

Washington State Department of Ecology

Spill Prevention, Preparedness, & Response Program

Standard Operating Procedure for Collecting Samples from Fish Kills

Version 1.0

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Please note that the Washington State Department of Ecology's Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical and administrative experts. Their primary purpose is for internal Ecology use, although sampling and administrative SOPs may have a wider utility. Our SOPs do not supplant official published methods. Distribution of these SOPs does not constitute an endorsement of a particular procedure or method.

Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the Department of Ecology.

Although Ecology follows the SOP in most instances, there may be instances in which Ecology uses an alternative methodology, procedure, or process.

Washington State Department of Ecology

Spill Prevention, Preparedness, & Response Program

Standard Operating Procedure for Collecting Fish Kill Samples

1.0 Purpose and Scope

1.1 This document is the Spill Prevention, Preparedness, & Response (Spills) Program Standard Operating Procedure (SOP) for collecting samples from fish kills.

1.2 **Objectives** – 1) To determine the cause of a fish kill. 2) To maintain the integrity of the sample(s) during sampling, transport, and storage.

1.3 The main purpose for collecting specimens associated with a fish kill is to provide evidence for determining the cause of the fish kill. To be legally valid, samples must be carefully collected to ensure that the specimens are properly preserved, and strict chain-of-custody must be maintained throughout collection, transport, and analysis.

1.4 Three types of specimens are usually collected for fish kills; Fresh, chilled specimens used for identifying pathogens or harmful algae, chemically preserved specimens used for histological analysis, and frozen specimens used for evaluation of toxic chemicals.

2.0 Applicability

2.1 This procedure is based on a protocol developed by the Washington Department of Fish and Wildlife.

3.0 Definitions

3.1 WDFW – Washington Department of Fish and Wildlife.

3.2 NWIFC – Northwest Indian Fisheries Commission.

3.3 GC/FID – Gas Chromatograph/Flame Ionizing Detector

3.4 GC/MS – Gas Chromatograph/Mass Spectrometer

3.5 TOC – Total Organic Carbon

3.6 TRAP – Trustee Resource Assessment and Protection.

3.7 NRDA – Natural Resource Damage Assessment.

3.8 SOSOC – State on Scene Coordinator

4.0 Personnel Qualifications/Responsibilities

4.1 Any Ecology employee with basic sampling skills can follow this SOP and collect samples for a fish kill, provided that they possess the necessary materials. There is little skill required to collect fresh chilled or frozen specimens. However, some skill is necessary and some caution is warranted for collection of the chemically preserved specimens due to the use of a sharp knife or scalpel and tissue fixative. Anyone

encountering a fish kill should use caution; if there is any indication that the fish kill is the result of a spill or release of a toxic or unidentified substance, do not enter the area until it has been determined to be safe. All Spills Program employees (including managers and administrative staff) are encouraged to attend basic sampling training, which will provide familiarization with basic skills and safety. Due to safety, at least two people are recommended for any field sampling and anyone that has taken basic sampling training, with or without field experience, can assist an experienced sampler.

5.0 Equipment, Reagents, and Supplies

5.1 Equipment

- 5.1.1 Collection of specimens from a fish kill does not require any specialized equipment.
- 5.1.2 GPS (set datum to WGS-84, Latitude/Longitude to degrees.decimal degrees).
- 5.1.3 Digital camera (synched to GPS time).
- 5.1.4 Ice chests with wet or blue ice (preferably equipped with chain of custody security cables).

5.2 Reagents

- 5.2.1 Pesticide grade acetone and hexane for decontamination.
- 5.2.2 Davidson's Fixative, prepare by mixing the following ingredients:
- 5.2.3 2 parts formalin (37%)
- 5.2.4 3 parts reagent alcohol
- 5.2.5 1 part glacial acetic acid
- 5.2.6 3 parts distilled water
- 5.2.7 Enough eosin to color

5.3 Supplies

- 5.3.1 Sample tags and labels
- 5.3.2 Field notebook
- 5.3.3 Chain-of-custody seals
- 5.3.4 Chain-of-custody forms
- 5.3.5 Nitrile disposable gloves
- 5.3.6 Safety glasses or goggles
- 5.3.7 Permanent marker
- 5.3.8 Bottles large enough to hold 4 specimens and twice as much fixative by volume
- 5.3.9 Ziploc bags (quart and gallon freezer bags)
- 5.3.10 Paper towels
- 5.3.11 Aluminum foil
- 5.3.12 Newspaper
- 5.3.13 Scalpel and blades or small sharp knife

- 5.3.14 Forceps
- 5.3.15 Syringe to inject fixative into shrimp

6.0 Summary of Procedure

6.1 Sample Collection Procedure

- 6.1.1 Safety is of greatest concern. If there is any indication that the fish kill is the result of a spill or release of a toxic or unidentified substance, do not enter the area until it has been determined to be safe. Be aware of physical and chemical hazards at the site. Get a safety briefing before entering the area. Do not enter confined spaces unless they have been determined to be safe. Use recommended safety equipment and procedures.
- 6.1.2 Develop a sampling plan. Identify the number and type of samples that are to be collected, the type of containers that will be used, and unique identifiers for each sample. Make a drawing of the area being sampled, including points of reference and the location of each site where samples are collected so the sites could be located later if necessary. Identify the sampling procedure(s) that will be used. Include any preservation techniques used for the samples, the type of analyses that will be done, and where the samples will be sent for analysis. The plan can be recorded in a field notebook or on a separate piece of paper; there is no required form or specific format that must be followed. Each plan will be different depending on the number and type of samples being collected and the complexity of sampling site.
- 6.1.3 **Collection of Fresh, Chilled Specimens**
 - 6.1.3.1 Collect only very freshly killed specimens (died within the last hour or two), preferably moribund specimens that are not quite dead yet (moving slowly near the surface of the water).
 - 6.1.3.2 Collect the specimens by hand, wearing disposable gloves
 - 6.1.3.3 Place the specimens in plastic bags
 - 6.1.3.4 Place the specimens in an ice chest with ice or blue ice
 - 6.1.3.5 Label each plastic bag. Use a waterproof marker to record the project or incident name, a station identifier, a unique sample identifier, the collection date and time, and the sampler's name or initials directly on the plastic bags.
 - 6.1.3.6 Transport the fish to the WDFW (Joan Thomas, 360-902-2667) or the NWIFC fish pathology lab as soon as possible (within 6 to 8 hours).
- 6.1.4 **Collection of Chemically Preserved Specimens**
 - 6.1.4.1 These animals will be used for histological analysis and will be preserved with Davidson's Fixative, which contains formalin and acetic acid.

- 6.1.4.2 Use caution when handling the fixative; wear gloves and eye protection, and do not use in a closed space.
- 6.1.4.3 Collect only very freshly killed specimens (died within the last hour or two), preferably moribund specimens that are not quite dead yet (moving slowly near the surface of the water).
- 6.1.4.4 **For Fish:** Cut the belly of the fish from the anal vent to just behind the gill plates.
- 6.1.4.5 Using forceps, GENTLY pull out the visceral mass so that it will be fully exposed to the fixative and to permit the fixative to permeate the body cavity; be careful not to tear open the intestine.
- 6.1.4.6 Carefully place the fish in a bottle and cover with fixative; agitate gently to make sure that there are no air bubbles trapped in the body cavity.
- 6.1.4.7 You may place up to four fish of the same species in one bottle if you ensure that there is at least twice as much fixative as fish tissues by volume. Use a bottle large enough so the fish are not packed closely together.
- 6.1.4.8 **For Shrimp:** Inject each side of the shrimp through the carapace (shell of main body) with fixative using the syringe. Use 0.5 ml per side for a 3" shrimp, less for a smaller animal and more for a larger animal.
- 6.1.4.9 Slit the shell on the under-side of the tail (length-wise).
- 6.1.4.10 Carefully place the shrimp in a bottle and cover with fixative; agitate gently to make sure that there are no air bubbles trapped in the body cavity.
- 6.1.4.11 You may place up to four shrimp of the same species in one bottle if you ensure that there is at least twice as much fixative as shrimp tissues by volume. Use a bottle large enough so the shrimp are not packed closely together.
- 6.1.4.12 Label each bottle with a paper tag and place the bottle and tag into a Ziploc bag. Record the project or incident name, a station identifier, a unique sample identifier, the collection date and time, and the sampler's name or initials.
- 6.1.5 **Collection of Frozen Specimens**
- 6.1.5.1 Collect only very freshly killed specimens (died within the last hour or two), preferably moribund specimens that are not quite dead yet (moving slowly near the surface of the water).
- 6.1.5.2 Wrap the specimens in aluminum foil.
- 6.1.5.3 Place the specimens in a Ziploc bag with a paper tag in the bag, then label the outside of the bag with a waterproof marker.

6.1.5.4 Record the project or incident name, a station identifier, a unique sample identifier, the collection date and time, and the sampler's name or initials.

6.1.5.5 Place the specimens on ice until they can be frozen.

6.1.5.6 Maintain strict chain-of-custody during sample storage and transportation.

6.1.6 **Data collection for all fish kills**

Record as much of the following information as possible:

6.1.6.1 1) Species affected (or at least the common name if known).

6.1.6.2 2) Other life forms affected (i.e. birds, mammals, reptiles, invertebrates, etc.).

6.1.6.3 3) Size(s) of the affected species.

6.1.6.4 4) Estimated number of animals affected.

6.1.6.5 5) Are they dying (moribund), freshly dead, or carcasses?

6.1.6.6 6) When were the affected animals first observed?

6.1.6.7 7) Over how many days did the event take place?

6.1.6.8 8) Description of fish/animal behavior or appearance (i.e. sucking air at surface, loss of equilibrium, bloody lesions, flared gills, eyes protruding, etc.).

6.1.6.9 9) Are there any irregular/unusual activities occurring in the area (i.e. spraying, construction, accidents, etc.)?

6.1.6.10 10) Have there been any unusual recent weather conditions or events?

6.1.6.11 11) Are there unusual water conditions (i.e. color, odor, flow level, etc.)?

6.1.6.12 12) Any other information that might help to identify the cause of the fish kill.

6.2 **Analytical Methods**

6.2.1 No specific analytical methods are identified for this SOP. The purpose of this SOP is to define how the specimens are collected for any fish kill. A wide variety of analyses could be performed depending on the suspected cause of the fish kill.

7.0 Records Management

7.1 Sampling Plan Template – Use this form as an aid for developing a complete and comprehensive sampling plan. This form is not required for developing a sampling plan, but provides ready access to guidelines and reminders.

7.2 The form is available at:
<http://teams/sites/SPPR/response/trap/Sampling/Forms/AllItems.aspx> (SharePoint)
or X:\Spills_Program\Response Section\TRAP-NRDA\Sampling\

7.3 Oil Spill Chain-of-Custody Form (ECY 050-42 (11/01))

8.0 Quality Control and Quality Assurance Section

8.1 There are no QA/QC requirements specific or unique to this procedure.

9.0 Safety

9.1 Chemically preserved specimens will be placed in Davidson’s Fixative, which contains formalin and acetic acid. Use caution when handling the fixative; wear gloves and eye protection, and do not use in a closed space.

9.2 Sample collection can present some unusual circumstances that could have equally unusual associated safety hazards. Samplers should consult with the Safety Officer or SOSC and review the incident safety plan or Hazard Assessment Worksheet (HAW) prior to developing a sampling plan so known hazards can be avoided. Samplers should also be aware that sampling will often take place in areas that may not be adequately addressed in the safety plan or HAW. If there is any question, then a separate HAW should be prepared prior to sampling. A Sampling HAW is available at:
<http://teams/sites/SPPR/response/trap/default.aspx> (SharePoint) or X:\Spills_Program\Response Section\TRAP-NRDA\Sampling, that includes action levels appropriate for sampling various petroleum products.

9.3 Some hazards that could be associated with sampling are:

9.4 Low areas that could collect fumes (vapors) or have reduced oxygen levels (confined space)

9.5 Higher concentrations of fumes at ground level where samples are collected

9.6 General water hazards when collecting samples on a shoreline or from a boat or dock, etc.

9.7 Increased risk of slips, trips, and falls

9.8 Traffic when sampling near a highway

9.9 Dangerous animals

9.10 Exposure to elements (hot or cold)

9.11 Eye damage from splashes or brush/branches

10.0 References - none