



**STANDARD OPERATING PROCEDURE FOR:
SAMPLING TREE SWALLOW EGGS AND NESTLINGS**

TVA-KIF-SOP-28

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1.0 PURPOSE

This standard operating procedure (SOP) describes methods for the collection and processing of tree swallow (*Tachycineta bicolor*) eggs and nestlings to determine exposure to metals from the ash spill at Kingston Fossil Plant (KIF). These procedures have been developed to help ensure that samples are collected, preserved, and prepared for shipping in a systematic manner and that appropriate documentation is maintained.

2.0 GENERAL CONSIDERATIONS

Potential hazards associated with the planned tasks are thoroughly evaluated prior to conducting field activities. A pre-job safety analysis (JSA) highlighting potential hazards is discussed at each new sampling event. Personnel use appropriate safety equipment to access nest boxes. Sample locations are walked by a member of the sampling crew and a Site safety representative prior to sample collection to identify potential safety hazards. Table 1 presents an example checklist of equipment and materials needed for avian egg collection and processing.

The following devices are generally used to sample tree swallow eggs and nestlings:

- Waders,
- Tupperware® or other similar plastic containers,
- Nitrile gloves,
- Cooler with ice if only collecting eggs and with dry ice if collecting nestlings, and
- Handheld or portable Global Positioning System (GPS) to identify sample location.

3.0 PROCEDURES

The following sections describe the procedures for sampling tree swallow eggs and nestlings. If deviations from these procedures are anticipated, the Project Manager and Quality Assurance (QA) Officer are contacted and the deviation is fully documented. Field work cannot progress until deviations are approved or resolved.

Investigators collect tree swallow eggs and nestlings from nest boxes (blue bird boxes) located in or adjacent to various localities near the KIF ash spill. Investigators also collect samples from nearby reference sites.

Nest boxes are placed in arrays that standardize their densities in order to control for the effects of birds competing for resources. When boxes are placed in single rows along the water's edge, they are spaced 12-15 meters apart. However, when boxes are placed in two or more rows (each row at least 10 meters apart), they are spaced 20-25 meters apart and rows are staggered. Boxes can be placed near tree lines and shrubs in fields (such as a narrow band of trees or shrubs separating the field from the water), but they are placed

2 meters from any structure (tree branch) that a predator could jump from onto the box. Nest boxes are installed in early April. The latitude and longitude of each nest box is documented during or immediately following the sampling event using a hand-held GPS unit. Mounting poles are equipped with predator guards.

Nest boxes are surveyed for occupancy over the course of the breeding season, including after installation, and then at appropriate intervals depending on activity in the nest box. During surveys, boxes are evaluated for presence/absence of adults, nesting material, egg clutch size, nestlings, and egg/nestling survival rates.

3.1 Pre-Sampling Preparation

The Project Manager is responsible for overall implementation of this SOP and ensuring that the SOP complies with current regulations and standards since these are subject to change. The Project Manager is also responsible for the following activities.

- a. Coordinate with the appropriate regulatory agencies to ensure that documentation and/or permits are obtained.
- b. Provide the Field Team Leader with sample localities and times.
- c. Coordinate with Field Team Leader to ensure that appropriate field equipment and safety gear are available.
- d. Review the site-specific *Quality Assurance Program Plan* (TVA-KIF-QAPP), *Site-Wide Safety and Health Plan* (SWSHP), and appropriate SOPs with the TVA Project Manager to determine appropriate field protocols.
- e. Develop contracts and interact with subject matter experts to review sampling protocols and results, ensure scientific rigor, and establish contaminant thresholds as necessary.
- f. Verify methods to be used to transport materials and identify appropriate laboratories to perform analyses.
- g. Identify appropriate laboratory space to store samples.
- h. Obtain legal right-of-entry to private properties prior to sampling.

3.2 Field Preparation

The Field Team Leader is responsible for implementation of the field collection process. The Field Team Leader ensures that samples are safely collected using sterile procedures to prevent cross-contamination and that associated data are accurate and well documented. The Field Team Leader ensures that the following activities have been completed prior to sampling initiation.

- a. Provide a summary (or JSA) of potential hazards and appropriate safety equipment to the field collection team.
- b. Identify sampling and documentation roles for personnel and thoroughly describe the sampling process.
- c. Distribute appropriate sampling and documentation equipment to the field collection team.
- d. Log collection activities in bound field logbooks using an indelible-ink pen for entries. Use permanent markers for hand-written labels on specimen bags and holding container labels.
- e. Work with appropriate personnel to produce computerized shipping labels.
- f. Ensure that QA and quality control (QC) protocols are followed.
- g. Maintain Chain-of-Custody (COC) documentation in accordance with the *Field Documentation SOP* (TVA-KIF-SOP-06).

3.3 Sampling Methods for Tree Swallow Eggs

Beginning in April, nest boxes are monitored weekly to identify initiation of nest construction. After noting the initiation of egg laying, the sampling is initiated by the sampling personnel by following the procedures listed below.

- a. As tree swallows typically lay one egg per day, monitor the nest every two days until all eggs are laid; as evident by two consecutive monitoring days with no additional eggs (nests usually contain four to seven eggs total). Complete the *Tree Swallow Nest Box Monitoring* form provided in Table 2 (form also available on TVA Kingston SharePoint drive at <http://sharepoint.tva.gov/sites/oer/KingstonEvent/KRP/default.aspx> under Environmental/Forms and Templates) including description of nest material.
- b. Note the number of eggs (clutch size) in each nest box on form.
- c. Collect one egg at random from 15 nest boxes at each study area while wearing nitrile gloves.

Note: If only one egg is observed in the nest, do not collect the egg.

- d. At one nest containing three or more eggs, collect a second egg for duplicate analysis.
- e. Place each egg in a small, pre-labeled resealable plastic bag. Verify that label on each bag includes the specimen ID number, date, and collector initials.
- f. Place the bags in a plastic container (such as Tupperware[®] or equivalent) lined with bubble wrap and seal the lid on the container.
- g. When egg collection is completed, remove each egg from the container being careful not to remove the egg from the resealable plastic bag (to avoid cross-contamination), rewrap the egg to help ensure that the egg will not break during transport, and place back in container.
- h. Label the containers on the outside with the following information: unique specimen ID number, river mile, time, date, and collector.
- i. Store containers in a cooler with wet ice.
- j. Take a GPS point at each sampling location during or following the sampling event.
- k. Upon completion of the sampling event, transport specimens to the appropriate processing laboratory at Kingston or TVA's Walnut Orchard Facility (Norris, Tennessee). At the laboratory, place the samples in a "Samples Only" refrigerator (with temperature maintained at < 6°C and above freezing) for holding until samples can be processed.
- l. Record information on the *Tree Swallow Egg Field Specimen Collection/Processing* form provided in Table 3 (form also available on the TVA Kingston SharePoint drive at <http://sharepoint.tva.gov/sites/oer/KingstonEvent/KRP/default.aspx> under Environmental/Forms and Templates).
- m. For egg sample processing, proceed to Section 3.5.1.

3.4 Sampling Method for Tree Swallow Nestlings

As eggs are sampled, nest boxes are monitored to determine the first hatch date (about 14 days after egg is laid) and when all nestlings are hatched; as evident by two consecutive days with no additional hatchlings. Nestling sampling is initiated by the sampling personnel by following the procedures listed below.

- a. Record first observed hatch event (hatch date) and total number of hatchlings (hatch success) on *Tree Swallow Nest Box Monitoring* form provided in Table 2.

- b. Once all hatchlings are ≥ 15 days old, record survival of nestlings and randomly select one hatchling from 15 monitored nest boxes at each study area while wearing nitrile gloves.
- c. Euthanize each nestling individually by placing each nestling in an open, pre-labeled resealable bag. (Do not seal bag until Step *f* below).
- d. Verify that the label on each bag includes the specimen ID number, date, and collector initials.
- e. Place the bag containing the nestling sample into an insulated box with dry ice (place small plastic bag or newspaper between ice and sample bag to keep from burning the specimens).
- f. Once the nestling has succumbed from exposure to carbon dioxide, seal the resealable plastic bag.
- g. Record information on the *Tree Swallow Nestling Field Specimen Collection/ Processing* form provided in Table 4 (and also located on the TVA Kingston SharePoint drive at <http://sharepoint.tva.gov/sites/oer/KingstonEvent/KRP/default.aspx> under Environmental/Forms and Templates).
- h. For nestling sample processing, proceed to Section 3.5.2.

3.5 Sample Processing

The procedures listed below are followed for egg and nestling samples regardless of collection times to ensure proper maintenance of sample temperature.

3.5.1 Egg Sample Processing

Eggs are processed individually while wearing nitrile gloves.

- a. Remove each egg from transport container and resealable bag and label the egg with an ID number on each end of the egg using a permanent marker.
- b. Weigh the egg on a digital analytical scale with an accuracy of ± 0.001 g.
- c. Measure the length and width of the egg to within 0.01 mm using a digimicrometer.
- d. Calculate volume of tree swallow eggs using the following equation (Hoyt, 1979).

$$\text{Volume} = \text{LengthWidth}^2 \times 0.51$$

- e. Record measurements on the *Tree Swallow Egg Field Specimen Collection/ Processing* form (Table 3).
- f. Replace egg into the original labeled collection resealable plastic bag, wrap in bubble wrap, place in a glass jar (provided by contract analytical laboratory), seal, and temporarily store back into a cooler with wet ice.

- g. Label the jar on the side and lid and seal with a labeled custody seal.
- h. Freeze processed samples at $< -10^{\circ}\text{C}$ until shipment to the approved laboratory for analysis.
- i. Complete the *Tree Swallow Egg Field Specimen Collection/ Processing* form (Table 3) documenting custody and sample integrity which accompanies the samples from the time of collection through temporary storage awaiting shipment to the laboratory.
- j. For shipping, place frozen samples in a cooler with dry ice and ship to the approved laboratories in accordance with the *Sample Labeling, Packing, and Shipping* SOP (TVA-KIF-SOP-07).
- k. Complete a COC form which accompanies the samples from time of collection through shipment to the laboratory. Refer to *Field Documentation* SOP (TVA-KIF-SOP-06) for proper COC procedures.

3.5.2 Nestling Sample Processing

Nestlings are processed in a similar manner as the egg samples.

- a. Remove each sample from the collection container and resealable bag.
- b. Weigh nestling on a digital analytical scale (0.001 g).
- c. Measure the length of the tarsus (on leg) and third primary feather using a digimicrometer (0.01 mm).
- d. Record measurements on the *Tree Swallow Nestling Field Specimen Collection/ Processing* form (Table 4).
- e. Transfer information from original resealable plastic bag to a new bag.
- f. Place specimen in the new bag; label and seal bag with a tamper-evident custody seal, and temporarily store in a cooler with dry ice.
- g. Once samples are processed, freeze samples at $< -10^{\circ}\text{C}$ for shipment to the approved laboratory.
- h. Complete the *Tree Swallow Nestling Field Specimen Collection/Processing* form (Table 4) to document custody and sample integrity which accompanies samples from time of collection through temporary storage awaiting shipment to the laboratory.
- i. For shipping, place frozen samples in a cooler with dry ice and ship to the approved laboratories in accordance with the *Sample Labeling, Packing, and Shipping* SOP (TVA-KIF-SOP-07).

- j. Complete COC form which accompanies samples from time of collection through shipment to the laboratory. Refer to TVA-KIF-SOP-06 and TVA-KIF-SOP-07 for proper COC procedures.

3.6 Field Logbook Documentation

In addition to the minimum requirements discussed in the *Field Documentation SOP* (TVA-KIF-SOP-06), the field logbooks document collection and sampling characteristics specific to this SOP. Field data are documented, using an indelible-ink pen, in a field logbook according to TVA-KIF-SOP-06 and the *Proper Field Documentation Procedures Memorandum* (Environmental Standards; 2009). Other specific field logbook documentation includes the following.

- Date, location or river mile, GPS coordinates, collection time, collectors, species, and number of individuals/species collected; and
- Additional comments associated with field access, weather conditions, predation of eggs or nestlings and anecdotal observations.

Similar information is recorded on the *Tree Swallow Egg Field Specimen Collection/Processing* form or the *Tree Swallow Nestling Field Specimen Collection/Processing* form (Tables 3 and 4).

Sampling information is provided to KIF QA/QC staff that create unique sampling labels for each specimen in accordance with *Sample Labeling, Packaging, and Shipping SOP* (TVA-KIF-SOP-07).

A completed project COC form and sample labels are provided post-processing and accompany the samples to the approved analytical laboratory. Refer to the *Field Documentation SOP* (TVA-KIF-SOP-06) for proper COC procedures.

4.0 REFERENCES

- Tennessee Valley Authority (TVA). *Field Documentation SOP* (TVA-KIF-SOP-06), 2009.
- TVA. *Quality Assurance Project Plan for the Tennessee Valley Authority Kingston Ash Recovery Project* (TVA-KIF-QAPP), December 18, 2009.
- TVA. *Sample Labeling, Packing, and Shipping SOP* (TVA-KIF-SOP-07), 2009.
- TVA. *Site-Wide Safety and Health Plan for the TVA Kingston Fossil Plant Ash Release Response* (SWSHP), 2010.

Table 1: Tree Swallow Egg and Nestling Sampling Equipment and Materials Checklist	
Item Description	Check
Health & Safety	
Nitrile gloves	
Hard hat	
Field first-aid kit	
U.S. Coast Guard-approved flotation device if working from boat	
Hip waders	
Paperwork	
Job Safety Analysis (Generic)	
Field logbook	
Chain-of-Custody forms	
Equipment/Materials	
GPS unit	
Collection containers	
Bubble wrap	
Resealable plastic bags	
Laboratory provided jars and lids	
Digimicrometer and digital analytical scale	
Indelible ink pen, permanent marker	
Cooler with ice (eggs) or dry ice (eggs and nestlings)	
Decontamination and Waste Management Equipment	
Deionized water	
Laboratory-grade detergent (such as Alconox [®])	

