealed with tape and a chain of custody label.

j. The cooler with the 500 ml volume sample will be shipped overnight directly to the EML Lab for bacteria analyses.

k. The cooler with the remaining litter sample will be shipped overnight to the soil/litter processing lab.

5. Exiting the Property

a. Once the field team has sampled a Poultry House, the team will approach the decontamination station maintained at the boundary of the grower's property and the public right-of-way, unless a location has otherwise been designated by the grower or integrator.

b. Samples will be handed across the decon station line into clean receiving bags as noted above.

c. All electronic equipment will be passed from the sampling team into a resealable plastic bag held by the decon team member. The electronic equipment will be wiped down with an antibacterial wipe followed by a cloth moistened with dionized water.

d. Sample trowels, shovels, and empty collection bucket will be offered to the grower. If the grower does not want these tools, they will be included with the protective coveralls and gloves to be discarded to a sanitary landfill or a municipal incinerator.

e. All disposable Personal Protective Equipment (PPE) to be disposed will placed into double bagged plastic bags held by the decon team member. These bags will be disposed at a dumpster serviced by a municipality that either disposes of the trash at a sanitary landfill or by incineration.

f. The rubber boots of the sampling team will be subject to decontamination by a phosphate free detergent rinse, followed by a bleach solution rinse, followed by a tap water rinse.

g. Once the boots have been decontaminated, the sampling team may cross the decontamination line onto the public right-of-way.

5.5 Field QA/QC Samples (Manure/Litter)

1. Field Duplicate Samples may be created in the soil/litter processing lab.
2. Decontamination Blank (created in the field): a sample of the final decontamination rinsate may be collected and forwarded to the processing lab to send to the analytical lab for analysis at a frequency of one decon rinsate for every facility. A decon rinsate would only be generated in the event that sampling equipment were to be reused. Currently, the plan is that all sampling equipment for manure/litter sampling is disposed after a single use.

6.0 Identification of Samples

Identifying information to be recorded on the sample label for soil samples:

1. Alphanumeric identification of the LAL or CL: LAL1 – LAL24, CL1 – CL8. The log book will be used to record the farm and location of each LAL or CL.
2. Alphanumeric identification of the Sampling Area: A – D
3. Alphanumeric identification of the Sub-sample location: 1 - 20
4. Alphanumeric identification of the depth of collection (i.e. –2, -4, -6)
5. The following sample number is an example of the soil sample taken from LAL field number 5, sampling area B, sub-sample location 18, and a depth of 2 inches:

   LAL5-B-18-2

6. For samples submitted to the analytical lab, additional alphanumeric identification of the type of sample will be added to the end of the identification number:
   a. A= laboratory sample
   b. B = laboratory duplicate
   c. C = reference soil (standard)

d. D = decontamination blank (added to field samples)

e. E = laboratory QA/QC (extra volume)

7. Date of sample collection (only on chain-of-custody),
8. Time of sample collection (only on chain-of-custody),
9. Initials of the person collecting the sample (only on chain-of-custody).

6.1 Identifying information to be recorded on the sample label for litter/manure samples:

1. Alphanumeric identification of the Facility: FAC1 – FAC8.
2. Alphanumeric identification of the Poultry House: A – C
3. The following sample number is an example of the litter sample taken from facility number 5 and poultry house B:

   FAC5-B

4. Samples sent to the analytical laboratory will have alphanumeric identification of the type of sample added to the end of the number:
   a. A= laboratory sample
   b. B = laboratory duplicate
   c. C = reference soil (standard)
   d. D = decontamination blank (added in the field)
   e. E = laboratory QA/QC (extra volume)
5. Date of sample collection (only on chain-of-custody),
6. Time of sample collection (only on chain-of-custody),
7. Initials of the person collecting the sample (only on chain-of-custody).

7.0 Shipment of Samples to the soil/litter processing laboratory and to the analytical laboratory

1. Once placed in sampling containers (plastic bags or jars), samples will be placed on ice (double bagged and sealed in plastic bags) within insulated protective containers.
2. If possible, samples will be shipped immediately via overnight shipment to the analytical laboratory.

3. In no event, will FAC samples be held more than 24 hours before shipment. Depending upon circumstances, LAL samples may be held as much as 48 hours before shipment.
4. Samples will be sent to the laboratory under a Chain-of -Custody.
5. A custody seal will be placed on the outside of the container across the area between the lid and the container. The custody seal will be signed.
6. The Chain-of-Custody will be sealed in a plastic bag and placed within the insulated protective container holding those samples to which it refers.
7. Samples shipped to the EML laboratory will be shipped to the following address:

   Environmental Microbiology Laboratory
   1150 Bayhill Drive, Suite 100
   San Bruno, CA 94066
   Contact: Cole Mackelprang, 858-268-2762
   e-mail: cmackelprang@emlab.com
   Contact: Megan S. Tatreau, 858-268-2770

8. Samples shipped to the CDM Prep laboratory will be shipped to the following address:

   CDM
   2714 Walnut Street
   Denver, CO 80205
   Contact: Todd Burgesser, 303-298-1311
   e-mail: burgesserte@cdm.com

8.0 Analytical

8.1 Laboratory

The laboratory conducting the analyses will be experienced in conducting the specified analyses and will have certifications to conduct the specified analyses.

All analyses and sample preparation will be conducted using accepted and published protocols and/or methods.

8.2 Analytical Protocols

Soil samples will be analyzed for one of two sets of parameters (short list or long list). Table 1 provides the parameters and analytical methods for the short and Table 2 provides the parameters and analytical methods for the long list.

Litter samples will be analyzed for Table 2 parameters.

Table 1: Short List Parameters – Soil

Parameter	Method
Moisture content (%)	Gravimetric (105C)
Organic matter	Walkley-Black (Modified)
Soil pH	Water 1:1
Soil Conductivity	Water 1:2
Total Nitrogen	Kjeldahl, modified
Total Aluminum (Al)	EPA SW-3050/6020
Total Phosphorus (P)	EPA SW-3050/6020
Total Arsenic (As)	EPA SW-3050/6020
Total Copper (Cu)	EPA SW-3050/6020
Total Zinc (Zn)	EPA SW-3050/6020

Table 2: Long List Parameters – Manure and Soil

Parameter	Method
Moisture content (%)	Gravimetric (105C)
Organic matter	Walkley-Black (Modified)
Texture (% sand, silt and clay)	Hydrometer ASTM-D422
Soil pH	Water 1:1
Soil Conductivity	Water 1:2
Total Phosphorus (P)	EPA SW-3050/6020
Mehlich-III Phosphorus (Mehlich-III P)	Mehlich III (ICP)
Soluble Phosphorus	Water 1:10, Bull.396, pg 17
Soluble nitrate	Water 1:10
Total Nitrogen	Kjeldahl, modified
Soluble ammonium	Water 1:10
Soluble sulfate	Water 1:10
Soluble chloride	Water 1:10
TAL Metals	EPA SW-3050/6020
Total Molybdenum (Mo)	EPA SW-3050/6020

Bacteria:	
Total coliform	SM-9221B
Enterococcus	SM-9230B
Fecal coliform	SM-9221E
e-coli	SM-9221F
Staphylococcus	BAM 12
Campylobacter	BAM-Chap. 7
Salmonella	BAM 5
17β-estradiol, estrone, estriol	LC-MS-MS

8.3 Data Reporting

1. Data from the laboratory shall be reported in both electronic and paper reports.
2. Data reports shall include all quality control data generated, including results for duplicates, blanks and spikes, as applicable. If applicable, a level 3 data quality report will be provided by the laboratory.
3. Data reports shall include a copy of the Chain of Custody accompanying each set of samples submitted

9.0 Bio-security, Decontamination of Equipment and Personal Protective Equipment

All persons engaged in sampling, observing sampling or documenting sampling under this protocol shall follow appropriate bio-security precautions. All persons doing sampling will receive bio-security training from the State of Oklahoma.

9.1 Soils

To the extent possible, disposable sampling equipment should be used.

All reusable sampling equipment shall be decontaminated using a non-phosphate detergent, a 6% (minimum) bleach solution, and three de-ionized water rinses between Sampling Areas.

9.2 Litter/Manure

To the extent possible, disposable sampling equipment should be used.

All reusable sampling equipment shall be decontaminated using a non-phosphate detergent, a 6% (minimum) bleach solution, and three de-ionized water rinses between poultry houses.

9.3 Health and Safety Plan:

A health and safety plan that is specific to this sampling protocol will be prepared and reviewed by all samplers.

10.0 References

Zhang, H. and Johnson, G. 2003. How to get a good soil sample. Oklahoma State University Cooperative Extension Service Fact Sheet F-2207. Available at http://osuextra.okstate.edu/pdfs/F-2207web.pdf

Zhang, H., Hamilton, D. W. and Britton, J. G. 2002. Sampling Animal Manure. Oklahoma State University Cooperative Extension Service Fact Sheet F-2248. Available at http://osuextra.okstate.edu/pdfs/F-2248web.pdf

Eucha/Spavinaw Watershed Management Team. Undated. Soil Sampling Protocol.

Eucha/Spavinaw Watershed Management Team. Undated. Steps for Pulling Litter Samples.

11.0 Revised Dates*

The following are other revision dates applicable to this SOP.

Revision 8 – February 5, 2007
Revision 7 – April 24, 2006
Revision 6 – May 11, 2005
Revision 5 – April 20, 2005
Revision 4 – March 25, 2005
Revision 2, 3 – March 16, 2005
Revision 1 – January 25, 2005

SOP: 5-2
Standard Operating Procedure
Litter and Soil Sample Compositing

Revision: 3*
Initial Date: 05/03/05
Last Revised: 02/05/07
Page 1 of 7

Prepared: Todd Burgesser Review: Kim Zilis

Approved: Roger L. Olsen Date Approved: 2/06/07

1.0 Overview and Application

This standard operating procedure (SOP) describes field procedures used for compositing of soil and poultry litter samples from the Illinois River watershed of eastern Oklahoma and western Arkansas. This will include handling, mixing, and shipment of soil and litter samples.

2.0 Handling and Compositing of Soil and Litter Samples

All individual soil samples from each sub-location will be placed in individual plastic bags (double bagged), packed in a cooler with blue ice and shipped over night under chain-of-custody to the CDM processing laboratory in Denver, Colorado. The sample number will be located between the inner and outer plastic bag. Each sampling area (up to four sampling areas per LAL) will have 60 individual samples (20 sub-sample locations x 3 sample depths = 60 samples). All samples will be received by the CDM processing laboratory for compositing. Each of the 20 sub-samples will be composited into one homogeneous sample using the protocol described below.

Litter samples will be received by the CDM processing laboratory under chain-of custody in a 5-gallon bucket. The litter sample will be contained in a plastic bag inside of the 5-gallon bucket will be closed with a tie. A unique sample number will be written on the outside of the bucket.

Upon receipt of the samples, the cooler/bucket temperature will be measured using a NIST traceable thermometer. The samples soil will then be removed from the cooler and checked against the chain-of-custody to ensure that all samples have been received.

The twenty sub-samples associated with the individual sample depths or the entire litter sample will be poured into a stainless steel bowl or 2.5-gallon bucket ready for mixing. All equipment will be decontaminated/sterilized with laboratory grade distilled water and 10 percent bleach (see procedure below).

2.1 Mixing of Soil Samples

- All health and safety protocol will be followed as described in the Health and Safety Plan for the Illinois River Basin Project. This is includes wearing nitrile gloves and processing soil in the hood.
- All feathers, rocks, twigs, debris and vegetation will be removed before sieving and mixing.
- Mixing will be accomplished using a disposable, plastic sampling scoop or a decontaminated stainless steel spoon.
- All clods over 0.5 inches in diameter will be disaggregated into smaller particles by hand or the use of a decontaminated stainless steel spoon or mortar.
- If the moisture content is too high to allow homogenization or disaggregation of the particles, the sample will be placed in steel drying pan and air dried over night.
- The sample will be hand mixed using the plastic scoop or stainless steel spoon for at least five minutes or until particles are uniform in size.
- If a plastic bucket is used, the bucket will then be sealed and inverted or rotated at least 10 times.
- After mixing, the sample will be sieved to remove particles sizes of greater than 2 mm using a decontaminated US Sieve no. 10 (gravel size particles will be removed).
- Each fraction (greater than 2 mm and less than 2 mm) will be weighted. The less than 2 mm fraction will be placed in a plate grinder and reduced in size to 0.074 mm (US sieve no. 200, very fine sand).
- The ground sample will be split using a riffle splitter and sent to the various laboratories (see splitting procedure in section 1.3.1, Duplicate Samples).

2.2 Mixing of Litter Samples

The same procedure as described above for the soil will be used for the litter. However, grinding may not be necessary if the litter can be sieved directly through a US sieve no. 200.

2.3 Laboratory QA/QC Samples (Soil)

Laboratory QA/QC samples may consist of duplicate samples, decontamination blanks, and blind standards. The following describes each type of QA/QC sample.

2.3.1 Duplicate Samples (created at the soil processing lab)

After sample mixing, sieving and grinding, two split samples will be collected. The sub-sample splits should be collected using a nonbiased riffle splitter. The sample is poured through the riffle splitter and into the decontaminated collection pans. The amount of soil or litter contained by the sample container shall be sufficient for the chemical and physical analyses to be conducted.

2.3.2 Blind Standards

A blind standard of a certified reference soil will be sent to the analytical laboratory for approximately every 50 samples send to the laboratory. The blind standard will be sent by the CDM soil processing lab. Blind standards will be for metals, arsenic, and phosphorus.

2.3.3 Decontamination Blanks

A sample of the final decontamination rinsate will be collected and forwarded to the analytical laboratory for analysis. The decontamination rinsate blank will be generated in the CDM processing laboratory using a final rinse of laboratory grade distilled water. All parameters will be analyzed.

3.0 Shipment of Samples to the Analytical Laboratory

- Once placed in sampling containers (plastic bags or jars), samples will be held at 4° C on blue ice (sealed in plastic bags) within insulated protective containers.
- If possible, samples will be shipped immediately after compositing via overnight shipment to the analytical laboratory.
- After compositing, samples should not be held more than 24 hours before shipment.
- Samples will be sent to the laboratory under a Chain-of -Custody.
- A custody seal will be place on the outside of the cooler between the lid and the body of the cooler. The custody seal will be signed.
- The Chain-of-Custody will be sealed in a plastic bag and placed within the insulated protective container holding those samples to which it refers.

4.0 Decontamination of Processing Equipment

All nondisposable equipment (bowls, sieves, spoons, and grinders) will be decontaminated/sterilized after each composite sample is created. Decontamination will include washing with phosphate free water followed by rinsing with laboratory grade distilled water. A final rinse of 10 percent bleach will be performed. The equipment will be air dried.

5.0 List of Analytes and Bottle Requirements

5.1 Analytical Parameters

Soil samples will be analyzed for one of two sets of parameters (short list or long list). Table 1 provides the parameters and analytical methods for the short list and Table 2 provides the parameters and analytical methods for the long list.

Litter samples will be analyzed for Table 2 parameters.

Table 1: Short List Parameters - Soil

Parameter	Method
Moisture content (%)	Gravimetric (105C)
Organic matter	Walkley-Black (Modified)
Soil pH	Water 1:1
Soil Conductivity	Water 1:2
Total Nitrogen	Kjeldahl, modified
Total Aluminum (Al)	EPA SW-3050/6010/6020
Total Phosphorous (P)	EPA SW-3050/6010/6020
Total Arsenic (As)	EPA SW-3050/6010/6020
Total Copper (Cu)	EPA SW-3050/6010/6020
Total Zinc (Zn)	EPA SW-3050/6010/6020

Table 2: Long List Parameters – Manure and Soil

Parameter	Method
Moisture content (%)	Gravimetric (105C)
Organic matter	Walkley-Black (Modified)
Texture (% sand, silt and clay)*	Hydrometer ASTM-D422
Soil pH	Water 1:1
Soil Conductivity	Water 1:2
Total Phosphorous (P)	EPA SW-3050/6020
Mehlich-III Phosphorous (Mehlich-III P)	Mehlich III (ICP)

Soluble Phosphorous	Water 1:10, Bull.396, pg 17
Soluble nitrate	Water 1:10
Total Nitrogen	Kjeldahl, modified
Soluble ammonium	Water 1:10
Soluble sulfate	Water 1:10
Soluble chloride	Water 1:10
TAL Metals	EPA SW-3050/6020
Total Molybdenum (Mo)	EPA SW-3050/6020
Bacteria:	
Total coliform	SM-9221B
enterococcus	SM-9230B
Fecal coliform	SM-9221E
e-coli	SM-9221F
staphylococcus	BAM12
campylobacter	BAM7
salmonella	BAM5
17β-estradiol, estrone, estriol	LC-MS-MS

*split before sieving and grinding

5.2 Bottle Requirements

Soil samples will be analyzed for one of two sets of parameters (short list or long list). Table 3 provides the parameters, bottle requirement and laboratory for the short list and Table 4 provides the parameters, bottle requirement and laboratory for the long list.

Litter samples will be analyzed for Table 4 parameters.

Table 3: Short List Parameters - Soil

Parameter	Bottle	Laboratory
Moisture content (%)	1 quart glass	A&L
Organic matter	1 quart glass	A&L
Soil pH	1 quart glass	A&L
Soil Conductivity	1 quart glass	A&L
Total Nitrogen	1 quart glass	A&L
Total Aluminum (Al)	1 quart glass	A&L
Total Phosphorous (P)	1 quart glass	A&L
Total Arsenic (As)	1 quart glass	A&L
Total Copper (Cu)	1 quart glass	A&L
Total Zinc (Zn)	1 quart glass	A&L

Note: 1 bottle for all of the above analysis

Table 4: Long List Parameters – Manure and Soil

Parameter	Bottle	Laboratory
Moisture content (%)	1 quart glass	A&L
Organic matter	1 quart glass	A&L
Texture (% sand, silt and clay)*	1 quart glass (separate from the other bottles)	A&L
Soil pH	1 quart glass	A&L
Soil Conductivity	1 quart glass	A&L
Total Phosphorous (P)	1 quart glass	A&L
Mehlich-III Phosphorous (Mehlich-III P)	1 quart glass	A&L
Soluble Phosphorous	1 quart glass	A&L
Soluble nitrate	1 quart glass	A&L
Total Nitrogen	1 quart glass	A&L
Soluble ammonium	1 quart glass	A&L
Soluble sulfate	1 quart glass	A&L
Soluble chloride	1 quart glass	A&L
TAL Metals	1 quart glass	A&L
Total Molybdenum (Mo)	1 quart glass	A&L
Bacteria	1 - 250 mL plastic (sterilized) or 1-8 oz. Whirl bag	EML
PCR	1-8 oz. Whirl bag	ISU
17β-estradiol, estrone, estriol	1 – 4oz. glass	GEL

*split before sieving and grinding

6.0 Analytical Laboratories

Bottles for estrogen metabolites (all samples) will be shipped to:

General Engineering Laboratories, LLC
201 Pine Ridge Road, Unit 5
Golden, CO 80403
Contact: Paul Winkler, 720-253-3093
Paul.winkler@gel.com

Bottles for nutrients, metals, etc (all samples) will be shipped to:

A&L Analytical Laboratories, Inc.
2790 Whitten Rd.
Memphis, TN 38133
Contact: Scott McKee, 800-264-4522
smckee@allabs.com

Bottles for bacteria analyses from soil and litter will be shipped to:

Environmental Microbiology Laboratory
1150 Bayhill Drive, Suite 100
San Bruno, CA 94066
Contact: Megan S. Tatreau, 858-268-2770
mtatreau@emlab.com

Bottles for PCR will be shipped to:

Idaho State University
Department of Biological Sciences-MRCF
Attn: Erin O'Leary-Jepsen
640 Memorial Drive
Pocatello, ID 83209-8007
Contact: Erin O'Leary-Jepsen, 208-282-4890

7.0 Documentation

Bound laboratory logbooks should be used for the maintenance of field records. All aspects of sample compositing and handling as well as visual observations will be documented in the field logbooks. Supplemental information may be documented on the field data sheets provided. All entries in laboratory logbooks should be legibly recorded and contain accurate and inclusive documentation of an individual's project activities.

8.0 Revised Dates*

The following are other revision dates applicable to this SOP.

Revision 2: 02/09/06

Revision 1: 05/10/05

Standard Operating Procedure
Manure Sampling for DNA Analysis

SOP: 5-3
Revision: 2*
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Approved: [signature] Date Approved: 2-06-07

1.0 Overview and Application

This standard operating procedure (SOP) describes field procedures used for collection of fecal matter for identifying the types and abundance of bacterial DNA. The bacterial DNA is first amplified by polymerase chain reaction (PCR), then digested with a restriction enzyme. The enzyme cuts DNA strands into different size fragments whose length is dependent upon the DNA sequence, and the last (terminal) fragment is labeled for detection. Each terminal fragment length is represents approximately one bacterial species. This program is designed to identify DNA fragments from bacteria that reside in fecal material from various animals, including cattle, swine, ducks, geese and humans.

2.0 Selection of Sampling Locations

Sample locations will be selected from farms, wildlife areas, septic clean-out trucks, or wastewater treatment plants as appropriate. The following sources of fecal matter will be targeted for collection.

1. A total of 10 fields where beef cattle are actively grazing; preferably five fields within the basin and five fields outside the basin,
2. A total of 2 dairy cattle milking barns; preferably in the basin, but could be outside of the basin (close to the basin as possible),
3. A total of 2 swine facilities; preferably in the basin, but could be outside of the basin (close to the basin as possible),
4. A total of five active geese landing areas; preferably in the basin, but could be outside of the basin (close to the basin as possible),
5. A total of five active duck landing areas; preferably in the basin, but could be outside of the basin (close to the basin as possible),
6. A total of three septic clean out trucks; preferably all in the basin, but at a minimum at least one sample in the basin,
7. A total of three small wastewater treatment plan influent locations; preferably all in the basin, but at a minimum at least one in the basin.

The locations should contain the following information for each associated Farm/Facility:

1. Name of Farm/Facility owner and Farm/Facility contact person,
2. Physical address and location (section-township-range) of Facility,
3. Contact address of Farm/Facility owner or Farm/Facility contact person,
4. Contact phone number of Farm/Facility owner or Farm/Facility contact person,
5. Whether or not one or more samples can be accessed at the Farm/Facility,
6. The physical location of each sample collection site(s) - record coordinates (latitude and longitude) of documented location (eg, corner of a field),
7. Estimate of number of animals at sample collection site or number of facilities serviced by wastewater treatment plant or septic clean out truck,
8. Estimate of the amount of feces available at the sampling site,
9. Estimate of when the feces was deposited; e.g., was the animal observed while it was defecating,
10. Observation as to whether any chicken litter application has occurred at the sampling field/site,
11. Estimates of amount, rate, and date of litter treatment applied to the site, if applicable, and information as to amount, rate and dates of application.

Site selections will be made based upon availability.

3.0 Sampling Documentation

3.1 Sampling Log Book and Sampling Forms

1. A Sampling Log Book and Sampling Forms shall be maintained.
2. Pages in the Sampling Log Book will reference specific sampling forms by use of the Sample Identification.
3. The Sampling Log Book shall be bound and shall be constructed of waterproof paper.
4. Entries in the Sampling Log Book or on the sampling form shall be made in black permanent ink.
5. Each page of the Sampling Log Book shall be dated.
6. The preparer shall initial each page of the Sampling Log book.

7. For each location sampled, the following information shall be recorded in the Sampling Log Book or on the sampling forms:
    a. Name, address and phone number of the Property/Facility owner,
    b. Identification of the Property/Facility (MAN),
    c. Name, address and phone number of the Property/Facility operator,
    d. If applicable, name, address and phone number of the Integrator responsible for the Property/Facility,
    e. If applicable, the amounts, rates and dates of prior litter/manure applications to specific fields at the Property/Facility (confirm State Reports),
    f. If applicable, the existence of prior soil sampling data for the property (yes or no),
    g. The water supply for the Property/Facility,
    h. The legal description (qtr-qtr-qtr-sec-twp-rng) of the property related to the Property/Facility,
    i. Information as to any fertilizers, chemicals or soil amendments added during the last five years,
    j. Specific information listed within this protocol,
    k. Sketch map of each property/facility with approximate dimensions; indicate local features on the sketch (vegetation, water bodies, adjacent fields, location of poultry houses, roads, old fence rows, livestock feeding areas, livestock grazing areas, etc); dimensions and features can also be placed on the aerial photographs,
    l. Land slope of property/facility,
    m. Distance to nearest water body,
    n. Notes on weather (temperature, wind, last precipitation event, etc),
    o. Type of vegetation currently on the LAL, if any, and any known vegetation grown in past 5 years,

p. Use of adjacent fields, and;

q. Other information as appropriate or relevant.

3.2 Photographic Record

A photographic record shall be made and maintained for all sampling activities on the MAN. All photographs made shall be time and date stamped.

3.3 Chain-of-Custody

A Chain-of-Custody shall be prepared for each set of samples transferred to the analytical laboratory, North Wind, Inc. in Idaho Falls, ID (see section 7).

The Chain-of-Custody shall, at a minimum, contain the following information:

1. The project name, ***Illinois River Watershed Manure DNA Sampling,***
2. Name of person or entity collecting samples,
3. Signature blocks with dates and times for all persons having custody (sampler, shipper, processing laboratory, etc),
4. For each sample related to a Chain-of-Custody:
    a. The unique numeric identifier on the submitted sample container/bag (see subsequent section 6)
    b. The date and time the sample was collected,
    c. The sample "matrix" (Manure).

4.0 Manure Sampling

4.1 Manure Locations (MAN)

4.1.1 Permissible Manure and Weather Conditions

1. Manure must be fresh. Sample should be from the interior of manure piles.
2. Manure should not be sampled during precipitation events.

4.1.2 Beef Cattle Sampling Areas

Manure samples will be collected from a total of ten fields actively grazed by cattle. Five locations will be from fields within the IRW. If available, both fields with and without litter application will be sampled. Five locations will be from fields outside the IRW and, if

possible, from fields with no litter application. Two composite samples will be collected from each field. Each composite sample will consist of samples from ten fresh manure piles. In all, twenty composite samples will be collected.

4.1.3 Dairy Cattle Sampling Areas

Manure samples will be collected from the clean out slurry of four milking barns. If possible, two barns handling cattle fed by grazing and two barns handling grain-fed cattle will be sampled. The clean out slurry must consist of that day's droppings. The samples must be collected from waste stream before the collection ponds. In all, four samples will be collected.

4.1.4 Swine Sampling Areas

Manure samples will be collected from the clean out slurry from two swine facilities. The clean out slurry must consist of that day's droppings. The samples must be collected from waste stream before the collection ponds. In all, two samples will be collected.

4.1.5 Duck Sampling Areas

Manure samples will be collected from up to five landing or residence areas. Sampling locations will be from wildlife areas, golf courses, or local ponds. Two composites will be collected from each landing/residence area. Composites will consist of ten swabs or direct fecal samples each, if possible. In all, ten samples will be collected.

4.1.6 Geese Sampling Areas

Manure samples will be collected from up to five landing or residence areas. Sampling locations will be from wildlife areas, golf courses, or local ponds. Two composites will be collected from each landing/residence area. The locations may be co-located with the duck locations; however, the samples have to be distinctly separate between species. Composites will consist of ten fecal samples each, if possible. In all, ten samples will be collected.

4.1.7 Human Waste Samples

Human sewage samples will be collected at two sources: septic clean out trucks and influent to wastewater treatment plants. Sewage samples will be collected from three separate septic clean out trucks. The samples should be collected at the pump out facility after at least several homes have been visited. The sample should be collected after the pumping has been in progress and the waste in probably mixed.

Sewage samples will be collected from the plant influent at three different wastewater treatment plants. The plant operator will determine the best way to collect a representative influent sample which has not been subject to treatment. Wastewater treatment plants will be selected that do not have contribution from industries which could contribute poultry or other animal waste products (i.e. processing plants).

In all, six human waste samples will be collected.

4.2 Collection and Handling of Samples

Sampling personnel will wear disposable, sterile gloves at all times when collecting fecal samples and will change gloves before they collect each new fecal sample. Samples will either be pre-composited samples (i.e. dairy cattle, swine, and human samples) or will be composited in the field (beef cattle, duck, and geese). All samples will be collected into 20 milliliter, sterilized, polystyrene, round bottom tubes. Each tube will contain 10 mL of 20 % glycerol solution (added to the tube by the laboratory). Pre-composited samples will be collected directly into the tubes (approximately 2 - 10 grams). For the samples to be composited in the field, ten aliquots will be sampled using a sterilized, disposable, polystyrene spatula. A similar sized sample (1 -2 grams) from each individual stool will be placed into one tube. The contents will then be mixed in the field by shaking the tube containing the glycerol/waste mixture. If swabs (sterile, cotton-tipped applicators) are used to collect duck feces, all the swab tips (ten) will be placed into the same round bottom tube. The tips will be cut from the attached plastic tube (or stick) using scissors (sterilized by cleaning with an alcohol wipe before use). Labels will be placed on the tubes and secured with transparent tape. The tubes will be placed inside individual resealable plastic bags. The bags will be placed in a cooler containing dry ice before leaving the property/facility where the sample was collected. The samples must be frozen prior to being shipped to the analytical laboratory. If the samples have not been frozen by exposure to the dry ice, they shall be placed in a freezer until freezing is complete. Samples will remain frozen until immediately prior to shipping. Samples shall be placed in a cooler with standard ice and shipped priority overnight to the analytical laboratory.

4.3 Field QA/QC Samples (Manure)

1. Duplicates: no field duplicate samples will be created since samples will be composite samples.
2. Blind Standard: no blind standards will be submitted for this particular program.
3. Decontamination Blank: no decontamination blanks will be generated for this particular program as all collection equipment will not be reused between samples.
4. Field Blanks: field blanks will be collected at a rate of one per twenty or per sample shipment. Field blanks will be collected by one of three methods.
    a. Dairy Cattle, Swine, and Humans - one field blank associated with one of these locations will be collected by opening the screw top cap and immediately replacing the cap. The tube will contain the glycerol from the laboratory.

b. Beef Cattle and Geese – one field blank associated with one of these locations will be collected by opening a packet containing a sterilized collection spatula and placing it directly into the screw cap tube containing the glycerol.

c. Duck – one field blank associated with one of these locations will be collected by placing a swab tip directly into the screw cap tube containing the glycerol.

4.4 Decontamination Procedures

Sampling equipment will be one time use. No equipment decontamination is anticipated. Only the scissors will be reused and these will be cleaned with an alcohol wipe between sampling sites.

If appropriate, bio-security decontamination measures will be implemented. All waste generated during the sampling procedure will be placed in disposable trash bag and placed in a container where the waste will be transported to a sanitary landfill.

5.0 Person(s) Collecting Samples and Observing Sampling

Personnel from CDM or Lithochimeia will conduct the manure sampling from each MAN. CDM personnel will process samples, chain-of-custody, coordinate shipping, etc.

6.0 Identification of Samples

Identifying information to be recorded on the sample label for DNA Manure samples:

1. Beef Cattle: Alphanumeric identification will consist of MAN-BC-1, MAN-BC-2 etc. The log book will be used to record the facility/property and location of each composite sample.
2. Dairy Cattle: Alphanumeric identification will consist of MAN-DC-1, MAN-DC-2 etc. The log book will be used to record the facility/property and location of each composite sample.
3. Swine: Alphanumeric identification will consist of MAN-SW-1, MAN-SW-2 etc. The log book will be used to record the facility/property and location of each composite sample.
4. Duck: Alphanumeric identification will consist of MAN-DK-1, MAN-DK-2 etc. The log book will be used to record the facility/property and location of each composite sample.
5. Geese: Alphanumeric identification will consist of MAN-GS-1, MAN-GS-2 etc. The log book will be used to record the facility/property and location of each composite sample.

6. Human: Alphanumeric identification will consist of MAN-HM-1, MAN-HM-2 etc. The log book will be used to record the facility/property and location of each composite sample.
7. If necessary, an alphanumeric identification will be assigned to a subarea if more than one sample is collected from the same facility/property: A, B, C, D etc.
8. The following sample number is an example of a manure sample taken from Beef Cattle field number 5, sampling area B:

   MAN-BC-5-B

9. For samples submitted to the analytical lab, additional alphanumeric identification of the type of sample will be added to the end of the identification number:

   a. F = Field Blank

10. Date of sample collection (only on chain-of-custody),
11. Time of sample collection (only on chain-of-custody),
12. Initials of the person collecting the sample (only on chain-of-custody).

7.0 Shipment of Samples to the analytical laboratory

1. Shipping coolers will be packed such that samples are stored with standard ice placed in double-bagged resealable plastic bags. The shipping coolers shall be insulated protective containers.
2. If possible, samples shall be shipped immediately via overnight shipment to the analytical laboratory. The laboratory address is:

   Idaho State University

   Department of Biological Sciences- MRCF
   Attn: Erin O'Leary-Jepsen

   650 Memorial Drive
   Pocatello ID 83209-8007
   208-282-4890

3. In no event, shall samples be held more than 24 hours before shipment unless they are frozen.
4. Samples shall be sent to the laboratory under a Chain-of -Custody.
5. A custody seal will be place on the outside of the container across the area between the lid and the container. The custody seal will be signed.

6. The Chain-of-Custody shall be sealed in a plastic bag and placed within the insulated protective container holding those samples to which it refers.

8.0 Analytical

8.1 Analytical Protocols

Analyses are being conducted by Tamzen W. Macbeth (208-528-8718), North Wind, Inc., 1425 Higham St., Idaho Falls, ID 83402. Analytical protocols are provided in a separate document.

8.3 Data Reporting

1. Data from the laboratory shall be reported in both electronic and paper reports.
2. Data reports shall include all quality control data generated, including results for duplicates, blanks and spikes, as applicable.
3. Data reports shall include a copy of the Chain of Custody accompanying each set of samples submitted

9.0 Bio-security, Decontamination of Equipment and Personal Protective Equipment

All persons engaged in sampling, observing sampling or documenting sampling under this protocol shall follow appropriate bio-security precautions.

9.1 Manure

To the extent possible, disposable sampling equipment should be used.

Any reusable sampling equipment shall be decontaminated using a non-phosphate detergent, bleach and three de-ionized water rinses between Sampling Areas. No reusable equipment is currently anticipated.

9.2 Health and Safety Plan:

The overall health and safety plan for the project will be used for this sampling protocol and will be reviewed by all samplers.

10.0 Revised Dates*

The following revision dates are applicable to this SOP:

Revision 1 -July 11, 2006

Standard Operating Procedure
Water Sampling of Rivers and Streams

SOP: 6-1
Revision: 2*
Initial Date: 01/11/06
Last Revised: 02/05/07

Prepared: Brian Bennett Review: Ronald French

Approved: Roger L Olsen Date Approved: 2/06/07

1.0 Overview and Application

This standard operating procedure (SOP) describes field procedures used for collection of surface water samples from rivers and streams in the Illinois River watershed of eastern Oklahoma and western Arkansas. This will include water chemistry, nutrients, bacteria, and other water sampling protocols. Procedures for on site measurement of various water quality parameters such as temperature, conductivity, turbidity, pH, and dissolved oxygen will also be discussed. This SOP will be used during field work and will follow the guidelines described in the *Standard Operating Procedures (SOP) for Field Sampling Efforts of the Oklahoma Water Resources Board's Beneficial Use Monitoring Program.* (ORWB, 2001).

The Illinois River watershed in eastern Oklahoma and western Arkansas has been receiving inputs from agricultural runoff, including inputs from fields where poultry waste has been applied. These wastes, along with the chemical constituents that make up the poultry wastes, have apparently impacted the water quality and sediments in the streams and lakes in the Illinois River Watershed. The purposes of the work being performed are to evaluate and document 1) the linkage and relationship, if any, between the disposal of poultry wastes and environmental contamination within the Illinois River Watershed, and; 2) the resulting harm/injury to natural resources that may have resulted from the disposal of poultry wastes within the Illinois River Watershed.

2.0 Sampling Methods Summary

Water samples of rivers and streams will be collected periodically at various locations throughout the Illinois River watershed and in several streams in surrounding watersheds. Multiple water chemistry parameters may be analyzed by qualified laboratories and on site water quality measurements may be taken. Due to the wide range of parameters being analyzed, multiple samples may be taken from each location and shipped to separate laboratories. **Table 1** describes the parameters, containers, and laboratories to be used.

For certain parameters, samples may be preserved immediately after collection. This is accomplished by placing the samples in bottles distributed by the analytical lab which already

contain the necessary types and amounts of preservatives (sulfuric acid, nitric acid, hydrochloric acid, etc.). In some cases, it may be necessary to add certain preservatives after sample collection (e.g. formalin in zooplankton samples). Some samples may be filtered prior to preservation in the appropriate sample bottles by using a peristaltic pump to force the sample water through a filter and into a beaker or flask.

2.1 Sampling Locations

Three large tributaries to Tenkiller Ferry Reservoir will be sampled on dates coinciding with reservoir sampling events. Additional water samples may be collected from each of 13 biological sampling stations during biological sampling events. Water sampling may also be conducted during base flow conditions at each of the 12 automated high flow sampling stations. Several other locations may also be sampled at various times throughout the course of this project.

2.2 Sample Types

Water samples can either be discrete or composite samples. A discrete sample is defined as a single aliquot from of a specific location or depth at a given point in time. Composites are samples composed of two or more specific discrete samples collected from one or several sampling location. This type of sample represents an average value and can, in certain instances, be used as an alternative to analyzing a number of individual discrete samples and calculating an average value. It should be noted, however, that compositing can mask the presence of contaminants by diluting isolated concentrations of an analyte that may be present in the environmental matrix.

2.3 Sampler Selection

The choice of samplers is dictated by sampling objectives, site accessibility, and parameters to be examined. Each sampling technique presents various advantages and disadvantages for its application. For example, sample disturbance, sample volume, chemical and physical reactivity between potential contaminants and sampling tool materials, and ease of decontamination vary from technique to technique.

Discrete samples will be collected using a Van Dorn water sampler or similar device. These samplers consist of a PVC tube that can be lowered into the water and then closed to collect a discrete sample. In some cases, discrete samples may be collected by a surface dip method in which the sample containers or an intermediate container such as a churn splitter are submerged and filled directly from the source water. However, sample bottles containing preservative should not be filled by the direct surface dip method so that any preservative is not lost into the water source. Composite samples can be collected by combining several discrete samples into a churn splitter and mixing prior to filling the sample containers.

2.4 Data to be Collected

At each sampling location, a variety of physical and water quality parameters may be assessed and recorded. The exact location of the sampling should be measured with a handheld GPS unit and recorded. Water quality parameters may be measured using a YSI model 650 multi-meter which should be calibrated and verified before and after each sampling event as described in Standard Operating Procedure 9.1: *Water Quality Meters*. At each sampling location the YSI meter should be lowered into the water and a reading should be recorded for each of the following parameters:

- Temperature in degrees Celsius
- pH
- Dissolved Oxygen (DO) in milligrams per liter
- Specific Conductance in micro-Siemens per cm
- Turbidity in Nephelometric Turbidity Units (NTU)

When an YSI-650 meter is not available, other electronic measurement devices may be used. Consult the devices' operation manuals for instructions on maintenance, calibration, and use.

2.5 Sample Nomenclature

In general, water samples collected from rivers and streams should be labeled according to the identification scheme: **AA-BB-CC-DD.**

AA is defined by the location type. Biological stations should use BS and high-flow stations should be denoted by HFS.

BB is defined by a two digit station identification number. In some cases, additional digits may be required.

CC is defined by a two digits which identify the sample medium; typically SW for surface water.

DD is defined by a two digit number which identifies the type of sample i.e. the original sample (01), blank (02), or a duplicate sample (03).

2.6 General Supplies

The following is a list of the minimum supplies needed for surface water sampling in streams and rivers:

- YSI 650 Multi-meters for collecting water quality parameters
- Cable for YSI 650 meter
- Van Dorn water sampler
- Churn splitter or similar mixing container
- Beakers/flasks
- Peristaltic or similar water pump
- Battery to power pump
- Filtering apparatus
- Filters (0.45 micron)
- Sample bottles with labels
- Clear tape
- Aluminum foil

- Coolers with ice
- De-ionized water
- Phosphate-free detergent
- Nitrile gloves
- Data Sheets
- Field Notebooks with water resistant paper
- Handheld GPS Unit, with extra batteries
- Writing utensils (waterproof)
- Digital camera with extra batteries
- Sunscreen
- Drinking water/snacks/lunches for the crew

3.0 Sampling Procedures

Due to the relatively large number of analyses that may be conducted throughout the course of the sampling, different numbers and types of samples may be collected during each event. Therefore, the procedures for collecting water samples may vary based on parameters to be analyzed at each location during a specific sampling event. All samples should be collected and preserved in accordance with both the OWRB guidelines and the specific analytical laboratory sampling requirements.

3.1 Sample Collection

Procedures for collecting the water samples are somewhat different depending on the type of sample desired. The following procedures should be employed.

3.1.1 Discrete Sample Collection

Discrete samples may be collected by the dip method wherever feasible, provided that the sample bottle does not contain preservatives and no filtering is necessary. This can be

accomplished by submerging the sample container below the surface and allowing it to fill with water. Care should be taken to avoid disturbing the sample by allowing air bubbles to form inside the bottle while filling. Nitrile gloves should also be worn to prevent contamination.

Discrete water samples may also be collected with the use of a Van Dorn sampler so that a sample may be collected and transferred to the appropriate sample container with a minimum of disturbance and aeration. The steps required to take a discrete water sample using a Van Dorn sampler are listed below:

- Calculate the number of samples you will be collecting at that location and prepare all the necessary bottles.
- If filtering is required, set up a decontaminated filtering apparatus and insert a new filter (wear nitrile gloves when handling the filters).
- Prepare the Van Dorn sampler for use by rinsing it and setting the spring-loaded stoppers. Make sure the messenger is ready to be deployed.
- Lower the sampler into the water and then release the messenger. This should trigger the stoppers to close, thus sealing the water inside the device.
- Bring the sampler to the surface and slowly drain the contents through the attached hose into the churn splitter.
- Keep the hose under the surface of the water in the container or direct the stream at an angle against the inside wall of the container to limit the amount of aeration that may occur.
- If necessary, filter the sample by slowly pouring it into the filtering apparatus and allowing the pump to force it through the filter.
- Carefully fill the appropriate sample bottles with the sample water.
- Store the samples in a cooler with plenty of ice.

3.1.2 Composite Samples Collection

During the main river sampling, three points equally distributed along a transect across the water body should be sampled and mixed into one composite sample. This can be done by combining a number of discrete samples collected with the Van Dorn device into a churn

splitter. Mix the samples thoroughly and pour into the sample bottles. Composite sampling may be conducted at other locations as well.

3.2 Filtering Samples

Some parameters require that the sample be filtered in the field prior to being preserved. This is accomplished with the use of a filtering apparatus, filters, and a peristaltic pump. Some types of filters need to be prepared in advance of a sampling event. Generally, this involves rinsing them with laboratory-grade, de-ionized water two times and allowing them to soak for several hours. Refer to instructions supplied with the filters or in the analytical procedure for specific details. Procedures for filtering in the field are described below:

- Set up the filtering apparatus by attaching the pump to a filtering flask and inserting the appropriate filter.
- Turn the pump on and begin slowly adding the sample water to the filtering apparatus. Again, try not to aerate or disturb the water.
- Allow the water to move through the filter and into the flask until the required amount of sample water has accumulated in the flask. In some cases, the filter may need to be changed before the necessary amount can be filtered for that sample.
- Pour the filtered water into the appropriate sample bottles, label, and store on ice.

3.3 Chlorophyll *a* Samples

The procedure for collecting chlorophyll *a* samples is somewhat different than most other sample types. This type of sampling involves preserving the sample prior to filtering. Instead of sending the filtered water to the laboratory, the filter pad is preserved and sent to the lab for analysis. Procedures for chlorophyll *a* sampling are outlined below:

- Collect a discrete water sample from the required depth as described in previous sections of this document.

- Set up the filtering apparatus and insert the glass filter pads distributed by the laboratory. These filters are different from the ones used for other parameters and should be inserted with the rough side of the pad facing up.
- Add 1ml of $MgCO_3$ preservative to a 100ml sample of water and stir gently.
- Pour the water with preservative into the filtering apparatus and allow the entire contents to be flushed through the filter.
- Remove the filter and fold it in half so that the bottom of the pad is on the outside.
- Wrap the filter in a piece of aluminum foil and place in an opaque sample bottle. This will ensure that no light can reach the sample material.
- Label the bottles and store on ice.

3.4 Base Flow Sampling

During periods of seasonal base flow, water samples may be collected from each of the 12 automated high flow sampling locations. Such samples may be collected with the standard collection methods or by pumping the water from the stream with the ISCO automatic sampler. Prior to beginning the grab sample, the input hose should be disconnected from the ISCO sampler at the connection between the peristaltic pump and the internal distribution arm. Start the pump and allow the water to run through the hose for several seconds before filling bottles. When filling a container, hold the hose at an angle against the side or below the surface of the water to minimize aeration and mixing. Filter samples as needed.

Once all samples have been collected, power down the ISCO sampler. Reattach the hose to the internal distributing arm connection. Reprogram the ISCO sampler as required to initiate monitoring for high flow sampling as described in CDM Standard Operating Procedure 2-1: *High Flow Sampling.*

4.0 Sample Containers, Preservation Techniques, Quality Control

Refer to **Table 1** for the specific analyses and analytical methods. Periodically throughout the sampling, samples should be packed and shipped in coolers to one of several different analytical laboratories depending on analyses required (contact information below). A description of sample containers required for each parameter is also available in **Table 1.**

Field control samples may be collected by the sampling team and/or created by the CDM laboratory (Denver) to determine whether data are of suitable quality. Control samples may include trip blanks, duplicates, decontamination (rinsate) blanks, or split samples. Duplicates collected as co-located or split samples should be collected at least once for every twenty samples

4.1 Decontamination Procedures

To ensure that samples are not contaminated by equipment or containers, it is necessary to follow certain procedures for cleaning or decontaminating equipment. All sampling equipment which is in direct contact with the sample water should be cleaned between each sample collection. Equipment which should be decontaminated may include, but is not limited to: Van Dorn samplers, churn splitters, filtering apparatus, beakers or flasks, and volumetric measurement devices.

Procedures for decontamination are as follows:

- Rinse all surfaces with de-ionized or distilled water.
- Using a spray bottle, apply a layer of phosphate-free detergent to all surfaces.
- Rinse all surfaces again with de-ionized or distilled water until all detergent has been removed.
- If possible, rinse the container with water from the sample source.

4.2 Laboratory Contact Information

The following is a list of contact information and shipping addresses for all analytical laboratories used for water samples. Refer to Table A-1 for a list of samples that should be sent to each laboratory.

General Engineering Laboratories, LLC
701 Pine Ridge Road
Unit 5
Golden, CO 80403
Contact: Paul Winkler, 720-253-3093
E-mail: Paul.winkler@gel.com

Aquatic Research Inc
3927 Aurora Avenue North
Seattle, WA 98103
Contact: Steve Lazoff, 206-632-2715

A&L Analytical Laboratories, Inc.
2790 Whitten Rd.
Memphis, TN 38133
Contact: Jimmy Ferguson or Scott McKee, 800-264-4522
E-mail: smckee@allabs.com

Environmental Microbiology Laboratory
1150 Bayhill Drive, Suite 100
San Bruno, CA 94066
Contact: Cole Mackelprang, 858-268-2762
E-mail: cmackelprang@emlab.com
Contact: Megan S. Tatreau, 858-268-2770
E-mail: mtatreau@emlab.com

Idaho State University-Department of Biological Sciences
650 Memorial Drive
Pocatello, ID 83209-8007
Contact: Erin O'Leary-Jepsen (208) 282-4890

Aquatec Biological Sciences
273 Commerce St.
Williston, VT 05495
802-860-1638
Contact: Jennifer Gallant or Phil Downey
E-mail: jgallant@aquatecb.com

5.0 Documentation

Bound field logbooks should be used for the maintenance of field records. All aspects of sample collection and handling as well as visual observations should be documented in the field logbooks. Supplemental information may be documented on the field data sheets provided. All entries in field logbooks should be legibly recorded and contain accurate and inclusive documentation of an individual's project activities.

6.0 Additional Information

Other contact information:

Bert Fisher, PhD
Lithochimeia, Inc.
222 South Kenosha Ave.
Tulsa, OK 74120
Telephone: 918-382-9784

Ronald French
CDM
100 North Tucker Blvd.
Suite 550
Saint Louis, MO 63101
314-241-8510

7.0 References

Oklahoma Water Resources Board (OWRB). 2001. *Standard Operating Procedures (SOP) for Field Sampling Efforts of the Oklahoma Water Resources Board's Beneficial Use Monitoring Program.* Oklahoma Water Resources Board; Water Quality Programs Division; Oklahoma City, Oklahoma.

Herlihy, A.T. 1998. Water chemistry. pp. 57-65 IN: J.M. Lazorchak, D.J. Klemm, and D.V. Peck (Eds.). Unpublished draft. Environmental Monitoring and Assessment Program - Surface Waters: Western Pilot Study Field Operations Manual for Wadeable Streams. EPA/620/R-94/004F. U.S. Environmental Protection Agency, Washington, D.C.

8.0 Revised Dates*

The following are other revision dates applicable to this SOP:

Revision 1 - April 14, 2006

Table 1. Summary of sample parameters, bottles used, preservatives, filtering required, and analytical laboratories used during water sampling of rivers and streams.

Parameters	Bottles		Preservative	Filtered	Analytical Lab
Cu, Zn, As, P (6010/6020)	500 mL	plastic	Nitric Acid	Yes	A&L
Cu, Zn, As, P (6120/6020)	500 mL	plastic	Nitric Acid	No	A&L
TKN, Total P (365.2)	500 mL	plastic	Sulfuric Acid	No	A&L
Total P (365.2)	500 mL	plastic	Sulfuric Acid	Yes	A&L
Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Total Ortho P, Nitrate+Nitrite	1 liter	plastic	None	No	A&L
Dissolved Ortho P, Anions	500 mL	plastic	None	Yes	A&L
Total Organic Carbon (TOC)	2 x 40mL	VOA vials	Hydrochloric Acid	No	A&L
Total P (duplicate)	60 mL	plastic	None	No	Aquatic Research
Total P, Ortho P (365.1) (duplicate)	60 mL	plastic	None	Yes	Aquatic Research
Chlorophyll *a*	250 mL	brown plastic	$MgCO_3$	Yes- send filters	Aquatec
Trihalomethane Formation Potential (TFP)	1 liter	glass	None	No	A&L
Estrogen Metabolites	1 liter	amber glass	None	No	GEL
Bacteria (7 types)	1 liter	sterile plastic	None	No	EML
Polymerase Chain Reaction (PCR)	1 liter	sterile plastic	None	No	Idaho State University

Standard Operating Procedure
Fish Community Sampling

SOP: 7-1
Revision: 3*
Initial Date: 8/5/05
Last Revised: 2/5/07

Prepared: Brian Bennett Review: Tony Gendusa

Approved: [signature] Date Approved: 6-Feb-2007

1.0 Overview and Application

This standard operating procedure (SOP) describes field procedures used for collection of resident fish from rivers, lakes, and streams. This SOP will be used for collecting samples using electrofishing gear and by seining and will be based upon the USEPA Rapid Bio-assessment Protocols (RBP) for use in wadeable streams and rivers (Barbour, 1999).

The Illinois River watershed in eastern Oklahoma and western Arkansas has been receiving inputs from agricultural runoff, primarily from fields where poultry waste has been applied. These wastes, along with the chemical constituents that make up the poultry wastes, have impacted the water quality and sediments in the streams and lakes in the Illinois River Watershed. The purposes of the work being performed are to evaluate and document 1) the linkage and relationship, if any, between the disposal of poultry wastes and environmental contamination within the Illinois River Watershed, and; 2) the resulting harm/injury to resident fish populations that may have resulted from the disposal of poultry wastes within the Illinois River Watershed.

2.0 Sampling Methods Summary

The collection of fish will involve the use of both electrofishing and seining as capture techniques. Due to variations in water chemistry, stream type, and fish morphology, different sampling techniques must be employed to ensure that representative samples of the resident fish populations are collected. The relative advantages of each sampling method are discussed in detail in Fisheries Techniques (Murphy and Willis, 1983).

Electrofishing is the process of introducing a high voltage/low amperage electric charge into the water which stuns the fish and allows for easy collection, commonly with the use of dip nets. This sampling technique is selective for deep-bodied fish with large surface areas. Electrofishing is typically used in habitats where seining is not possible, such as around woody debris, in very shallow riffles, and near undercut banks. Electrofishers are only effective in water with a specific conductance of 40-1700uS with a maximum specific conductance of roughly 1000uS for the less powerful backpack units (OWRB, 2004).

Seines are nets of various dimensions and mesh sizes which are dragged through the water at a certain speed in order to encircle and trap the fish. The seines are pulled along the bottom from both ends of the net and are long enough to produce a pocket or bag behind the operators that will capture and hold fish. Seining is selective for collecting smaller fish then electrofishing and is only effective in areas without large quantities of woody debris or other obstacles that may become entangled in the net.

2.1 Sampling Locations

Fish sampling will be conducted at 10 impacted locations in various streams and tributaries within the Illinois River watershed. Additional fish sampling will be conducted at 3 predetermined reference locations within the USEPA Eco-region III. Sample areas will be 100 meters in length and consist of riffles, runs, and pools in proportions that are reflective of the overall characteristics of the stream.

2.2 Sampler Selection

In an effort to achieve a representative sample of the resident fish populations, both electrofishing and seining may be conducted at each sampling location. The amount and proportion of each method used will vary depending on site-specific factors that are limiting to the effectiveness of each sampling type.

2.3 Site Data to be Collected

At each fish sampling location, a variety of physical variables should be recorded in order to quantify factors that may have an influence on the resident fish populations and/or the efficacy of the sampling techniques employed. Variables may include, but are not limited to:

- Average stream width, depth, and velocity within the sampling reach.
- Water temperature, conductivity, pH, and dissolved oxygen (DO) content.

2.4 General Supplies

The following is a list of the minimum supplies needed for a resident fish sampling event:

- Backpack and bank electrofishing units
- Shocking wands (anodes) with kill switch
- Plastic five-gallon buckets with handles
- Block nets with ¼ inch mesh size
- Seines
- Dip nets of various sizes
- Fish cages for live fish storage
- Gas powered electric generators
- Variable voltage pulsator units (VVP)
- Tow boat to hold generator and VVP
- Waders for each crew member
- Non-conductive rubber gloves for each crew member
- Polarized glasses for each crew member
- Multi-meter for collecting water quality parameters
- Marsh-McBernie water velocity meter
- Tape measure (for measuring width and length of station)
- Magnifying glasses to aid in fish identification
- Fish identification keys

- Extra gasoline/oil mixture for generators
- Spark plugs and soldering iron for equipment repairs
- Voltmeter
- 100' extension cord
- Handheld GPS Unit, with extra batteries
- One meter fish measuring board
- 1 pound capacity weight scale
- 5 pound capacity weigh scale
- Data Sheets on water resistant paper
- Field Notebooks with water resistant paper
- Writing utensils (waterproof)
- Digital camera with extra batteries
- Applicable scientific collection permits with team member who appears on permit
- Sunscreen
- Insect repellent
- Drinking water/snacks/lunches for the crew

3.0 Safety

Electrofishing involves running a high-powered electric current through water can be very dangerous if the proper safety procedures are not followed. Since electric shock can occur, crewmembers should be trained in first-aid and CPR prior to sampling. Some general safety guidelines include:

- While electrofishing, avoid contact with the water unless sufficiently insulated from electric shock. Only waders and watertight, non-conductive rubber gloves should ever touch the water.
- Do not reach into the water at anytime during electrofishing.
- Avoid contact with the anode.
- All electrofishing wands have kill switches that will stop the flow of electricity if released during sampling. Do not make any modifications to these switches.
- Be aware of other members of the crew. If another crewmember falls or makes contact with the water, release the kill switch to stop the electric current immediately.
- If waders or gloves develop leaks or become wet on the inside, they may no longer serve as effective insulators and should be replaced.
- Do not electrofish in heavy rain or around other people, pets, or livestock.
- If stream conditions are not fit for electrofishing (i.e., flow is too high, conductivity is <10uS or >1000uS, water is too deep or too turbid), the crew may choose not to sample the site at that time.
- Gasoline and preservatives should be handled and stored properly.

4.0 Sampling Procedures

Fish will be collected across a 100-meter stretch of stream with block nets (¼ inch mesh) placed on each end of the sampled reach to ensure that the sampling is limited to a closed population and the data collected is not influenced by emigrant or immigrant individuals. A multiple-pass depletion methodology based on a closed population will be used to provide the data necessary for a reasonable population estimate of all fish species present at each location (White et al. 1982).

4.1 State Notification

Prior to sampling the appropriate state agency must be contacted to inform them of the location of the sample site and when sampling will occur. A copy of the collecting permits must be present at all times. The contact information differs for each state.

For sites in Oklahoma contact:

Jim Burroughs
N.E. Region Fisheries Supervisor
Oklahoma Department of Wildlife Conservation
9097 N. 34th Street West
Porter, Oklahoma 74454
918-683-1031

For sites in Arkansas contact:

Capt. Luther Hungate
Arkansas Game and Fish Commission
Northwest Regional Office
455 Dam Site Road
Eureka Springs, AR 726314
866-253-2506
1-800-482-9262

4.2 Site Preparation

Once the location of each site has been established and verified by GPS, the site must be prepared prior to sampling. The 100m reach of stream to be sampled should be measured and block nets should be placed at both ends of the reach. The block nets should extend across the entire width of the stream and be tall enough to extend approximately 1 meter above the surface of the water while maintaining firm contact with the substrate. The block nets should be weighed down with a lead line or available rocks so that fishes cannot move past them. Fish cages should be placed at regular intervals along the stretch of stream to hold captured fish.

4.3 Procedures

Following the completion of necessary site preparations, the first sampling pass can begin. A sampling pass will consist of a thorough collection via electrofishing and/or seining in all feasible locations within the sampling reach. A minimum of two complete passes will be conducted at each location, and fish will not be returned to the site between passes. This will enable statistical calculations to be made so that a valid population estimate can be calculated.

4.3.1 Electrofishing

The backpack electrofisher is essentially a small gasoline powered generator attached to a variable -voltage pulsator (VVP) which has outputs for both an anode and a cathode. The cathode is a stainless steel cable that is trailed behind the operator. The anode consists of a horizontal metal ring or diamond shaped electrode attached at the end of a fiberglass pole with a safety kill switch mounted on the handle. In most cases, either electrode type may be used. However, in waters with extremely low conductivity (<40uS) or in deep water, the ring electrode is often more effective. In waters with a conductivity >500uS, the diamond-shaped electrode should be used (OWRB, 2004).

In larger streams a shore-based electrofishing unit may be used. This unit involves the use of a more powerful generator and VVP unit that can be placed on the shore or floated through the current on a small tow boat. The VVP is connected to the generator with a 100-foot waterproof extension cord to allow for greater mobility. The basic procedures remain the same as with a backpack unit.

Prior to shocking a safety overview of electrofishing should be conducted and crew members should read and understand the operating manuals for the backpack electrofishing units prior to use. To avoid the risk of electric shock, persons involved in sampling should wear rubber, non-conductive gloves and waders at all times. All safety precautions must be observed.

The following procedures should be followed:

- A minimum of two crew members are required per electrofishing unit, one to operate the unit and at least one person to net stunned fish. Additional netters may also be employed to increase the catch rate of stunned fishes. One crew member will be responsible for regularly

transporting collected fish in buckets to live wells to prevent the adverse effects of overcrowding in the collection buckets. Crew members must wear the appropriate safety gear at all times.

- Sampling begins at the downstream limit of the 100m site and will proceed in an upstream direction. All available habitat types should be sampled within the stream.
- Once the electrofishing unit is powered on, the operator should depress the safety kill switch, causing the electric field to be produced. The operator should gradually pass the anode back and forth across the stream width and around any areas that could provide cover. Special attention should be given to areas surrounding root wads, brush piles or undercut banks.
- As the electrode approaches fish, they will become stunned and will roll become visible to the netters. The netters can then collect the fish and move them to buckets or live wells until the collection is completed.
- In some cases, it may be more effective to insert the probe into an area containing fish prior to depressing the switch. This will allow the fish to become accustomed to the probe and will prevent them from fleeing. When the field is turned on, more fish will be stunned and will be easier to capture.
- Once the entire reach has been sampled, the crewmembers may power off the electrofishing units and return to the live wells to examine and enumerate the fish.

4.3.2 Seining

Seines consist of nets of various lengths attached to vertical poles on either end and are pulled through the water while maintaining contact with the bottom. The height of the net should be greater then the depth of the water to prevent fish from escaping. Obstructions within the stream will often make seining impossible, so only certain areas will be sampled by this method.

- Seining will typically be done by two crewmembers pulling the net through the water in a downstream direction. Enough slack should be maintained behind the operators in order to capture and hold fish. The lead-lines should maintain contact with the bottom and the float-lines should be above the surface at all times.

- The seine should be pulled through the water for at least 10 meters and then dragged up a gradually sloping bank. Then the fish captured can be collected and stored in live wells or buckets for identification and eventual release.
- If a gradually sloping bank is not available, the dip-method can be utilized. This involves keeping a wide bag in the net and then pulling it up a steeper bank by turning the poles horizontally while following the bottom as closely as possible.
- In some cases, a fast moving current will make seining in a downstream direction impossible. In this event, seining should be conducted perpendicular to the current with the downstream operator moving slightly ahead to form a "j" shape in the net.
- Other seining techniques may also prove effective and may be employed by the field crew in some cases.

5.0 Data Collection and Quality Control

After each pass, all fish captured will be counted and stored in a live well or bucket until the final pass at each site is completed. The specimen collected will then be keyed out and identified to the species level. Various fish identification manuals will be available including: *Fishes of Oklahoma* (Miller and Robinson, 2004), *Fishes of Arkansas* (Robinson and Buchanan, 1984), and the *Peterson Field Guide to Freshwater Fishes of North America.* Any specimen that cannot be positively identified in the field will be preserved and brought back to the lab for identification.

Where applicable, all captured fish will also be weighed and measured for total length and any physical abnormalities will be noted. All fish population data will be recorded on the supplied data sheets using the fish species codes (**Table 1**) and any additional information will be recorded in field notebooks. If a large number of fishes of the same species are collected at a site, they may be counted and grouped into size classes so that a representative specimen may be measured from each group.

6.0 Documentation

Bound field logbooks should be used for the maintenance of field records. All aspects of sample collection and handling as well as visual observations shall be documented in the field logbooks. Supplemental information may be documented on resident fish population sampling

field sheets provided. All entries in field logbooks should be legibly recorded and contain accurate and inclusive documentation of an individual's project activities.

7.0 Additional Information

Other contact information:

Bert Fisher, PhD
Lithochimeia, Inc.
222 South Kenosha Ave.
Tulsa, OK 74120
Telephone: 918-382-9784

Ronald French
CDM
100 North Tucker Blvd.
Suite 550
Saint Louis, MO 63101
314-241-8510

8.0 References

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, 2nd Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Murphy, B.R., and D.W. Willis, editors. 1996. Fisheries techniques, 2nd Edition. American Fisheries Society, Bethesda, Maryland.

Oklahoma Water Resources Board (OWRB). 2004. Water Quality Monitoring Program: Field Sampling Protocol for Water Quality Assessments of Streams and Rivers, Draft Copy. Oklahoma Water Resources Board; Water Quality Programs Division; Oklahoma City, Oklahoma.

White, G.C., D.R. Anderson, K.P.Burnham, D.L. Otis, 1982. Capture-Recapture and Removal Methods for Sampling Closed Populations. LA-8787-NERP. U.S. Department of Energy; Los Alamos National Laboratory; Los Alamos, New Mexico.

9.0 Revised Dates*

The following revision dates are applicable to this SOP:

Revision 1 – November 29, 2005

Revision 2- January 19, 2006

Table 1: List of 3-Letter Codes for Captured Fish Species

Common Name	Scientific Name	Species Code
Banded darter	*Etheostoma zonale*	BND
Banded Sculpin	*Cottus carolinae*	BDS
Bigeye chub	*Hybopsis amblops*	BCH
Bigeye shiner	*Notropis boops*	BES
Black Bullhead	*Ameiurus melas*	BBH
Black Redhorse	*Moxostoma duquesnei*	BRH
Blackstripe Topminnow	*Fundulus olivaceus*	BTM
Bluegill Sunfish	*Lepomis macrochirus*	BSF
Bluntnose minnow	*Pimephales notatus*	BNM
Brook Silverside	*Labidesthes sicculus*	BSS
Cardinal Shiner	*Luxilus cardinalis*	CDS
Central Stoneroller*	*Campostoma anomalum*	CSR
Channel Catfish	*Ictalurus punctatus*	CCF
Creek Chub	*Semotilus atromaculatus*	CCH
Fantail Darter	*Etheostoma flabellare*	FTD
Fathead Minnow	*Pimephales promelas*	FHM
Gizzard Shad	*Dorosoma cepedianum*	GSD
Golden Redhorse	*Moxostoma erythrurum*	GRH
Green Sunfish	*Lepomis cyanellus*	GSF
Greenside Darter	*Etheostoma blennioides*	GSD
Largemouth Bass	*Micropterus salmoides*	LMB
Largescale stoneroller*	*Campostoma oligolepis*	LSR
Logperch	*Percina caprodes*	LGP
Longear Sunfish	*Lepomis megalotis*	LES
Mosquito Fish	*Gambusia affinis*	MOF
Northern Hogsucker	*Hypentelium nigricans*	NHS
Northern Studfish	*Fundulus catenatus*	NSF
Orangethroat Darter	*Etheostoma spectabile*	OTD
Ozark Minnow	*Notropis nubilus*	OZM
Redspot Chub	*Nocomis asper*	RCH
Rock bass	*Ambloplites rupestris*	RKB
Shadow Bass	*Ambloplites ariommus*	SHB
Shorthead redhorse	*Moxostoma macrolepidotum*	SRH
Slender Madtom	*Noturus exilis*	SMT
Smallmouth Bass	*Micropterus dolomieu*	SMB
Southern Redbelly Dace	*Phoxinus erythrogaster*	SRD
Spotted Bass	*Micropterus punctulatus*	SPB
Stippled Darter	*Etheostoma punctulatum*	STD
Warmouth Sunfish	*Lepomis gulosus*	WSF
Yellow Bullhead	*Ameiurus natalis*	YBH

Prepared: Brian Bennett Review: Tony Gendusa

Approved: _____ Issued: _____

1.0 Overview and Application

This standard operating procedure (SOP) describes field procedures used for collection of resident fish from rivers and streams during the 2007 sampling event. This SOP will be used for collecting samples using electrofishing gear and kick seining and is primarily based on the USEPA Rapid Bio-assessment Protocols (RBP) for use in wadeable streams and rivers (Barbour, 1999), with modifications based on (1) protocols summarized from various natural resource agencies (**Table 1**) and (2) intended data uses.

The Illinois River watershed in eastern Oklahoma and western Arkansas has been receiving inputs from agricultural runoff, primarily from fields where poultry waste has been applied. These wastes, along with the chemical constituents that make up the poultry wastes, have impacted the water quality and sediments in the streams and lakes in the Illinois River Watershed. The purposes of the work being performed are to evaluate and document 1) the linkage and relationship, if any, between the disposal of poultry wastes and environmental contamination/nutrient enrichment within the Illinois River Watershed, and; 2) the resulting harm/injury to resident fish populations that may have resulted from the disposal of poultry wastes within the Illinois River Watershed.

2.0 Sampling Methods Summary

The collection of fish from rivers and streams will involve the use of both electrofishing and kick seining as capture techniques. Due to variations in water chemistry, stream type, and fish life history, different sampling techniques must be employed to ensure that representative samples of the resident fish populations are collected. The relative advantages of each sampling method are discussed in detail in Fisheries Techniques (Murphy and Willis, 1983).

Electrofishing is the process of introducing a high voltage/low amperage electric charge into the water which stuns the fish and allows for easy collection, commonly with the use of dip nets. This sampling technique is selective for deep-bodied fish with large surface areas. Electrofishing is typically used in habitats where seining is not possible, such as around woody debris, in very shallow riffles, and near undercut banks. Electrofishers are only effective in water with a specific conductance of 40-1700uS with a maximum specific conductance of roughly 1000uS for the less powerful backpack units (OWRB, 2004).

Seines are nets of various dimensions and mesh sizes which are dragged through the water at a certain speed in order to encircle and trap the fish or, for kick seines, are placed at locations for a short time period to trap fish dislodged by kicking the substrates in which these species live. Kick seines are specifically employed for the collection of riffle-dwelling fish species that are not successfully collected using electroshocking techniques. These commonly include small bottom dwelling species such as madtoms and darters. The kick seines are placed more or less perpendicular to the flow of water and held in place by two persons. A third person stands immediately (i.e., one to two meters) upgradient of the seine and kicks the stream bottom substrates (sand, gravel, cobble) to dislodge bottom dwelling or sediment-associated fish. Dislodged fish are then carried by the current into the seine, where they are collected, identified, and counted.

2.1 Sampling Stations

Fish sampling will be conducted at approximately 30 selected locations in various streams and tributaries within the Illinois River watershed. Additional fish sampling may be conducted at predetermined reference locations within the USEPA Eco-region III. In wadeable streams, the sampling area will consist of a stream length equal to 30 times the mean wetted stream width at the time of sampling, but in any case not less than 100 meters in length. The mean wetted stream width will be measured by averaging the mean wetted width of two riffles and two pools. The mean wetted width of four representative transects will be used where habitat variability is low (i.e., if two pools and two riffles are not present). Stream length will be measured along the descending left bank (on the left, facing downstream). The minimum stream length to be sampled is 100 meters, and the maximum length is 800 meters. The maximum will be most applicable to larger non-wadeable rivers such as the mainstem Illinois River. Block nets will not be used but natural barriers or habitat type boundaries (e.g., beginning or end of a riffle) will be used to define the beginning and end of each sampling reach.

2.2 Sampling Procedures - Overview

In an effort to achieve a representative sample of the resident fish populations, both electrofishing and kick seining will be conducted at each sampling location. The amount and proportion of each method used will vary depending on site-specific factors that are limiting to the effectiveness of each sampling type. Electrofishing will be employed for sampling runs and pools, and kick seining will be used to sample all riffles. Pools and runs At least 3 pools and 3 runs within the selected reach of wadeable streams will first be sampled using backpack electroshocking. At least 3 riffles of the same reach will be additionally sampled using kick seines. Sampling using both methods will be based on a predetermined unit of time, initially set at 3 minutes for pools and runs and 0.5 minutes (30 seconds) for riffles, but subject to field modification. Each unit of effort will therefore initially be a 3 minute (pools and runs) or a 0.5

minute (riffles) period of time. Some habitats (e.g., a small riffle) may require only one unit of effort (30 seconds) to completely sample, while others (e.g., a deep pool with logs and vegetation) may require 3 or more units of effort (9 + minutes, in this example). At the end of each unit of effort, the sampling will pause and another sample will be collected. The "pause" may simply be switching buckets to keep each "unit's" catch separate or, in cases where a habitat type has been completed, may consist of identifying and counting the fish collected during that "unit". Fish data (e.g., numbers of each species collected) will be kept separate for each unit of effort, even if multiple units are required to sample a single habitat unit. Units of effort will apply to both electroshocking (actual "pedal on" time of 3 minutes or 180 seconds) and kick seining (30 seconds of substrate disturbance). Habitat units will be limited to one of three habitat types: pool, riffles, and runs/glides. Each will be defined using U.S. Forest Service (USFS) guidelines (McCain et al. 1990). For this sampling effort, runs and glides are assumed to be equal, and no distinction is made between these two habitat types. USFS graphical and textual descriptions for each of the three habitat types will be maintained by each field team.

Electroshocking in wadeable streams will be conducted using one pass, proceeding from downstream to upstream, moving from shore to shore in a zigzag pattern, until the minimum number of habitat types has been completely sampled. Kick seining of riffle habitats will also proceed from downstream to upstream, following completion of backpack electroshocking. Electroshocking by boat in non-wadeable streams will proceed from downstream to upstream along one bank, then in the same direction along the opposite bank, making one pass per bank. Section 4.3 provides detailed procedures for each sampling method.

2.3 Data Collection

At each fish sampling location, a variety of physical variables should be recorded in order to quantify factors that may have an influence on the resident fish populations and/or the efficacy of the sampling techniques employed. Variables may include, but are not limited to:

- Average stream width, depth, and velocity within the sampling reach.
- Amount and type of vegetation along each bank and instream (e.g., 60% vegetated, primarily with grasses and shrubs)
- Water temperature, conductivity, pH, and dissolved oxygen (DO) content.
- Dominant substrate type and size for each of the four habitat types (pool, riffle, run/glide)
- Numbers of each type of fish collected by unit of effort for each habitat unit (i.e., for each 3 minute unit of effort for a given pool or run and for each 30 second effort for each riffle).

2.4 Supplies

The following is a list of the minimum supplies needed for a resident fish sampling event:

- Smith-Root LR-24 Electrofishing Equipment
- Boat and boat-mounted electroshocking equipment
- Shocking wands (anodes) with kill switch
- BC-24ps Battery Charger
- Portable generator
- Plastic five-gallon buckets with handles
- Kick seines (1/8 inch mesh)
- Dip nets of various sizes (1/8 inch mesh)
- Waders for each crew member
- Non-conductive rubber gloves for each crew member
- Polarized glasses for each crew member
- Habitat identification keys
- Multi-meter for collecting water quality parameters
- Marsh-McBirney water velocity meter
- Tape measure (for measuring width and length of station)
- Magnifying glasses to aid in fish identification
- Fish identification keys
- Extra gasoline/oil mixture for generators (if applicable)
- Extra batteries for electrofishing units
- Spark plugs and soldering iron for equipment repairs

- Voltmeter
- 100′ extension cord
- Handheld GPS Unit, with extra batteries
- Data Sheets on water resistant paper
- Field Notebooks with water resistant paper
- Writing utensils (waterproof)
- Digital camera with extra batteries
- Applicable scientific collection permits with team member who appears on permit
- Sunscreen
- Insect repellent
- Drinking water/snacks/lunches for the crew

3.0 Safety

Electrofishing involves running a high-powered electric current through water can be very dangerous if the proper safety procedures are not followed. Since electric shock can occur, crewmembers should be trained in first-aid and CPR prior to sampling. Some general safety guidelines include:

- While electrofishing, avoid contact with the water unless sufficiently insulated from electric shock. Only waders and watertight, non-conductive rubber gloves should ever touch the water. Non-conductive rubber gloves will be worn by all team members while in the water.
- Do not reach into the water at anytime during electrofishing.
- Avoid contact with the anode.
- All electrofishing wands have kill switches that will stop the flow of electricity if released during sampling. Do not make any modifications to these switches.
- Be aware of other members of the crew. If another crewmember falls or makes contact with the water, release the kill switch to stop the electric current immediately.

- If waders or gloves develop leaks or become wet on the inside, they may no longer serve as effective insulators and should be replaced.
- Do not electrofish in heavy rain or around other people, pets, or livestock.
- If stream conditions are not fit for electrofishing (i.e., flow is too high, conductivity is <10uS or >1000uS, water is too deep or too turbid), the crew may choose not to sample the site at that time.
- All field personnel should read the User Manual for the Smith-Root LR-24 Electrofisher, Pages 45 through 49 cover safety features for this equipment.

4.0 Sampling Teams

Sampling teams will consist of 4 persons per team. For electroshocking in wadeable streams, each team will consist of 1 shocker, 2 netters, and 1 recorder (follows closely behind the electroshocking team). For kick seining, each team will consist of 1 "kicker" (disturbs the substrate), 2 persons stabilizing the seine, and 1 recorder. For boat electroshocking, each team will include 1 shocker/netter, 1 recorder and boat driver. The recorder may have to follow along the shore if the boat capacity does not allow for 4 people.

4.1 State Notification

Prior to sampling the appropriate state agency must be contacted to inform them of the location of the sample site and when sampling will occur. A copy of the collecting permits must be present at all times. The contact information differs for each state.

For sites in Oklahoma contact:

Jim Burroughs
N.E. Region Fisheries Supervisor
Oklahoma Department of Wildlife Conservation
9097 N. 34th Street West
Porter, Oklahoma 74454
918-683-1031

For sites in Arkansas contact:

Capt. Luther Hungate
Arkansas Game and Fish Commission
Northwest Regional Office
455 Dam Site Road
Eureka Springs, AR 726314
866-253-2506
1-800-482-9262

4.2 Initial Tasks / Site Preparation

Once the location of each site has been established and verified by GPS, several tasks must be completed prior to sampling. Water quality samples, if required, should be collected prior to disturbing the bottom substrates. One team member should begin filling out the data collection/field forms which generally describe the site, as discussed in Section 2.3. Other team members can begin determining the specific reach to be sampled. This begins by determining if at least 3 runs, 3 riffles, and 3 pools are present within the minimum reach length of 100m. If yes, then the upper boundary of reach is flagged at 100m and the reach to be sampled is 100 meters in length. If at least 3 of each habitat units does not occur within 100m of the starting location, then the upper boundary of the reach is flagged at the point determined by 30 times the mean wetted stream width, as discussed in Section 2.1, but not to exceed 800m in length. In this case, the reach to be sampled should be measured and recorded in the field data form. The upper or most upstream limit of the reach may be extended slightly beyond the marked limit to ensure complete sampling of a specific habitat unit. For example, do not end the sampling in the middle of a large pool but extend the sampling to ensure complete sampling of the pool. The final length sampled should be recorded on the field data sheets, with notification if it exceeds the length beyond that defined by either the 100m minimum or 30 times the mean wetted stream width.

4.3 Detailed Sampling Procedures

4.3.1 Electrofishing

Wadeable Streams

The LR-24 backpack electroshocker consists of a trailing stainless steel electrode cable and a ring electrode mounted on a yellow wand that has a red operating/kill switch. A minimum of three people should be used for each electroshocking event. A fourth person may be identified as a recorder, and this person remains on the bank during electroshocking. One person carries the shocking unit and wand, and two people each carry both a large dip net and a smaller dip net.

Team members should not switch tasks during the sampling effort at each site (i.e. the person carrying the anode will not switch with a netter and vice versa during the pass). A netter should be on each side of the shocking wand. Each netter will have two nets, one smaller one for difficult to reach fish and one larger net. Collection begins at the downstream end of the segment, moving upstream. The wand holder should move in a zigzag pattern across the habitat unit, with the goal of sampling all habitat types within the specified habitat unit. The amount of electroshocking time as shown on the backpack unit (actual "pedal-on" shocking time) should be recorded on the field sheet. Prior to shocking, a safety overview of electrofishing should be conducted and crew members should read and understand the operating manuals for the backpack electrofishing units prior to use. To avoid the risk of electric shock, persons involved in sampling should wear rubber, non-conductive gloves and waders at all times. All safety precautions must be observed. All fish will be returned to the stream except those that cannot be reliably identified in the field. These will be retained in formalin for laboratory identification.

The following procedures should be followed:

- A minimum of four crew members are required per electrofishing unit, one to operate the unit, two persons to net stunned fish, and a team member to record data as sampling proceeds—this person follows the shocking crew. Upon the completion of sampling a habitat type (e.g. riffle, pool, etc), sampling team members will identify the fish collected and record on the appropriate data sheets. Fish will be released back to the stream, downstream of the habitat type sampled. The amount of electroshocking time for that habitat type will be recorded. Electroshocking time can be obtained from the LR-24 unit. Electroshocking time in each habitat type will be for 3 minutes.

- Sampling begins at the downstream limit of the reach to be sampled and will proceed in an upstream direction. All available microhabitats will be sampled within the identified habitat unit.

- Once the electrofishing unit is powered on, the operator should depress the safety kill switch, causing the electric field to be produced. The operator should gradually pass the anode back and forth across the habitat unit (e.g., pool) and around any areas that could provide cover. Special attention should be given to areas surrounding root wads, brush piles or undercut banks. After 3 minutes have passed and the electrode has been turned off, do not turn it back on to re-shock fish that may have been missed during the netting process, unless they are actively swimming away. Every effort should be made to keep the pedal on time to exactly 3 minutes.

- As the electrode approaches fish, they will become stunned and will roll or float to the surface where they become visible to the netters. The netters can then collect the fish and move them to buckets or live wells until the collection is completed. In some cases, it may be more effective to insert the probe into an area containing fish prior to depressing the switch. This will allow the fish to become accustomed to the probe and will prevent them from fleeing. When the field is turned on, more fish will be stunned and will be easier to capture.

- A brief description of the physical characteristics (e.g. substrate type, pool depth) of the habitat will be recorded on the field data sheets.

Unwadeable Streams

In larger streams a shore-based or boat-mounted electrofishing unit may be used. For this study, the Smith-Root 2.5 GPP electroshocking unit is mounted to a 14' boat. The pulsating unit is connected to the generator with the electroshocking wands and cable extending from the boat. The boat crew shall consist of one person driving the boat, one of the net persons directing the boat driver, and a third person netting (as needed) and recording. One additional person can remain on shore to aid in fish identification.

As per wadeable streams, the length of river reach to be sampled is based on the minimum of 100m or 30 times the mean wetted stream width. If this value is calculated to exceed 800 meters, then the maximum length of stream to be sampled is set to 800 meters. Both banks will be shocked, and any available instream habitat will be sampled as well. A single pass will be made along each bank, proceeding from downstream to upstream. Netted fish will be kept in a live cage/well until identified and enumerated. All fish will be returned to the stream except those that cannot be reliably identified in the field. These will be retained in formalin for laboratory identification.

Sampling by boat will be used for all pools and runs within the predetermined sampling reach when that reach contains a mix of wadeable and unwadeable portions. In such reaches, kick seines will be used to sample all riffles, as described below.

4.3.2 Kick Seining

Kick seines consist of nets about 6 to 8 feet in length, 4 feet in height, with a mesh size of 1/8 inch. At each end of the seine is a vertical pole used to maintain the net in an upright position. The net is placed at the downstream end of a riffle perpendicular to the flow, and kept more or less upright by two persons (one at each pole). A third person stands about two meters immediately upgradient of the seine and kicks/disturbs the bottom substrate to dislodge small bottom dwelling fish. Kicking continues for 30 seconds, then the seine is removed and the fish are identified and counted. If the riffle has been completely sampled with this single 30 second

sampling event, the seining continues on to the next riffle upgradient. If the riffle is large or includes multiple microhabitat types, it may require additional 30-second collections. All microhabitat types within a riffle will be sampled regardless of how many "30-second time units" it takes to complete the sampling. For example, some riffles may be sampled using 3 separate "transects" to ensure that unique microhabitats within the riffle are sampled.

5.0 Data Collection and Quality Control

After each unit of time (initially set at 3 minutes), all fish captured will be either identified and counted or stored in a separate bucket or live well until the collection is complete for that habitat unit (e.g., a pool). The fish collected will be identified to the species level. Various fish identification manuals will be available including: *Fishes of Oklahoma* (Miller and Robinson 2004), *Fishes of Arkansas* (Robinson and Buchanan 1984), and the *Peterson Field Guide to Freshwater Fishes of North America* (Page and Burr 1991). Any specimen that cannot be positively identified in the field will be preserved and brought back to the lab for identification.

All captured fish will also be observed for any physical abnormalities, and any findings will be recorded on the field data sheets. All fish population data will be recorded on the supplied data sheets using the fish species codes (Table 2). Any additional information relevant to this study will be recorded in field notebooks.

6.0 Documentation

Bound field logbooks should be used for the maintenance of field records. All aspects of sample collection and handling as well as visual observations shall be documented in the field logbooks. Supplemental information may be documented on resident fish population sampling field sheets provided. All entries in field logbooks should be legibly recorded and contain accurate and inclusive documentation of an individual's project activities.

<u>Other contact information:</u>

Ronald French
CDM
100 North Tucker Blvd.
Suite 550
Saint Louis, MO 63101
314-241-8510

8.0 References

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, 2nd Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

McCain, M., D.Fuller, L.Decker and K.Overton. 1990 . Stream habitat classification and inventory procedures for northern California. FHC Currents. No.1. U.S. Department of Agriculture. Forest Service, Pacific Southwest Region.

Murphy, B.R., and D.W. Willis, editors. 1996. Fisheries techniques, 2nd Edition. American Fisheries Society, Bethesda, Maryland.

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U.S. Forest Service (USFS). July 2005. Stream Condition Inventory Technical Guide. United States Forest Service. Ecosystem Conservation Staff. Vallejo, CA 111p.

White, G.C., D.R. Anderson, K.P.Burnham, D.L. Otis, 1982. Capture-Recapture and Removal Methods for Sampling Closed Populations. LA-8787-NERP. U.S. Department of Energy; Los Alamos National Laboratory; Los Alamos, New Mexico.

Table 1. Summary of Protocols for Fish Sampling / Proposed Protocols

Item / Issue	ADEQ	ORWB	ODWC	OCC	NAWQA	USEPA	Proposed
Sampling reach length (ft)	700 - 1,500 ft (used in at least 1 study but not a specific protocol)	General = 400 m (sm streams = 200 m, rivers = 800 m)	not stated	30X mean stream width	not stated	Fixed distance and proportional distance (e.g., 40X mean width) acceptable. Max time acceptable for large rivers (e.g., 3 hrs).	**30X mean wetted stream width (measured along left descending bank), determined by mean of widths at two riffles and two pools, min = 100 m and max = 800 m (natural barriers, no block nets)**
Alternate (to electroshock) method employed	yes, seine	yes, seine	requires electroschock + seine	Requires electroschock + seine	requires electroschock + seine	electroshock single best method	**electroschock (backpack for wadeable streams; boat for non-wadeable streams or portions of streams) + kick net seine ; kick net seine follows electroshock-ing and covers range of microhabitats in all available riffles (e.g., 1-3 samples per riffle)**
Number of passes	not stated	not stated	min = 1, multiple at restoration sites, min = 3 for depletion estimates	Not stated	2	not stated	**1 (4 person team; two netters, 1 shocker, one recorder; each netter has both large and small nets)**
Battery powered backpack	yes	no, gas with Honda generator	not specific	no, gas with Honda generator	not specific	not specific	**Yes**
Habitat sampled	all available	all available	all available	All available	all available	all available	**all available**

Comparison Station	Upstream of stressor source preferred (comparison to another water body or to "least disturbed" sites often used)	not stated	not stated	Not stated	not stated	not specific but implied via use of various metrics (comparison to reference)	**Little Lee Cr for small streams;**
Species focus	all fish (for assessing water quality impacts)	not stated	sport fish	All	all	all, but fish <20 mm in length are not included	**all, but fish <20 mm in length are not included in ID or counts**
Fish released	large fish	large fish, species of concern if ID verified, easily identified taxa	all sport fish following wt and length	Only large with field ID (others preserved for lab ID)	all T&E species, all post ID except voucher specimens	all T&E species, all post ID except voucher specimens	**all T&E species, all post ID except those not positively identifiable in the field**
Dip net mesh size	Not stated	not stated	not stated	Not stated	not stated	not stated	**1/8" (for both large and small nets)**
Catch per unit effort / Recording of Effort	Not stated	not stated	not stated	Not stated	not stated	not specific	**record min. of shock time, total stream length; record fish data per discrete habitat unit (e.g., per each run, riffle, pool); target sampling time unit is 3 minutes of "pedal-on" time (180 seconds, for pools and runs) or 30 seconds of "kicking" for kick seine collections. In all cases, multiple units may be required for large or complex habitat units (each habitat unit = pool or or run or riffle, as defined by**

							USFS guidelines. Note that glides and runs are defined here to be equal, with no distinction between these two habitat types)
Summary Comment	Appears flexible	less specific but generally similar to EPA	focus on sport fish	Less specific but generally similar to EPA	less specific but generally similar to EPA	most specific and detailed, multiple options presented	**The methods/details shown in this column have been discussed and accepted by all parties (CDM, ODWC, June 2007)**

Table 1. References

ADEQ	Arkansas Department of Environmental Quality	**Pages 71-72 in***:* Illinois River Water Quality, Macroinvertebrate and Fish Community Survey, Benton and Washington Counties, Arkansas. ADEQ Water Division, 1997, 90 p. **AND Page 63 in***:* 2002 Integrated Water Quality Monitoring and Assessment Report*.* Prepared pursuant to section 305(b) and 303(d) of the Federal Water Pollution Control Act, ADEQ Water Division, 476 p.
ORWB	Oklahoma Water Resources Board (2004)	Oklahoma Water Resources Board (OWRB). 2004. Water Quality Monitoring Program: Field Sampling Protocol for Water Quality Assessments of Streams and Rivers, Draft Copy. Oklahoma Water Resources Board; Water Quality Programs Division; Oklahoma City, Oklahoma.
ODWC	Oklahoma Department of Wildlife Conservation	Oklahoma Department of Wildlife Conservation (ODWC). Date unknown. Protocols for Assessing Fish Population Response to Stream Restoration Projects. ODWC, 5 pages.
OCC	Oklahoma Conservation Commission	Oklahoma Conservation Commission (OCC). 1996. Standard Operating Procedure: Sampling Procedures used by the Oklahoma Conservation Commission for Fish Collection in Streams. OCC, 7 pages.
NAWQA	USGS- National Water Quality Assessment Program	Moulton, S.R., J.G. Kennon, R.M. Goldstein, J.A. Hambrock. 2002. Revised Protocols for Sampling Algal, Invertebrate, and Fish Communities as Part of the National Water-Quality Assessment Program. USGS Open-File Report 02-150. US Geological Survey; Reston, VA.
USEPA	U.S. Environmental Protection Agency, Rapid Bioassessment Protocols (RBP), second edition	Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Table 2: Fish Species in Illinois River Watershed (OK/AR)

Common Name	Scientific Name	Species Code	Trophic Guild	Tolerance
Shadow Bass	*Ambloplites ariommus*	SHB	-	-
Rock bass	*Ambloplites rupestris*	RKB	P	M
Black Bullhead	*Ameiurus melas*	BBH	I	M
Yellow Bullhead	*Ameiurus natalis*	YBH	I	T
Freshwater Drum	*Aplodinotus grunniens*	FWD	V	M
Central Stoneroller	*Campostoma anomalum*	CSR	H	M
Largescale stoneroller	*Campostoma oligolepis*	LSR	H	M
White Sucker	*Catostomus commersoni*	WHS	O	T
Banded Sculpin	*Cottus carolinae*	BDS	I	M
Grass carp	*Ctenopharyngodon idella*	GCP	H	M
Red Shiner	*Cyprinella lutrensis*	RSH	O	T
Steelcolor shiner	*Cyprinella whipplei*	SCS	I	M
Common Carp	*Cyprinus carpio*	CCP	O	T
Gizzard Shad	*Dorosoma cepedianum*	GSD	O	M
Gravel chub	*Erimystax x-punctata*	GCH	I	M
Greenside Darter	*Etheostoma blennioides*	GSD	I	M
Fantail Darter	*Etheostoma flabellare*	FTD	I	M
Stippled Darter	*Etheostoma punctulatum*	STD	-	-
Orangethroat Darter	*Etheostoma spectabile*	OTD	I	M
Banded darter	*Etheostoma zonale*	BND	I	I
Northern Studfish	*Fundulus catenatus*	NSF	I	I
Blackstripe Topminnow	*Fundulus olivaceus*	BTM	I	M
Mosquito Fish	*Gambusia affinis*	MOF	I	M
Bigeye chub	*Hybopsis amblops*	BCH	-	-
Northern Hogsucker	*Hypentelium nigricans*	NHS	I	I
Channel Catfish	*Ictalurus punctatus*	CCF	P	M
Smallmouth Buffalo	*Ictiobus bubalus*	SBF	I	M
Black Buffalo	*Ictiobus niger*	BBF	I	M
Brook Silverside	*Labidesthes sicculus*	BSS	I	M
Longnose Gar	*Lepisosteus osseus*	LNG	P	M
Green Sunfish	*Lepomis cyanellus*	GSF	I	T
Warmouth Sunfish	*Lepomis gulosus*	WSF	P	M
Bluegill Sunfish	*Lepomis macrochirus*	BSF	I	M
Longear Sunfish	*Lepomis megalotis*	LES	I	I
Redear Sunfish	*Lepomis microlophus*	RES	I	M
Cardinal Shiner	*Luxilus cardinalis*	CDS	I, other *Luxilus*	M, other *Luxilus*
Duskystripe Shiner	*Luxilus pilsbryi*	DSS	I, other *Luxilus*	M, other *Luxilus*
Redfin Shiner	*Lythrurus umbratilis*	RFS	I	M
Smallmouth Bass	*Micropterus dolomieu*	SMB	P	M
Spotted Bass	*Micropterus punctulatus*	SPB	P	M
Largemouth Bass	*Micropterus salmoides*	LMB	P	M
Spotted Sucker	*Minytrema melanops*	SPS	I	M
Black Redhorse	*Moxostoma duquesnei*	BRH	I	I
Golden Redhorse	*Moxostoma erythrurum*	GRH	I	M
Shorthead redhorse	*Moxostoma macrolepidotum*	SRH	I	M
Redspot Chub	*Nocomis asper*	RCH	I, other *Nocomis*	I, other *Nocomis*
Bigeye shiner	*Notropis boops*	BES	I	I
Ozark Minnow	*Notropis nubilus*	OZM	H	I
Rosyface Shiner	*Notropis rubellus*	RYS	I	I
Slender Madtom	*Noturus exilis*	SMT	I	I

Logperch	*Percina caprodes*	LGP	I	M
Southern Redbelly Dace	*Phoxinus erythrogaster*	SRD	H	M
Bluntnose minnow	*Pimephales notatus*	BNM	O	T
Fathead Minnow	*Pimephales promelas*	FHM	O	T
Bullhead Minnow	*Pimephales vigilax*	BHM	O	M
White Crappie	*Pomoxis annularis*	WCR	P	M
Black Crappie	*Pomoxis nigromaculatus*	BCR	P	M
Flathead Catfish	*Pylodictis olivaris*	FCF	P	M
Creek Chub	*Semotilus atromaculatus*	CCH	G	T

Trophic Guild:
- P-piscivore
- H-herbivore
- O-omnivore
- I- insectivore
- V-intertivore

Tolerance Designation (non-specific stressors):
- I-intolerant
- M-intermediate
- T-tolerant

Reference for Trophic Guild and Tolerance Designations:

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish. Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency: Office of Water: Washington, D.C.

Standard Operating Procedure
Periphyton Sampling

SOP: 7-2
Revision: 2*
Initial Date: 7/22/05
Revised Date: 2/5/07

Prepared: Brian ~~Bennett~~ Review: Renee Mulcrone

Approved: [signature] Date Approved: 6-Feb-07

1.0 Overview and Application

This standard operating procedure (SOP) describes field procedures used for collection of benthic algae (periphyton) from streams in the Illinois River watershed in eastern Oklahoma and western Arkansas. Periphyton are a vital part of many stream communities and are often used as indicators of water quality conditions (Windell, date unknown). By comparing the composition, density, and growth rates of benthic algal assemblages between affected and reference streams at similar times of year, a valuable assessment of the environmental impact of various forms of pollution can be formulated. This SOP will be used during sampling and will follow the standard laboratory based approach for periphyton sampling as outlined in the USEPA's Rapid Bioassessment Protocols (RBP) for Use in Wadeable Streams and Rivers (Barbour et al., 1999).

The Illinois River watershed has been receiving inputs from agricultural runoff, primarily from fields where poultry waste has been applied. These wastes, along with the chemical constituents that make up poultry waste have impacted the water quality and sediments in the streams and lakes in the Illinois River Watershed. The purpose of this work is to evaluate and document 1) the linkage and relationship, if any, between the poultry waste disposal and environmental contamination within the Illinois River Watershed, and; 2) the resulting harm/injury to resident periphyton communities that may have resulted from the disposal of poultry wastes within the Illinois River Watershed.

2.0 Sampling Methods Summary

Observations of benthic algae will involve collecting samples from multiple habitat types within each stream. Three types of substrate will be sampled: natural substrates pre-existing at the site, manufactured artificial surfaces (microslides) placed at the site, and from clean stones placed at the site. All substrates introduced to the site will remain on location for 18-21 days before collection to allow for periphyton to colonize the surface. If a high flow (scouring) event occurs during this period, a longer sampling time will be required. Each substrate type will be collected separately, for a total of three individual sample types for each site.

Once collected, the samples will be preserved in Lugol's solution and stored in labeled 250 ml plastic jars. The samples must be kept in the dark until laboratory analysis is conducted. Each sample will be shipped to a qualified lab for analysis. A chain of custody (COC) document will be completed and shipped with each sample.

2.1 Sampling Locations

Seven periphyton samples will be collected from a 100 m reach of stream at each of 10 impacted locations in various streams and tributaries within the Illinois River watershed. Additional periphyton sampling will be conducted at three predetermined reference locations within the USEPA Eco-region III.

2.2 Data to be Collected

At each sampling location, a variety of physical variables should be recorded to quantify factors that may have an influence on the resident periphyton communities and/or the efficacy of the sampling techniques employed. Variables may include, but are not limited to:

- Average stream width, depth, and velocity.
- Water temperature, conductivity, pH, and dissolved oxygen (DO) content.

2.3 General Supplies

The following is a list of the minimum supplies needed for a periphyton sampling event:

- Scraping tools (e.g., stainless steel teaspoons, toothbrushes, razor blades)
- Two inch sections of PVC pipe (1.5" diameter)
- Artificial substrate (micro-slides)
- Clean natural substrate (rocks) to place at site
- Fencing to contain placed natural substrate
- Frame to hold artificial substrata (Periphytometer)

- Forceps, suction bulb, and disposable pipettes
- Preservative (Lugol's solution)
- Chain of Custody forms and labels
- Data Sheets on water resistant paper
- Field Notebooks with water resistant paper
- Multi-meter for collecting water parameters
- Tape measure (for measuring width and length of station)
- Handheld GPS Unit, with extra batteries
- Writing utensils (waterproof)
- Digital camera with extra batteries
- Applicable scientific collection permits with team member who appears on permit
- Sunscreen
- Insect repellent
- Drinking water/snacks/lunches for the crew

3.0 Sampling Procedures

Periphyton will be collected across a 100-meter stretch of stream in accordance with the USEPA Rapid Bio-assessment Protocols (RBP) multi-habitat approach. Three sample types will be collected from each location. Appropriate metrics that are relevant to the Illinois River watershed will be used to measure the ecological health of the periphyton community.

Prior to sampling, it is necessary to:

- Locate the predetermined site using a hand-held GPS unit.
- Measure out and mark the appropriate length of stream to be sampled (100 m).

- Use the multi-meter to measure the various in-stream parameters for the site.
- Identify substrates to be sampled and decide on appropriate sampler locations.

3.1 Collection from Existing Natural Surfaces

When sampling the natural surfaces existing at a site, three randomly selected rocks will be scraped. These samples will then be combined into a single, composite sample for each site (Barbour, 1999). The total sampled area should be equal for each site. Collection methods are outline below:

- Collect 10 various sizes of in-stream rocks that show demonstrated algae growth. Place rocks in a row and assign them numbers from 1-10, beginning on the left. Write the numbers 1 through ten on small pieces of paper and mix them up. Random number selection can then be made by blindly drawing three numbered pieces of paper out of the ten. These numbers will represent each rock that will be sampled.
- On the three rocks selected, mark a 1 ½ inch area using a plastic PVC pipe. Trace a circle around the pipe using a sharp nail. Gently remove the algae within the circle area with a stiff brush or razor blade and siphon off the contents with a syringe. Place the contents in a 250 ml amber bottle, that contains 100 ml of site water and preserve with five (5) drops of Lugol's solution.

After collection from all three surfaces is complete, label the bottle with station number, location, substrate type, date, collector's name, and type of preservative. Record any additional information in field notebook. Place the sample in a cooler and fill out a chain of custody (COC) form.

3.2 Collection from Introduced Surfaces

The collection of benthic algae from artificial surfaces will require two visits to the site. During the first visit, the artificial substrates (microslides) and cleaned stones will have to be placed on site. The microslides will be placed in a device known as a periphytometer which holds the slides in place within the water. Cleaned stones will be placed in a wire cage to keep them separate from the pre-existing substrate. The cage will then be placed in the stream and clearly

marked. The substrates will then be collected 18-21 days later during the return visit and the periphyton that has accumulated on the surfaces can be sampled.

3.2.1 Placement of Periphytometers

The periphytometers will consist of glass microslides that are placed in a stand which is placed in the stream at an appropriate location. The microslides must be thoroughly cleaned before being placed on location. Periphytometers should be placed in the water with the shield facing upstream. They should be secured to the bottom by attaching them to a brick or large stone and marked so that they can be recovered. Each periphytometer will contain 16 microslides and 1 periphytometers will be placed at each site. If a high-flow or scouring event occurs during the 18-21-day incubation period, additional time may be necessary.

3.2.2 Retrieval of Periphytometers

After an incubation period of 18-21 days, the microslides may be collected and analyzed. Upon returning to the site, record the relevant stream conditions and parameters (see Section 2.2) in the field notebook. To determine if an individual microslide is suitable for collection, the following criteria should be reviewed:

- The slide should still be completely immersed in water.
- The slide should be free of any floating debris (trash, leaves, etc.).
- The periphytometer should not have been subjected to a high-flow event.
- The slides should not have had >10% of the surface area cleaned by grazing, abrasion, or any other means (OCC, 2002)

The slides in each periphtyometer will be numbered and a computer program will be used to randomly select which slide numbers will be sampled. Collect suitable microslides and remove the randomly selected slides from each site. Each slide collected should be carefully removed from the periphytometer and placed in a 250ml sample container completely filled with water and an adequate amount of preservative. Label the bottle with station number, location, substrate type, date, collector's name, and type of preservative. Record any additional information in field notebook. Place the sample in a cooler with ice and fill out a chain of custody (COC) form.

3.2.3 Placement of Stone Surfaces

These introduced natural substrates will consist of locally gathered rocks that have been cleaned thoroughly to ensure that no periphyton is attached to the surface prior to placement. The stones will be placed in a small wire cage (approximately 1 ft on each side) with 1" mesh. This cage will be placed in shallow, lotic water so that the stones will remain submerged for 18-21 days to allow periphyton to accumulate on the surface of the stones. The cage should be marked and the location recorded so that retrieval will be possible. If a high-flow or scouring event occurs during the incubation period, additional time may be necessary.

3.2.4 Retrieval of Stone Surfaces

After an incubation period of 18-21 days, the stones will be collected and analyzed. Upon returning to the site, record the relevant stream conditions and parameters (cited in Section 2.2) in the field notebook. For the stones to be used as suitable samples the following criteria should be met:

- The stones should still be completely immersed in water.
- The stones should be free of any floating debris (trash, leaves, etc.).
- The location should not have been subjected to a high-flow event.

Once the stones and cages have been located, they can be removed from the stream and a sample of attached periphyton can be taken. A 1.5-inch diameter circle should be randomly marked on the stones. Scrape or brush the benthic algae attached to the surface of the stones within the circle and rinse accumulated algae into one 250 ml sample container and add preservative. Label the bottle with station number, location, substrate type, date, collector's name, and type of preservative. Record any additional information in field notebook. Place the sample in a cooler with ice and fill out a chain of custody (COC) form.

4.0 Preservation Techniques and Quality Control

To ensure that all samples are in sufficient condition for analysis of the periphyton when received by the laboratory, certain procedures must be followed.

- After collecting each sample, be sure to fill the jars with water and an adequate amount of preservative (Lugol's solution) immediately to prevent decay.

- Keep jars out of the sunlight until received by laboratory.
- Label all samples on the outside of the jar with sample ID, date, location, time, type of sample, and sampler's name.
- Complete a chain of custody form for each sample location and ship to laboratory for analysis.

5.0 Documentation

Bound field logbooks will be used for the maintenance of field records. All aspects of sample collection and handling as well as visual observations should be documented in the field logbooks. Supplemental information may be documented on periphyton sampling field sheets provided. All entries in field logbooks should be legibly recorded and contain accurate and inclusive documentation of an individual's project activities.

6.0 Additional Information

Laboratory contact information:

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7.0 References

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, 2nd Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Kentucky Department of Environmental Protection (KDEP). 2002. Methods for Assessing Biological Integrity of Surface Waters. Kentucky Department of Environmental Protection, Division of Water, Frankfort, Kentucky.

Oklahoma Conservation Commission (OCC). 2002. Periphytometers & Processing for Chlorophyll-a Measurement. SOP No: IIA-06.1. Oklahoma Conservation Commission, Water Quality Division, Oklahoma City, Oklahoma.

8.0 Revised Dates*

The following are other revision dates applicable to this SOP:

Revision 1 – January 17, 2006