



**STANDARD OPERATING PROCEDURE FOR:**

**MAYFLY SAMPLING**

**TVA-KIF-SOP-29**

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## 1.0 PURPOSE

This Standard Operating Procedure (SOP) describes methods for collecting aquatic nymphs of the mayfly *Hexagenia* for contaminant analysis relative to the ash spill at Kingston Fossil Plant (KIF) including proper sample handling to ensure sample integrity. Additionally, the procedures necessary to maintain appropriate documentation are detailed. This procedure is applicable to sampling mayflies in surface waters requiring access by and collection from a boat.

## 2.0 GENERAL CONSIDERATIONS

Potential hazards associated with the planned tasks are thoroughly evaluated prior to conducting field activities. The *Site-Wide Safety and Health Plan (SWSHP)* provides a description of potential hazards and associated safety and control measures. A pre-job safety analysis (JSA) highlighting potential hazards are discussed at each sampling event. Sampling is only conducted by properly trained personnel. When collecting mayfly nymphs, the field collection team(s) is lead by personnel experienced with the boat mounted boom, motorized winch, and the dredges.

Sampling personnel wear powder-free nitrile gloves while performing the procedures described in this SOP. Specifically, powder-free nitrile gloves must be worn while preparing sample bottleware, preparing and decontaminating sampling equipment, collecting samples, and packing samples. At a minimum, nitrile gloves are changed prior to the collection of each sample, or as necessary to prevent the possibility of cross-contamination with the sample, the sample bottleware, or the sampling equipment.

Field sampling equipment that may come in contact with mayflies that have been collected are decontaminated in accordance with *Decontamination of Equipment SOP (TVA-KIF-SOP-08)* procedures prior to use.

Additionally, a Float Plan is completed prior to traveling anywhere on a water vessel. A Float Plan includes a description of the water vessel, specifies who is on board, provides a description of the safety equipment being carried, and indicates destination and expected time of return.

## 3.0 PROCEDURES

This section documents general operating procedures and methods associated with collecting samples of aquatic nymphs and adults of the mayfly *Hexagenia*. Any variation in these procedures is approved by the Project Manager and Quality Assurance/ Quality Control (QA/QC) Lead and must be fully documented. Field work cannot progress until deviations are approved or resolved.

### 3.1 Pre-Job Preparation

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

- a. Coordinate with appropriate regulatory agencies to ensure that appropriate documentation and/or permits are obtained.
- b. Review project work control documents including the *Quality Assurance Project Plan* (QAPP), SWSHP, and appropriate SOPs to determine suitable field protocols.
- c. Develop a Sampling and Analysis Plan (SAP) that ensures project objectives are met such as sample type (individual animal, composite), sample size (necessary biomass/volume of material needed and number of specimens needed to meet those requirements), number of replicates, sample locations (transects, random, stratified).
- d. Identify approximate sample-station locations. If necessary, make a reconnaissance visit of perspective sampling locations to evaluate their suitability.
- e. Obtain legal right-of-entry from appropriate landowners and jurisdictions as needed.
- f. Discuss project objectives and potential hazards with project personnel.
- g. Coordinate with the Field Team Leader to verify that appropriate field equipment and safety gear are available.
- h. Identify appropriate laboratories to perform analyses and confirm that they have been contacted and are prepared to receive the samples.
- i. Make sure that training requirements and needs have been identified and that project personnel complete the necessary training.

### 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, that decontamination protocols are used to maintain sample integrity, and that the associated data are accurate and well documented. The Field Team Leader is also responsible for the following activities.

- a. Gather equipment necessary for completing the collection and sampling activities (refer to Table 1 for an example checklist of equipment for mayfly sampling).

- b. Provide a summary of potential hazards (or review appropriate JSA worksheet) and provide appropriate safety equipment to the field sampling team.
- c. Verify that QA/QC protocols are followed.
- d. Maintain documentation of field activities and Chain-of-Custody (COC) records in accordance with the *Field Documentation* SOP (TVA-KIF-SOP-06).

### 3.3 Sampling Methods for Mayfly Nymphs

Mayfly nymphs are collected from the substrate using a boat-mounted boom and motorized winch with attached Peterson or Ponar dredge (see *Benthic Macroinvertebrate Sampling* SOP, TVA-KIF-SOP-35). Mayfly nymphs that will not have their digestive systems cleared (or deperated) before analysis are sampled following steps *a* through *h* of this section and the steps in Section 3.3.1. Nymphs that will be deperated are sampled following steps *a* through *h* of this section and the steps in Section 3.3.2.

- a. Prior to the collection of each set of samples at a site, wash dredges, sieves, forceps, and plastic containers with a Liqui-Nox<sup>®</sup> wash/scrub (or similar phosphate-free detergent) and rinse with surface water.
- b. At each sample location, establish a line-of-sight transect perpendicular to the channel and collect dredge grab samples at no less than five locations dispersed along the transect.
- c. Transfer the contents of each dredge sample to a stainless steel sieve fitted with appropriately sized stainless steel, Nitex, or Teflon<sup>®</sup> mesh/netting to retain the desired organisms (mess size  $\leq 2$  mm).
- d. To expose the nymphs in the samples, rinse the sample with surface water from the site using a water pump and attached hose.
- e. Remove the nymphs from the sieve using decontaminated stainless steel, plastic, or Teflon<sup>®</sup>-coated forceps and place into a decontaminated plastic container filled with surface water from the site (or surface water bath) to allow some preliminary removal of substrate adhering to the organism. Discard nymphs if they appear to have been damaged (such as severed head/abdomen) during the sampling process.
- f. Obtain geographic coordinates using a portable GPS unit at the beginning and end of each sampling transect.
- g. Record the number of organisms collected in each grab sample on a data sheet, along with date, time, water depth, and general characterization of the type of substrate collected.
- h. Label clean sample jars with project name, site (location) name and replicate number, date and time of collection, and name or initials of collector.

### 3.3.1 Sampling Methods for Mayfly Nymphs With No Depuration

Mayfly nymphs that will not have their digestive systems depurated before analysis are sampled according to the following steps, in addition to the steps detailed in Section 3.3. (Proceed to Section 3.3.2 for procedures for nymphs that will be depurated.)

- a. After the required number of nymphs has been collected, transfer them from the water bath (see Section 3.3.e.) directly into a properly labeled, unused, certified glass jar using stainless steel, plastic, or Teflon<sup>®</sup>-coated forceps, and immediately place jars on ice in a cooler. Replenish the surface water bath with fresh surface water each time nymphs are transferred into the glass sample jar.
- b. Maintain the jars containing samples on ice in a cooler until final sample processing (described in Section 3.4). Samples must be washed/rinsed and frozen within 24 hours of collection.

Steps *b* through *h* in Section 3.3 and steps *a* and *b* in Section 3.3.1 are repeated for each sample replicate at each sample site.

### 3.3.2 Sampling Methods for Mayfly Nymphs With Depuration

If the digestive system of the nymphs will be cleared in the laboratory prior to analysis, nymphs must be alive and therefore, handling stress to the nymphs must be minimized to improve survival while collecting and transporting nymphs to the laboratory by following the steps below.

- a. Regularly (such as every 10 to 30 minutes depending on temperature and sun intensity) count nymphs that have been collected and placed in the water bath (see Section 3.3.e.) and transfer them into a properly labeled, unused, certified glass jar filled with surface water from the collection site. To regulate the temperature of the water in the sample jar, submerge the sample jar with lid secured in a 5-gallon bucket of surface water, maintained in the shade, until sampling of transect is completed. Replace the surface water bath with fresh water from the collection site each time nymphs are transferred into the glass sample jar.
- b. After collecting the pre-determined number of nymphs along a transect, replace at least 50% of the water in the jar with fresh water from the collection site and replace the lid on the jar. Nymphs are transported live from the field to the laboratory in their original sample containers in a cooler containing ice to complete processing per Oak Ridge National Laboratory's (ORNL's) *Laboratory Procedures—Preparation of Invertebrates for Contaminant Analysis* SOP (BAA-SOP-06).

Steps *b* through *h* in Section 3.3 and steps *a* and *b* in Section 3.3.2 are repeated for each sample replicate at each sample site.

### 3.4 Field Sample Processing of Non-Depurated Nymphs

Mayfly nymphs that are not to be depurated are processed in the laboratory within 24 hours of collection as follows.

- a. Process each sample separately.
- b. Rinse each sample with deionized (DI) water following one of three methods:
  1. Triple rinse each sample with DI water in the initial sample jar followed by a final rinse in new, certified clean glass jar. A sample jar containing nymphs shall be partially filled with DI water, sealed, and gently agitated to remove residual substrate/sediment material. The DI water is poured from the jar by loosening the lid and holding the lid slightly askew to form a small opening for water to escape. Care is taken to avoid expelling nymphs from the jar with the discarded rinse water. Repeat the process three times or until visible particles are dislodged and removed, and then transfer the nymphs using clean forceps to a new, certified clean glass jar for a final rinse following the same aforementioned procedures.
  2. Triple rinse each sample with DI water in the initial sample jar. A sample jar containing nymphs is partially filled with DI water. Cover the opening of the jar with clean Nitex netting (0.5 – 2.0-mm mesh), gently agitate, and then pour the water out of the jar through the Nitex netting. Repeat the process three times or until visible particles are dislodged and removed.
  3. Pour the nymphs from a sample jar onto a piece of Nitex netting (0.5 – 2.0-mm mesh) and hold the net under a stream of distilled water until visible particles are dislodged or removed.
- c. After the final rinse, transfer nymphs to a new, certified, pre-weighed, 2- or 4-oz glass jar, obtain sample weight, and place jar in a secure freezer under appropriate chain of custody in accordance with *Sample Labeling, Packing, and Shipping* SOP (TVA-KIF-SOP-07) until shipment to the ORNL.
- d. Decontaminate forceps prior to processing each sample with a Liqui-Nox<sup>®</sup> wash/scrub (or similar), rinse with potable water, and then rinse with DI water following the general protocols in the *Decontamination of Equipment* SOP (TVA-KIF-SOP-08).

### 3.5 Sampling Methods for Mayfly Adults

The collection of adult mayflies will be made opportunistically by direct removal from vegetation or other structures along the shoreline or by use of sweep nets.

- a. Prior to the collection of each set of samples at a site, decontaminate forceps and sweep nets with Liqui-Nox<sup>®</sup> wash/scrub (or similar) and rinse with DI water. If sweep nets are decontaminated in the laboratory, allow them to air dry, and place each net in an individual plastic bag to prevent introduction of contaminants.
- b. Upon arrival at a collection site, conduct a visual search for mayflies.
- c. As mayflies are observed, collect by removing them from vegetation or other structures by hand while wearing nitrile gloves or by shaking/disturbing the vegetation and collecting them using a sweep net while they are in flight. When a sweep net is used, taken care to limit contact with other surfaces (such as boat deck) to prevent introduction of contaminants.
- d. Place collected organisms in contaminant-free jars in the field and immediately place on ice in a cooler. Maintain samples on ice until placed in a secure freezer (within 24 hours) under appropriate chain of custody in accordance with *Sample Labeling, Packing, and Shipping* SOP (TVA-KIF-SOP-07) until transfer to ORNL.
- e. Transport samples of adult mayflies to ORNL in a cooler containing dry ice to complete processing per ORNL's *Laboratory Procedures – Preparation of Invertebrates for Contaminant Analysis* (BAA-SOP-06).

### 3.6 Mayfly QA/QC Sampling

To collect samples for QA/QC, the steps below are followed.

- a. Create one duplicate sample set for every 20 investigative samples (such as adults and nymphs) according to the *Field Quality Control Sampling* SOP (TVA-KIF-SOP-11) and the QAPP. Create duplicates by dividing a sample into equal numbers of whole individuals and analyze independently.
- b. Collect sample container blanks for every 20 sample jars used according to the *Field QC Sampling* SOP (TVA-KIF-SOP-11) and the QAPP.
- c. Collect the equipment blank on the Nitex netting by pouring laboratory-provided DI water through the Nitex netting directly into the laboratory-provided glass sample jar.

### 3.7 Sample Handling, Packing, and Shipping

Samples from the field are processed, transferred to ORNL, and further transferred to the appropriate analytical laboratories in accordance to the labeling, chain of custody, and custody sealing procedures defined in the *Sample Labeling, Packing, and Shipping SOP* (TVA-KIF-SOP-07).

### 3.8 Field Logbook Documentation

Field logbooks to record daily activities, including sample collection and tracking information, are maintained by the Field Team Leader. Information is entered into the field logbook by the appropriate field team member using indelible ink. In addition to the minimum requirements discussed in the *Field Documentation SOP* (TVA-KIF-SOP-06), the field logbooks document those collection and sampling characteristics specific to this SOP and as defined in the applicable project work control documents.

The Field Team Leader and/or designee review the field logbook entries on a weekly basis at a minimum (daily review is preferred) for completeness and accuracy and indicate this review by initialing the entries. The Field Team Leader is also responsible for the completion of required data collection forms.

### 3.9 Decontamination and Waste Management

Sampling equipment decontamination is performed in a manner consistent with the *Decontamination of Equipment SOP* (TVA-KIF-SOP-08). Investigation-derived wastes (IDW) produced during field sampling or decontamination is managed in accordance with *Management of Investigation-Derived Waste SOP* (TVA-KIF-SOP-12).

## 4.0 REFERENCES

- Oak Ridge National Laboratory. *Laboratory Procedures—Preparation of Invertebrates for Contaminant Analysis SOP* (BAA-SOP-06), 2009.
- Tennessee Valley Authority (TVA). *Benthic Macroinvertebrate Sampling Using Ponar Dredge SOP* (TVA-KIF-SOP-35), 2010.
- TVA. *Decontamination of Equipment SOP* (TVA-KIF-SOP-08), 2010.
- TVA. *Field Documentation SOP* (TVA-KIF-SOP-06), 2009.
- TVA. *Field Quality Control Sampling SOP* (TVA-KIF-SOP-11), 2009
- TVA. *Management of Investigation-Derived Waste SOP* (TVA-KIF-SOP-12), 2010.
- TVA. *Quality Assurance Project Plan for the Tennessee Valley Authority Kingston Ash Recovery Project* (TVA-KIF-QAPP), Environmental Standards, Inc., 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), Jacobs, June 2009.
- U.S. Environmental Protection Agency. *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*. SW-846. EPA, Office of Solid Waste, Economic, Methods, and Risk Analysis Division, Washington, DC. (<http://www.epa.gov/epawaste/hazard/testmethods/sw846/index.htm>). 2007.

**Table 1. Suggested Mayfly Sampling Equipment and Material Checklist**

Item Description	Check or NA
<b>Health &amp; Safety</b>	
Motorized boat of sufficient capacity to carry field gear and up to 3 passengers	
U.S. Coast Guard approved life jackets (1 per passenger)	
U.S. Coast Guard approved boat cushion (at least 1)	
Paddles (at least 2)	
Waders	
Hard hat (if working in construction zone)	
Field first-aid kit	
Reflective safety vest (if working in construction zone)	
Safety glasses	
Steel-toed boots	
Hearing protection	
Disposable nitrile gloves	
<b>Equipment/Materials</b>	
Field logbook and pen with indelible ink	
Chain-of-Custody forms (if samples will be changing custody from original collector)	
Custody seals (if samples will change custody using a secondary carrier )	
Clean forceps, ~5 inches long (plastic, plastic or Teflon coated, or stainless steel) <sup>1</sup>	
Clean, glass sample containers (16 oz or larger) <sup>1</sup>	
Clean glass sample container (2 or 4 oz capacity) <sup>1</sup>	
Clean plastic or stainless steel pans (1 or more) <sup>1</sup>	
Sample labels	
Permanent markers	
5-gallon bucket	
5-gallon clean, plastic carboy filled with distilled water <sup>1</sup>	
Clean plastic squirt bottle for dispensing distilled water <sup>1</sup>	
Sufficient cooler capacity to hold ice and all sample containers	
Ice and/or dry ice	
Liqui-Nox <sup>®</sup> or other phosphate-free detergent	
Global positioning system (GPS) unit and spare batteries	
Digital camera	
Sweep nets (wooded or plastic handles recommended; aluminum handles are taped)	

<sup>1</sup>If these items cannot be certified as contaminant-free, they must be cleaned per *Decontamination of Equipment* SOP (TVA-KIF-SOP-08).

**End of Procedure**