

FISH KILL INVESTIGATION­ MANUAL, 3RD Ed.



Virginia Department of Environmental Quality

Water Monitoring and Assessment Program

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# CHAPTER 1. INTRODUCTION AND PURPOSE

The Virginia Department of Environmental Quality (DEQ) Fish Kill Investigation Manual guides field personnel through an investigation of a fish kill event. A fish kill is a sudden and unexpected or atypical death of fish or other aquatic animals in a clearly defined area. Fish kills can occur anywhere inhabited by fish and can be caused by water pollution or by natural events. This manual is for trained DEQ personnel to follow in response to a reported fish kill and should not be used as a substitute for formal training or for use by untrained personnel. DEQ personnel responsible for fish kill should receive adequate training before acting as a lead investigator of a kill. Elements of a fish kill investigation include:

* Defining the extent of the kill area,
* Locating and stopping the source of the kill (if safe and possible for the investigation team),
* Notifying other response partners and key stakeholders,
* Collecting the necessary information to substantiate the cause of the kill,
* Determining the numbers and kinds of fishes killed, and
* Presenting the data collected for potential enforcement action and cost recovery.

This document provides an overview on each of these elements to serve as a reference as needed. It is accompanied by Guidance Document [GM23-2006](https://townhall.virginia.gov/L/ViewGDoc.cfm?gdid=7728), which provides detailed instructions on conducting a formal fish kill investigation. All staff serving on the investigation team for a fish kill case should have reviewed GM23-2006 and been trained in the processes detailed there.

Fish kills may be obvious indicators of serious water quality degradation. In addition, the loss of the Commonwealth's natural resources merits investigation and, if possible, compensation for damages sustained. Procedures outlined in this manual are the product of many fish kill investigations undertaken since the creation of the State Water Control Board (SWCB) in 1946, which was a predecessor to the DEQ. Fish kill investigations have been a necessary function of the SWCB and DEQ since their inceptions. In 1970, the State Water Control Law was modified to make the SWCB responsible for investigating fish kills (§ 62.1-44.15 (11)). The modification also empowered the SWCB to recover the costs of investigations and the replacement costs of dead fish, and to impose penalties when appropriate. In 1993, DEQ was formed, and began conducting fish kill investigations on behalf of the SWCB. In 2022, the General Assembly made DEQ directly responsible for fish kill investigations, removing the SWCB role, and any resulting resolution and enforcement actions.

Fish kill investigations serve many important purposes, including determining the sources and causes of fish kill events in Virginia state waters, assessing environmental damage, and determining the parties responsible for such kills. Evidence gathered during investigations may become part of actions to seek reimbursement of investigation and fish replacement costs by the responsible party per [State Water Control Law §62.1-44.15](https://law.lis.virginia.gov/vacode/title62.1/chapter3.1/section62.1-44.15/) and to address contravention of [Virginia's Water Quality Standards (9 VAC 25-260)](https://law.lis.virginia.gov/admincodefull/title9/agency25/chapter260/) or other environmental laws. Fish kill investigations also serve as the impetus for further studies which may reveal environmental or human health concerns, in cases where Water Quality Standards are not being met, or where the consumption of contaminated or diseased fish is possible.

This document is based on information contained in the U.S. Fish & Wildlife Service Field Manual for the Investigation of Fish Kills (Meyer, F et al., ed., 1990) and the American Fisheries Society (AFS) Investigation and Monetary Values of Fish and Freshwater Mollusk Kills (Southwick and Loftus, ed. 2018) published in 2017 and revised in 2018 to reflect changes in the valuation of fishes for cost recovery. Investigators should review and be familiar with those two documents before conducting fish kill investigations and refer to those documents for detailed information as needed. Investigators are expected to set priorities and exercise varying degrees of best professional judgment in carrying out fish kill investigations.

# CHAPTER 2. RESPONSIBILITIES

Reports of fish kills to regional pollution response teams are investigated on the assumption that fish kills represent a potentially serious water quality impairment and loss of aquatic resources. Several DEQ program areas work together to manage fish kill events: Regional Biologists and Pollution Response Program (PREP) Coordinators in are the primary responders, and they work closely with other regional staff, including regional Enforcement Program, and staff from DEQ Central Office, including the Water Monitoring and Assessment Program (WMA), PREP, the Division of Enforcement (DE), the Office of Financial Responsibility and Waste Programs (OFRWP), the Division of Communications, and the Division of Policy.

Throughout the course of a large or unusual fish kill investigation, coordination among the regional offices, Divisions of Enforcement and Communications, and the Water Monitoring and Assessment program is essential. Immediate staff time requirements may exceed that which can be satisfied by personnel kept on call for these investigations. Since fish kills need an immediate response, this may result in the postponement or cancellation of other scheduled work.

## 2.1 Regional Office Water Monitoring, PREP, and Enforcement Staff

* Regional office water monitoring staff and pollution response teams conduct investigations of fish kill events. Staff included on the field investigation team may vary, depending on the nature of each fish kill, and on the knowledge, skills, and qualifications of available staff. Field investigation teams typically include regional PREP Coordinators and WMA staff, but may include staff from Central Office, other regions, and other programs, as needed. The field investigation team may determine that follow-up investigations involving additional staff with specialized expertise or specialized field equipment are required.
* The field investigation team collects field data during investigations and maintains a field logbook, data sheets, and photographs.
* Regional staff coordinate notifications to other federal, state and local agencies, and any needed follow-up investigations as appropriate, with assistance from Central Office WMA, PREP, and Enforcement staff as needed.
* Regional staff log the initial report in the PREP database and assign a pollution incident report number (IR#) to the investigation.
* The field investigation team should develop an initial operations plan at the office before embarking on the field investigation. The plan should include all necessary safety preparations, including checking in with the on-site Incident Commander for a safety briefing, and the inclusion of field staff with sufficient expertise for the investigation to the extent possible based on available information.
* The field investigation team may conduct initial interactions with media or public on site. Field staff should notify inquirers that an incident has been reported and an investigation is underway and should refer further inquiries to the Division of Communications (communications@deq.virginia.gov).
* The field investigation team should document all investigative costs and present justification for recovery of investigative and fish costs when appropriate. To obtain replacement costs of fish, DEQ provides final fish counts and associated data (e.g., fish length and conditions, as indicated in Appendix C) to the Department of Wildlife Resources (DWR). Costs of lab analyses are obtained from the Division of Consolidated Laboratory Services (DCLS) or other analytical laboratories, if needed. Cost recovery information is provided to the OFRWP to pursue cost recovery from a responsible party.
* The field investigation team, with collaboration from other agency staff, as needed, should prepare any needed reports outlining the results of the fish kill investigation and maintain report files. In finished form, these reports should be referenced for cost recovery, enforcement actions, and to provide to the party responsible for the kill. Each report should contain at least the information required to complete the "Virginia Department of Environmental Quality Fish Kill Report/Notification,” and its appropriate attachments (Appendix A). Staff preparing the reports should forward them to the regional enforcement staff or other appropriate programs if necessary.
* Regional water compliance staff should be provided with fish kill reports, cost recovery forms and evaluations, and any other supporting documentation when a fish kill event occurs to prepare compliance letters in accordance with the [Guidance Memorandum No. 02-2010 - Water Compliance Auditing Manual](https://townhall.virginia.gov/l/GetFile.cfm?File=C:%5CTownHall%5Cdocroot%5CGuidanceDocs%5C440%5CGDoc_DEQ_1432_v1.pdf)
* Regional enforcement staff should be provided with fish kill reports, cost recovery forms and evaluations, and any other supporting documentation when a fish kill event is referred for enforcement action.
* Regional office investigators, water compliance, and enforcement staff should request technical review and assistance from WMA staff, other DEQ staff, or outside experts when needed.
* Regional office investigators, water compliance, and enforcement staff should coordinate with CO Office of Financial Responsibility and Waste Programs to seek cost recovery of all fish kill investigative costs.
* Regional office enforcement staff will pursue enforcement actions or issues resulting from the fish kill investigation in accordance with DEQ enforcement policies and procedures.
* Establish official files, save records according to the specific program’s records management requirements, and maintain files in DEQ’s Enterprise Content Management (ECM) system as needed.

## 2.2 Central Office Division of Enforcement

The Central Office Division of Enforcement should:

* Provide interpretation and guidance on applicable statutory, regulatory, and policy requirements to regional and other DEQ staff as necessary.
* Coordinate judicial action on fish kills with the Office of the Attorney General and the regional office staff.
* Review cases involving statewide policy and precedent-setting issues.
* Assist in obtaining inspection warrants.

## 2.3 Central Office Water Monitoring and Assessment Program

The Central Office WMA program should:

* Update fish kill investigation guidance and manual as necessary.
* Maintain a position with fish kill coordination responsibilities to provide technical and policy assistance to regional office staff upon request.
* Provide technical review of fish kill reports for regional office staff upon request.
* Assist regional investigators with obtaining the appropriate literature for reference data.
* Assist regional office staff with unusual or large fish kill investigations (e.g., conducting fish counts).

## 2.4 Office of Financial Responsibility and Waste Programs

Staff from OFRWP should:

* Receive information from regional office investigators documenting the investigative and fish replacements costs provided by DWR to initiate cost recovery.
* Draft cost recovery correspondence to send to the responsible party. Submit to the Office of Financial Management to send to the responsible party.
* Track receipt of cost recovery requests.
* Address ability to pay or failure to pay issues from responsible parties.

## 2.5 Division of Communications - Media and Public interactions

Only after completing the initial investigation will DEQ have enough information to respond comprehensively to inquiries by the press or other concerned parties. The size and character of some fish kills necessitates a quick response; however, the Regional Director, along with the Division of Communications, are responsible for release of information. The Division of Communications:

* Responds to press inquiries about fish kills.
* Coordinates with regional staff who have conducted investigations to gather necessary information to communicate to the media, press or public.
* Posts information about the response and resolution to a fish kill investigation on DEQ website, press releases, or social media accounts.

# CHAPTER 3. RECEIPT OF FISH KILL REPORT

## 3.1 Collecting initial information

The regional points of contact for those reporting fish kills should be PREP or other staff experienced in receiving and documenting fish kill complaints. If inexperienced staff receive the initial contact, they should contact PREP or another experienced staff member, who will take over the initial interaction. Regional PREP and WMA staff should coordinate to ensure that experienced staff are available to receive and document initial fish kill reports to the extent feasible, based on staff availability.

Members of the public may report fish kills in a variety of methods, including by phone, email or online.

Fish kills reported through the PREP online reporting portal ( [https://portal.deq.virginia.gov/v2/prep/search](https://portal.deq.virginia.gov/v2/prep/search.)) should capture the information listed below. For reports made by other means (e.g., phone, email, in-person), and for any reports that are incomplete, the details below should be logged in the PREP database as soon as possible. All new staff are required to complete the DEQ – Intro to PREP CEDS Module and should be able to enter the incident report into the PREP module. If staff have questions, they can reach out to PREP for assistance.

Regardless of the reporting method, the following information should be collected when an initial fish kill report is received by DEQ:

* Contact information: Record the name, address, telephone number, email address, and any other pertinent information about the individual in case there is a need for later contact. Identify individuals as “anonymous” if they do not wish to be identified.
* Fish kill location: Obtain the exact location, directions, and description of the fish kill site, not only to find the area but also for trip preparation. Precise information on as many location details as possible (e.g., route numbers, intersections, railroad crossings, landmarks, latitude and longitude, or nearby street addresses) are essential. Record the water body type (e.g., lake, pond, river, stream) so that investigators know whether the kill site is a lake or river requiring a boat, or a small wadable stream.
* Safety concerns: **DEQ staff should advise those reporting fish kills that they should keep people and pets away from the affected area and avoid contact with the water and any affected animals until officials have investigated.** Staff should ask the individual if they have observed any evidence of effects on people, pets, wildlife near the site (e.g., unexpected behavior, signs of illness or injury), or if they have any reason to suspect a health risk to those near the site or to the field investigation team. **If oil, flammable, or hazardous substances are suspected, staff should contact the local fire department and the Virginia Department of Emergency Management (VDEM) for assistance**, to ensure that field investigators with the proper qualifications and proper safety equipment are available to manage these risks.
* to ensure that field investigators with the proper qualifications and proper safety equipment are available to manage these risks.
* Magnitude of the fish kill: Obtain the approximate size of the kill area in acres or stream length (feet or miles), the species and sizes of fish, and the approximate number of fish involved.
* Condition of fish: Record if the fish are still dying, which can help the investigator determine how recently the kill occurred and if the pollutant is still present or being released. Additionally, the individual should be asked if they observed any obvious external marks, lesions, or other abnormalities on the fish.
* Possible cause of the fish kill: Solicit information from the individual about the time and cause of the fish kill. For example, obtain information on recent watershed activities upstream of the fish kill, such as pesticide spraying, fuel or chemical spills, agricultural or construction activity, dumping of fish by commercial fisherman, unusual odors, color of water, foam, or oil sheen on the surface of the water.

## 3.2 Notifications

Since fish kills are often part of a larger pollution incident being investigated, all staff should work closely with PREP when notifying various agencies and individuals. Upon receipt of the fish kill report, the regional point of contact should notify the:

* Regional Water Quality Monitoring Manager and/or other regional staff that need to be notified following the office’s protocols for incident response and the nature of the incident.
* VDEM and local Fire Department if flammable or hazardous chemicals have been spilled or released.
* DEQ’s Communications Division.

In addition, the regional point of contact may also need to notify the following organizations depending on the situation:

* Local Emergency Management and/or Local Planning/Zoning/Environmental Compliance may be able to provide initial response in remote or distant locations. Local officials may be able to provide information on possible short-term projects or other sources in the area that might contribute to a fish kill.
* Contact the [Virginia Department of Health](https://www.vdh.virginia.gov/health-department-locator/) (VDH) Office of Drinking Water if public and/or private water supplies are at risk (or if this risk is unknown) so that water intakes can be closed.
* Contact the [VDH-Division of Shellfish Safety](https://www.vdh.virginia.gov/environmental-health/environmental-health-services/shellfish-safety/) if the fish kill is in a shellfish harvesting area. They can temporarily close leased shellfish bed harvesting if a fish kill is determined to be caused by a pollutant in shellfish harvesting areas.
* Notify the [Virginia Department of Wildlife Resources](https://dwr.virginia.gov/about/offices/) (DWR) and [U.S. Fish and Wildlife Service](https://www.fws.gov/northeast/virginiafield/pdf/PersonnelDirectory.pdf) (USFWS) for large or high-value fish or mussel kills, those for which a pollutant cause and a responsible party are suspected or if a fish kill occurs in waters containing native species of particular conservation concern (e.g. threatened or endangered species). In these cases, DWR and USFWS may request that specific field investigation protocols are followed and may conduct independent evaluations to derive the final value of the fish killed in the event. All fish kill reports should be considered in draft form, and not finalized until after DWR and USFWS have had a chance to provide input on the fish valuation and have indicated that they will not request changes to DEQ’s valuation. In cases where threatened and endangered species may be impacted, the DWR Division of Fisheries will conduct the final valuation. DWR may apply correction factors or other calculations in lieu of, or in addition to, the calculations presented in this document.
* If a harmful algal bloom (HAB) is suspected, the event should be reported to the VDH online [HAB report form](https://www.vdh.virginia.gov/waterborne-hazards-control/harmful-algal-bloom-online-report-form/#:~:text=Please%20contact%20the%20HAB%20Hotline,in%20or%20near%20the%20water.) and a HAB investigation should be conducted as soon as possible.
* In marine or estuaries of Virginia, the [VMRC Marine Police](https://mrc.virginia.gov/mp/leoverview.shtm) may be contacted for an initial response. They may also be able to provide boat transportation and can identify possible net dumps and illegal harvesting activities.
* [DWR Conservation Police](https://dwr.virginia.gov/conservation-police/) may also be contacted for an initial response, particularly in inland waters of Virginia.
* Notify other relevant state or federal agency that regulates the facility known or suspected to have caused the fish kill. For example, notify the Virginia Department of Energy if the fish kill is thought to be caused by coal or mineral mining or another activity regulated by that department. Virginia Department of Agriculture & Consumer Services may be contacted for pesticide related incidents.
* In special cases, where technical assistance in the investigation is required, the Central Office Fish Kill Monitoring Coordinator may be contacted for guidance.
* The regional office will originate any request for assistance. Notifications to the above parties does not necessarily indicate that the regional office desires assistance with field investigations.

# CHAPTER 4. CONDUCTING A FORMAL FISH KILL INVESTIGATION: FISH KILL INVESTIGATION GUIDANCE, THIRD EDITION (GM23-2006)

The DEQ Fish Kill Investigation Guidance provides detailed instructions on the key aspects of fish kill cases that are generally the responsibility of the DEQ Water Quality Monitoring Program. The guidance document follows this paragraph. Differences between this chapter and the official version of GM23-2006 posted on Virginia Regulatory Town Hall (available at: [https://www.townhall.virginia.gov/l/ViewGDoc.cfm?gdid=7728](https://www.townhall.virginia.gov/l/ViewGDoc.cfm?gdid=7728%20)) are limited to numbering of sections and appendices. Key elements of the formal investigation process include preparing staff and equipment, locating and accessing field sites, field measurements, observations and samples, and identifying, counting and evaluating the condition of killed fishes, determining causes, safety procedures, and key aspects of communication with the public, coworkers and agency partners associated with the case. All staff participating in a fish kill investigation should review be trained in all aspects of fish kill investigations as detailed in this chapter.

A quick response to a fish kill is essential and the incident should be treated as an emergency response situation; however, some office preparation should take place prior to departing for the field to ensure a safe and efficient investigation. Investigators should make every effort to get to the site and begin counts as soon as possible because predation, drifting or other factors may cause the number of visible dead fish to decrease with time. Estimates of the number of fish killed that are made 24 hours after the kill may be 50% lower as compared to estimates made immediately (Nielsen and Johnson, 1983).

The primary purpose of the fish kill investigation is to determine the cause of death (natural or otherwise), the mechanism of death (e.g., toxicosis, asphyxia, sepsis), conditions that lead to death, and the size and number of each species killed (Meyer and Barclay 1990). The investigation may entail conducting field tests, sampling, and additional observations (e.g., impacts to benthic invertebrates, amphibians, reptiles, birds, and mammals) with the objective of answering these basic questions. The investigator should also observe for possible triggering events, such as spills or other discharges, that led to the fish kill with effort to support the overall investigation. The response team will look to identify the responsible party(ies), and stop or contain any pollution discharged, and to prevent future fish kills. No document can cover all potential aspects of an investigation; however, the following sections will aid in the use of specific techniques that are often beneficial.

The Department of Environmental Quality (DEQ) [Pollution Response Program – Base Manual: PREP-2017-01](https://townhall.virginia.gov/L/GetFile.cfm?File=C:\TownHall\docroot\GuidanceDocs\440\GDoc_DEQ_6238_v1.pdf) (July 18, 2017) is a valuable reference explaining the basic techniques and requirements for conducting a pollution incident investigation.

* 1. **Preparation prior to visiting the site**

Before departing the office, check all field equipment and sample containers to determine if they are sufficient to conduct the investigation. Generally, staff should be prepared to collect the information listed in Sections 4.6, 4.7 and 4.10 (general observations, typical field measurements and fish counts respectively) when responding to any fish kill. However, investigators may need to collect additional data depending on site conditions and the suspected causes of a particular fish kill, as described in Sections 4.8 and 4.9. In planning prior to visiting the site, investigators should evaluate what is known about the kill already, through discussions with staff from DEQ’s Water Quality Monitoring & Assessment (WQM and WMA) programs, DEQ’s Pollution Response & Emergency Preparedness (PREP) program, or an onsite incident commander, particularly when less routine data collection is necessary. When investigating a fish kill, staff should generally follow the protocols established to prepare for a sampling run in the Water Quality Monitoring Standard Operating Procedures (SOP; <https://www.deq.virginia.gov/our-programs/water/water-quality/monitoring>). Whenever possible, the field investigation team should include staff that are proficient in the methodology described in this guidance and those in the SOP. When this is impractical, given site conditions, time constraints or staff availability, the field investigation team should conduct all the field activities for which they are qualified and equipped and review the collected data with qualified WQM staff to determine if additional investigation is necessary.

The investigator should locate the fish kill area on maps. DEQ’s Environmental Data Mapper (EDM), USGS topographical maps, and Virginia county maps are ideal for fieldwork and enable the investigator to perceive the terrain and water area involved in order to prepare appropriate equipment for the investigation. DEQ’s EDM also allows staff to quickly identify previous pollution complaint sites (PREP reports), nearby permitted facilities, and other spatial data to help determine if the fish kill is a continuing or recurring problem. The investigator should record the latitude and longitude of the fish kill as indicated on the digital map (approximate if necessary). A precise latitude and longitude of the site should then be recorded in the field, using a GPS receiver, especially if it is in a remote area. Staff should also use online maps to note if the fish kill is in a section of stream that has any special classifications such as being a trout water, 303(d) listed impaired water, having an approved TMDL or Implementation Plan, Exceptional state water, or any other special designation.

Prior to visiting the site, the investigator should determine if any rare, threatened or endangered species are in the vicinity of the fish kill by querying the Fish and Wildlife Service (USFWS) IPAC database (<https://ipac.ecosphere.fws.gov/>), Virginia Department of Wildlife Resources (DWR)Virginia Fish and Wildlife Information Service (VaFWIS) databases ([https://services.dwr.virginia.gov/fwis/)](https://services.dwr.virginia.gov/fwis/), and Natural Heritage Data Explorer (<https://vanhde.org/user>). If queries find that rare threatened, threatened, or endangered species are in the vicinity, then staff should notify the appropriate DWR and USFWS contacts about incident and DEQ’s investigation plans. Whenever possible, the field team should confirm what, if any, special investigation procedures are requested by DWR to account for high-value, threatened and endangered species.

Communication among DEQ staff and partner agencies is an essential but challenging component of every investigation. Communication procedures and key contacts change frequently, and therefore, are not covered in this guidance. These procedures and contacts are available to staff in procedural documents, which are revised regularly based on current information. All potential members of fish kill investigation teams should have a current contact list, and be familiar with current procedures for communications before, during, and after each investigation.

* 1. **Safety**

As initial responders to a fish kill, staff must ensure that they follow all safety protocols, as hazardous materials that cause the kill may be present at the site. DEQ maintains a [Safety Manual](https://covgov.sharepoint.com/sites/DEQ-Health) to promote safety in the field and office with protocols that staff must follow when responding to the fish kill. Responding staff must have taken appropriate safety training.

DEQ does not expect employees to risk their personal safety when conducting these investigations. At any point in an investigation, as soon as hazardous, explosives, or flammable materials are observed or suspected, staff must stand clear of the site and contact the Virginia Emergency Operations Center (Call: 1-800-468-8892) and the local fire department to report the incident and receive instruction before proceeding.

When accessing a site:

* Appropriate safety equipment should be assembled prior to conducting any site investigation, such as having proper PPE, high visibility vests, proper footwear, waders, safety glasses, gloves, first aid kits, or other equipment as needed.
* Staff should remain aware of often overlooked safety concerns when conducting investigations including: weather and tides, traffic (including delays in responding due to distance), time of day (do not work after dusk), and general situation awareness (e.g., entering private property, environmental conditions, terrain and wildlife).
* If a fish kill investigation is part of a known pollution incident, the investigation team should check in with the incident commander prior to accessing the site and exposing themselves to contaminants.
* The investigation team should obtain a safety briefing from a safety officer or other response personnel that understand any hazards that may be present in the fish kill area, including contaminants that may be present in the water. Staff should explain to the incident commander their need to access the site quickly to assess environmental impacts, count fish or collect samples, which could expose them to a pollutant.
* If contaminants are present in the water, investigation staff must consider the nature of the contaminants, and available personal protective equipment, when deciding whether to enter the water. If an incident commander is assigned to a case, the field team should obtain their approval before conducting field activities. Contact with the incident commander can be in person, remotely by phone, or through other DEQ staff (e.g., PREP staff already engaged).
  1. **Communicating with the public and media**

As with any pollution incident, a fish kill may generate public or press interest, where investigators onsite may need to respond to questions. Some general guidelines to follow if approached to discuss the incident:

* Staff should conduct initial interactions with media or public if approached onsite or contacted in the office.
* Investigators should only convey facts about what is known, and avoid any conjecture, speculation, or giving opinion about the incident or who or what is responsible. In the initial stages, often all that can be relayed is that an incident has been reported and an investigation is underway.
* After responding to initial inquiries, refer further inquiries from the public or press to the Division of Communications.
* Convey any interactions with the press to regional management and Communications (include names, phone numbers, and emails of individuals if possible).
* Be sure that responding to inquiries does not cause delays that interfere with the time sensitive nature of an investigation. Staff may need to indicate that they cannot respond or can only respond briefly so that they can focus on the investigation.
  1. **Location and confirmation of event**

The general steps, and the order in which they typically occur are described below. If emergency responders, an incident commander, or other PREP staff are engaged in the event, the field investigation team should follow any direction they provide on specific actions to take and the order of their execution. Primary steps:

1. Confirm the fish kill.
2. Determine if fish are still dying.
3. Determine the upstream and downstream extent of the fish kill.
4. Collect initial water quality measurements and samples.
5. Convey initial field test data to PREP staff to assist with the investigation. In cases where pollution and its apparent effects are severe, or where the causes and solutions of the fish kill are apparent, locating the source and stopping the pollution may occur before the water quality investigation.
6. Attempt to locate the source of the pollutant.
7. Take measures to stop and contain the pollutant with the local Fire Department, the Virginia Department of Emergency Management (VDEM), and assistance from the responsible party. Generally, PREP staff or other DEQ staff that are not members of the field investigation team lead efforts to stop and contain the pollutant.
8. Count and identify dead fish and other species.
   1. **Problems of site access**

Investigators on the scene have an obligation and a responsibility to report anything that appears harmful or damaging to the environment. If an investigator deems that an inspection of a facility or posted property is appropriate or essential to the investigation, permission to enter the facility or posted property should be requested from a company official or property owner. The owner or operator in charge at the time of the site investigation must give consent to for DEQ to inspect the property or the facility. If an investigator is denied entry for any reason, the investigator should immediately contact regional management and request direction on appropriate action. DEQ’s [Pollution Response Program – Base Manual: PREP-2017-01](https://townhall.virginia.gov/L/GetFile.cfm?File=C:\TownHall\docroot\GuidanceDocs\440\GDoc_DEQ_6238_v1.pdf) (July 18, 2017) provides instruction on steps to take when accessing a site where access is denied. Central Office Division of Enforcement may also be contacted for assistance in obtaining inspection warrants. The DEQ [Enforcement Manual (February 2023)](https://www.deq.virginia.gov/home/showpublisheddocument/16917/638109331921900000) provides direction on accessing private property for inspections and investigations, including situations where access is denied, and inspection warrants are required. Field investigation teams should only follow these PREP and Enforcement resources and access properties where permission is denied after receiving approval by and under the direction of a Regional Office or Division Director.

* 1. **General observations and additional tests**

Upon entering the site, the investigator should record general observations in a field logbook and take photographs and/or video of the fish kill. Prior to gathering detailed counts and gathering water measurements or samples, record general observations of the site (such as weather, site characteristics, apparent water quality issues etc.) as quickly as possible. If additional help is needed for further investigation, staff should request it at this time. After recording general observations:

* As quickly as possible, staff should establish the area of the kill and estimate the number, size and species of fish involved. Determine the upstream and downstream extent of the fish kill. The upstream extent usually coincides with the source of the fish kill, and the downstream extent is usually at a point at which the pollutant or other cause has been sufficiently diluted or neutralized, frequently at the confluence with a larger water body. Establishing these two points early on is critical to the remainder of the fish kill investigation.
* If fish are still dying, staff should observe and record their behavior; for example, whether they are listless, frantic, spiraling or suffering from a loss of equilibrium. Appendix G: Clinical Signs may be useful when identifying behavioral abnormalities.
* Staff should take note of any apparent abnormalities on the fish and remember to quantify these along with the count, as described below. Appendix G: Clinical Signs may be useful when identifying abnormalities.
* Collect field measurements of water quality (Section 4.7), water samples (Section 4.8) and conduct other tests as needed (Section 4.7).
* Count the number of dead fish using the techniques outlined in Section 4.10 and the American Fisheries Society (AFS) guidelines. The species of each individual (or lowest possible taxonomic level), along with a detailed accounting of the number of individuals that exhibit abnormalities, and the nature of those abnormalities should be included. Staff should examine the fish externally for gross abnormalities such as growths, lesions, hemorrhaging, or other unusual features using the Clinical Signs form (Appendix G). Staff should collect a few live fish and keep on ice.
* In nontidal streams and rivers, staff should conduct a macroinvertebrate benthic survey to provide important clues as to the cause of the kill and to document the total impact of the pollution event. In other systems, such as estuaries or lakes, benthic surveys are not typically conducted, but may be conducted on a case-by-case basis.
* Make algal observations. If an algal bloom is suspected, the case should be reported using the [Virginia Department of Health Online Report Form](https://www.vdh.virginia.gov/waterborne-hazards-control/harmful-algal-bloom-online-report-form/) and qualified staff should conduct a harmful algal bloom investigation. Dense algal blooms may change typical water quality characteristics and indirectly cause a fish kill or may produce toxins that directly kill fish and cause human health risks.
* During fish counts staff should document impacts to non-fish species (benthic invertebrates, amphibians, reptiles, birds, and mammals). Additional protocols for mollusk counts are presented in Section 4.11.
  1. **Field Tests**

If fish are found dead or dying, the investigator should collect common field measurements, including pH, dissolved oxygen, temperature, and conductivity, in the immediate area where dead fish are found, using properly calibrated instruments. Depending on the suspected cause of the kill, additional field measurements may be required. These vary widely among cases. If unsure, the field team should contact the Central Office WQM Fish Kill Coordinator or a WQM monitoring specialist to determine what additional data should be collected. Calibration data should be logged to ensure that any field test measurements are defensible in an enforcement case. Following the investigation, conduct post-calibration to ensure that all measurements remained within the acceptable margin of error. Conduct all calibrations and measurements according to the SOP (<https://www.deq.virginia.gov/water/water-quality/monitoring>). Document all field test locations, date and time, method, and measurements in a waterproof field notebook. Each page should be initialed by the investigator(s) collecting the data and information on that page. Field tests may be used to trace the source of a spill or pollutant discharge and to delineate the area impacted by a spill. Convey field test information to other DEQ staff or incident commanders to assist with the investigation as needed.

* 1. **Collect Samples**

Once initial field tests have been made, collect water column, sediment, and fish samples for lab analyses as needed. To obtain reliable results, ensure that the sample taken is truly representative of the stream, use proper sampling techniques, preserve and protect the samples until they are analyzed, and use proper sample chain of custody procedures. Collect all samples using the procedures outlined in the [DEQ Water Quality Monitoring Standard Operating Procedures](https://www.deq.virginia.gov/home/showpublisheddocument/4826/638518072363570000) (SOP). When necessary, Chain of Custody procedures should follow those outlined in section 4.11 of the SOP. Field teams should consult their regional management, who, in turn, may consult the Central Office Division of Enforcement if it is unclear whether Chain of Custody procedures should be followed. In cases where this consultation may delay the investigation, the delay should be avoided, and the field team should always follow Chain of Custody procedures. When samples are submitted to Virginia Division of Consolidated Laboratory Services that require chain of custody, contact the WQM Central Office Laboratory Liaison, or other WQM Central Office staff. The Liaison or other staff will make arrangements to ensure that the procedures are followed throughout the process and that, if needed, expedited analysis is ordered and funded. In cases where chain of custody samples are required of other laboratories, these arrangements should be made directly with the laboratory as soon as possible (ideally before the samples are collected).

Once safe procedures are established and qualified personnel are on site, a water sample at the point-source discharge or in the area where the effects of the suspected pollutant appear most severe should be collected as soon as possible. If possible, discharges should be sampled and measured at the location where the discharge enters the water body. The field team should consult with regional management, other PREP and WQM staff engaged in the case, and the Central Office WQM Fish Kill Coordinator to determine the number, types and locations of samples and measurements to collect. If field staff are unsure of the best location to sample, the discharge should be sampled at the discharge point, before entering State Waters, and the water body should be sampled in the immediate vicinity of the initial release. Additional samples and measurements should be collected such that the field team and other engaged staff are satisfied that the release has been sufficiently characterized. Samples and measurements taken in the waterbody upstream of the fish kill effect, downstream of the effects, or other samples in comparable water bodies that are not affected by the kill are often needed to identify the most likely causes. Extra samples may be discarded if there is no need for them to be analyzed with little wasted effort. In contrast, if too few samples and measurements are collected, missing data that might be needed to determine the cause, magnitude, and potential solutions for the kill, or to identify a responsible party, may not be recoverable at a later time. In all cases, before sampling potential pollutants the field team should understand the potential safety issues associated with them and be prepared with the knowledge and equipment necessary to safely investigate. In cases of unknown safety risks, the field team should defer to the incident commander and emergency responders if they are engaged in the event, and to PREP staff and regional management if not, to obtain sufficient guidance to safely investigate. DO NOT sample in or near discharges whose contents are unknown, or suspected to be hazardous, flammable or explosive without consulting with qualified staff in the field or the Virginia Emergency Operations Center (Call: 1-800-468-8892). Preserved (typically frozen) voucher specimens of fish submitted for testing should be kept to confirm data if required. If uncertain, consult the analytical laboratory to ensure that the preservation method will not interfere with the analysis.

Continued investigation (e.g., field testing, sampling, etc.) after initial observations and sampling may be necessary. The investigator should use the initial information as a starting point to begin a more detailed investigation/sample plan.

* 1. **Reference or control station**

Reference stations may be infeasible or unnecessary in some cases but should be established in most cases to provide a basis for comparison of the field test results, field samples, and observations of aquatic life. If the limits of the kill can be found, then a reference or control station should be selected upstream from the kill or, if the kill is in a tidal estuary, far enough away from the kill area so that tidal influence does not cause conditions in the kill area to affect the reference area. If the kill has occurred in the headwaters of a stream, then another, comparable stream may be used as a reference. Obtain a control or reference sample for each type of field test and/or sample collected. As with the fish kill site, the reference station locations, date and time, field test and sampling methods, and measurements should be documented in a waterproof field notebook or on prepared field data sheets and each page initialed by the investigator(s) conducting the procedures.

* 1. **Counting dead fish**

DEQ is responsible for assessing the damage to aquatic life caused by pollution incidents. The State Water Control Law, [§62.1-44.15 (11)](https://law.lis.virginia.gov/vacode/title62.1/chapter3.1/section62.1-44.15/), specifically states that DEQ shall have the duty "To investigate any large-scale killing of fish.” Thus, the counting of fish killed is a responsibility of the DEQ staff. Beyond a simple count of dead fish, staff must gather information to assess the impact comprehensively. Since DWR assigns the costs of fish replacement, the count must contain the information needed for DWR to make assessments, which DEQ also uses to determine the total natural resource damage. Staff should follow the established procedures presented in this guidance as well as in the AFS Guidelines so that fish counts or valuations are defensible if challenged. As all fish kills are unique, if the investigator needs to deviate from the standard procedures, they should follow established ecological sampling methods and consult with the Central Office WQM Fish Kill Coordinator as needed. Document the methods used and explain the reasons for deviations from established methods in field notes and the investigation report.

* Record final fish counts for the stream segments on the "Fish Kill Count Form" (Appendix B).
* In general, staff should identify all fish killed to the species level. In cases where identification of all individuals to species in a timely manner is especially challenging, identifications of problematic specimens to family or genus may suffice. However, this should be done only as a last resort when accurate species identifications cannot be made in a timely manner. Whenever possible, the investigation team should include personnel capable of identifying killed fish to species. Staff should contact a DWR nongame fish biologist, or a qualified DEQ WQM staff member if assistance with identifications is needed. This is especially important in waters where high-value, threatened or endangered species may have been killed. If the procedures to be used are unclear, or sufficient expertise on the field team is lacking, contact the Central Office WQM Fish Kill Coordinator to determine how to proceed.
* Measure the length of each dead or dying fish.
* The investigator should take photographs of each species present for future reference.
* Collect vouchers of specimens whose identities are uncertain or unknown. Remember to record the number of the unknown fish at each segment and their size. If multiple unknown species are encountered, identify each as thoroughly as possible (e.g., to genus or family) and make notes that allow you to determine the number of each species later. Staff should contact DWR or USFWS for assistance if they feel that a voucher specimen of a threatened or endangered (T&E) species should be collected. Collection of live or dead T&E specimens without consulting with these agencies may be a violation of DEQ's collection permit, state, or federal laws.
* In cases where only weight is acceptable, make a representative collection of species sizes. This collection is weighed in the field using accurate scales and the values extrapolated to get the total number of fish killed.
* Three publications specific to fishes in Virginia are referenced at the end of this guidance (Murdy et. al. 1997, Jenkins and Burkhead, 1993, and Bugas et. al. 2019). In addition, the DWR maintains an on-line Wildlife Information Database at <https://services.dwr.virginia.gov/fwis/> to enable users to quickly generate a list of fish and other aquatic and semi-aquatic animals including species listed as threatened, endangered, and special concern that are expected to occur within the fish kill area. The database is free to the public; however, users can formally register as subscribers to the site to access smaller (3-mile) geographical search ranges. Investigators should register for the Wildlife Information Database and be familiar with it and the other fish identification texts referenced above.

Non-DEQ fish kill counts: Counts used in fish kill reports can come from several non-DEQ sources, such as DWR, the Virginia Institute of Marine Science (VIMS), Virginia Marine Resources Commission (VMRC) or private companies. Staff must be cautious when accepting count information from other sources, as DEQ will ultimately be responsible for the accuracy of the information reported. Every count, no matter who makes it, must be conducted using an acceptable technique, such as, AFS or USFWS guidelines. Any count data received from outside sources must include a detailed description of the methods employed. If needed, DEQ staff, in coordination with management, may give written approval to outside entities to conduct fish kill counts that will be used by DEQ for cost recovery or enforcement purposes. Staff should review and approve the methods being employed prior to allowing outside entities to conduct the counts so that if they find the methods inadequate, they can then perform the count themselves in a timely manner. Whenever possible, DEQ personnel should be present at the investigation to ensure that proper procedures are followed. Regardless of who conducts the investigation, the fish count information referenced in Appendix B, to include the specific methodology used for the count and the species, condition and total length of the specimens observed, is essential.

The Central Office WQM Fish Kill Coordinator should be consulted to determine whether a non-DEQ dataset is appropriate to be used in a DEQ fish kill case.

Three primary methods may be employed to conduct fish kill counts: 1) total count, 2) standard procedural count, and 3) estimates. The following sections explain the methods and the information required to ensure accurate and representative fish kill counts.

* + 1. **Total count**

The preferred method of counting fish is to make a total count of every dead fish, starting downstream and working upstream, whenever possible. The total count is the most accurate method available, and least subject to challenge, since it is an exact count of every fish seen by the investigator. However, in many cases, a kill will be of such a large magnitude that an accurate total count is not possible within a single day. Counting over multiple days can reduce accuracy, as fish may be lost to predation, drifting, or other factors.

* + 1. **Standard procedural counts**

Lacking a total count, the next best method is a standard procedural count involving the counting of fish within sampling areas and extrapolating the total number of fish killed. DEQ uses the sampling techniques from the AFS Investigation and Monetary Values of Fish and Freshwater Mollusk Kills publication (Southwick and Loftus, ed. 2018). This reference is an update and expansion of the widely accepted counting techniques and monetary values of fish published by AFS since 1975. Standard procedural count methods exist for several types of waterbodies and situations, including narrow streams completely accessible, narrow streams incompletely accessible, narrow streams with drifting fish, lakes & wide streams, shoreline counts, open water counts, large meandering streams, and multiple-day counts. The AFS guidelines for common waterbodies encountered are summarized below however investigators should refer to the AFS guidelines directly for more detail and/or unique situations.

**4.10.2.1 Standard procedural count in a narrow completely accessible stream**

AFS defines a narrow stream as a stream where each sample section can be traversed by boat or wading and walking the banks and the investigator can count or collect every dead fish observed along both banks and in the stream. A completely accessible stream is a stream that an investigator can access for the entire stretch of the kill.

To conduct a standard procedural count in a narrow, completely accessible stream:

1. Determine the furthest upstream and downstream locations where dead fish are found.
2. Divide the stream segment with the fish kill into sample areas or reaches at regular intervals, for this example ½-mile intervals, between the upstream and downstream locations on a map (Figure 1). Begin at the downstream end and move upstream. The following examples use English units of measure to be consistent with AFS guidelines, but investigators may also use metric units. A long (50-300ft) measuring tapes or laser range finder should be used to measure distances along the stream channel, and pink or blaze orange flagging may be used to mark sample areas.

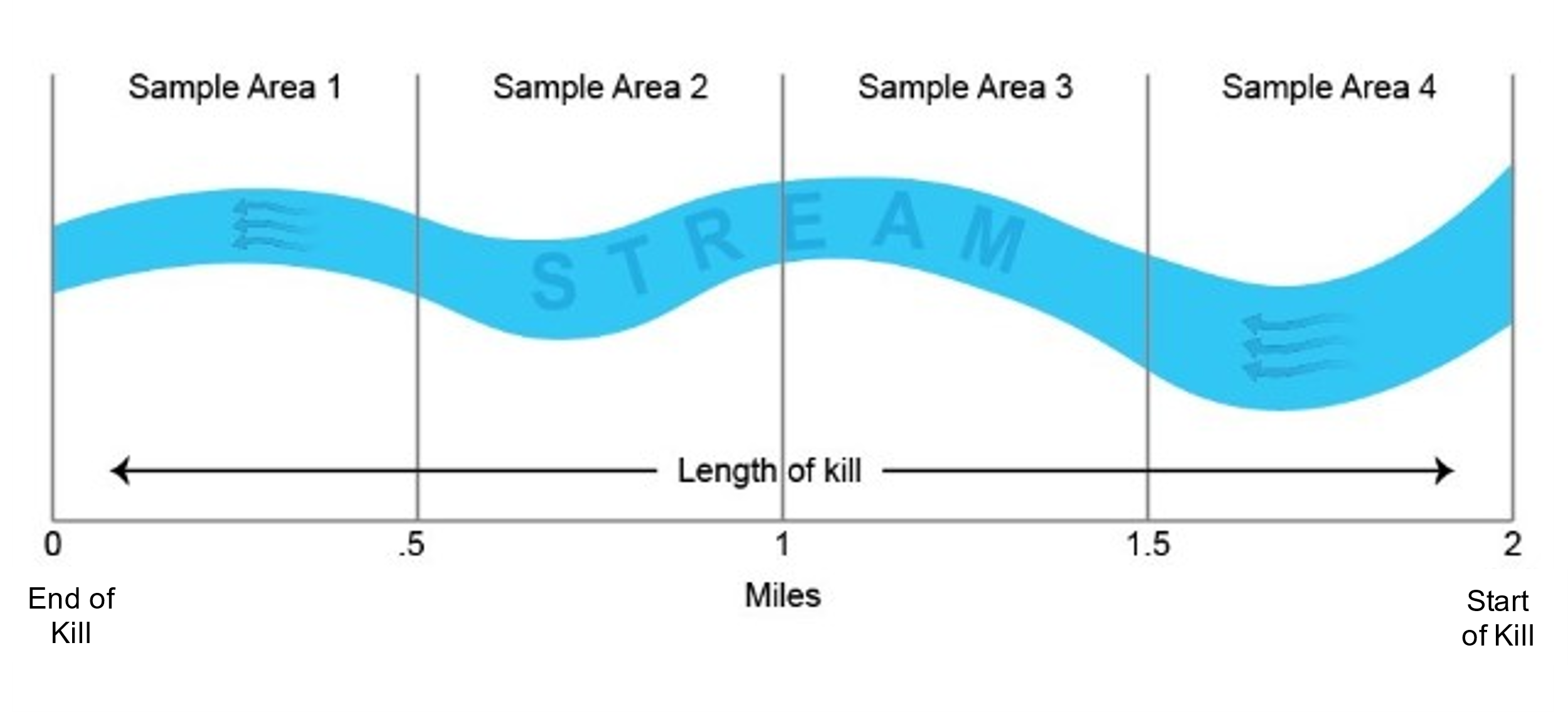


Figure 1. A two-mile fish kill in a stream (represented by blue line) has been divided into four (4) – 1/2-mile sample areas.

1. Randomly select a starting sample segment (also known as sample site in AFS guidelines) in the first sample area (Figure 2). An approximately 100-yd sample segment every ½ mile or a 100-meter sample segment every kilometer throughout the area of the kill is recommended. Use at least 3 sample segments to complete fish counts.

* As an example, a ½-mile sample area (880 yd) can be divided into eight-110 yd sample segments. The investigator randomly selects a number between 1 and 8. Suppose 7 was chosen, then the first 110 yd sample area starts in the 7th segment which starts at 660 yd and ends 770 yd upstream from the start of the kill (7 x 110 = 770). Next, using the same gap width (½ mile or 880 yd), the investigator marks the next 110-yd long sample segments in the other sample areas (Figure 2).
* Fish kill investigations may result in enforcement actions, and cost recovery from a responsible party, based on the number of dead fish observed. Therefore, choosing sampling segments randomly is critical to prevent investigator bias and to represent the total number of killed fish as accurately as possible.
* If a large kill covers many miles, extend the interval between sample segments appropriately. Similarly, smaller kills may require smaller sample segments at shorter intervals (e.g., 10 yd every 200 yd).
* The systematic sampling as described above and employed by AFS is a type of cluster sampling that provides an easier way to select sample sites compared to completely random sampling. This sampling method may increase precision when populations are clumped randomly and tends to eliminate count errors caused by clumps by spreading out sites evenly (Southwick and Loftus, ed. 2018).

Chart: A 2-mile fish kill in a stream (blue line) marked into four 110-yard sample segments in red in each sample area using a random start and the same gap width (0.5 miles or 880 yards). 
Identify and count killed fish in each sample segment (red areas in Figure 2) as described in Section 4.10.

Figure 2. A 2-mile fish kill in a stream (blue line) marked into four 110-yard sample segments in red in each sample area using a random start and the same gap width (0.5 miles or 880 yards).

Identify and count killed fish in each sample segment (red areas in Figure 2) as described in Section 4.10.

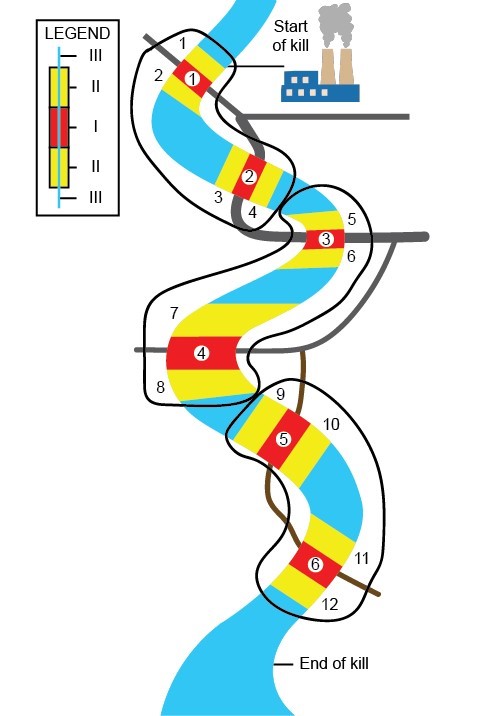
1. Exclusion zones: The investigator must be careful not to bias the count, particularly if dams or other obstructions, which accumulate fish, are present. A dam or obstacles where many fish carcasses are trapped and accumulate may greatly influence standard procedural or extrapolated counts. The sample segment count should be a representative of the whole, not an exception. To address accumulating fish, establish an Exclusion Zone if an obstruction is encountered in one of the randomly selected sample segments.

* Count all carcasses in the exclusion zone (obstruction area where carcasses accumulate).
* Complete the count in the remainder of the random sample segment selected.
* Do not include the counts from the exclusion zone in the total standard procedural count calculation. The exclusion zone count will be added to the total after the standard procedural count calculation is complete.

**4.10.2.2 Standard procedural count in a narrow incompletely accessible stream**

Streams may be incompletely accessible due to factors such as heavy vegetation, wetlands, logjams, fences, and trespass restrictions. When faced with a stream that is partially accessible the field team should adapt a modified version to the directions given previously where the fish kill is divided into strata. For example, suppose a stream has a fish kill starting at an industrial facility and ending at a larger river where the pollutant was diluted (Figure 3). The stream is only accessible at 6 road crossings, (see road crossings numbered 1-6). The kill is 4,000 yd (2.27 mi) long.

1. At each road crossing, divide the sample areas into strata.
2. Stratum I is the portion under the influence of road crossings due to culverts. Use best judgment to determine the length of Stratum I (a minimum of 50 yd in each direction is often necessary). Stratum I consist of six 100-yard segments (50 yd downstream & 50 yd upstream of road crossings). When possible, all fish in each Stratum I segment should be counted. If staff availability does not allow this level of effort, a random subsample of the segments may need to be selected (the field team should select as many Stratum I segments as can be completed in 1 day).



Text Box

1. Stratum II is the accessible portion beyond the influence of the road crossing. Again, use best judgement (100 yd immediately upstream and 100 yd downstream of Stratum I is typical). Stratum II consist of twelve 100-yd segments (Figure 4). Determine if fish in Stratum II segments can be counted with available staff. These 3-strata segments, consisting of 1 Stratum I and 2 Stratum II segments, should be established in all accessible areas along the reach affected by the kill. In the example in Figure 3, the strata can be combined to comprise 3 segments distributed along the entire fish kill; each containing 4 Stratum II segments (12 total Stratum II segments / 3 Stratum II segments that will be sampled = 4). With 3 groups of 4 Stratum II sample segments, one random sample out of each 4 segments is selected. The investigator randomly selects a number 1-4. Suppose the number 3 was chosen, then counts would be made at Stratum II segments 3, 7, and 11 (i.e., the 3rd Stratum II segment in each of the 3 12-strata segments in Figure 3). All killed fish in each selected stratum should be counted. The level of effort described in this example is typical; however, staff availability may not allow this number of Stratum I and Stratum II segments to be counted, and accessible areas may not occur at each road crossing within the kill area. In such cases, the maximum number of segments that can be counted in a day, distributed over as much of the kill area as possible, should be counted. In these cases, the procedure for selecting the strata to be counted will need to be modified by an experienced fish biologist on the field team. Contact the Central Office WQM Fish Kill Coordinator for assistance in selecting count strata.
2. The water quality field parameters described in Section 7 are typically measured at each road crossing sampling location; however, the frequency and types of measurements and observations (i.e., those described in sections 6 and 7) should be chosen by experienced field staff, based on the data needs for the particular case and on staff availability.
3. Stratum III is the inaccessible portion of the stream. An expansion factor is used to calculate the number of dead fish in Stratum III, assuming the number of killed fish in the Stratum III segments would be the same as the number killed along an equal length of Stratum II segments (accessible areas not affected by road crossings). Investigators should note any apparent differences between Stratum II and Stratum III segments that might make this assumption inaccurate (e.g., differences in channel morphology or flow, different land cover types in the surrounding watershed).

The expansion factor (E) is the total length of the kill in proportion to the length of all reaches surveyed.

Expansion factor E = Ltotal /L

Where:

Ltotal = Total length (stream yd or meters) of fish kill

L=Sum of the Lengths of the transects

In this example, a separate expansion factor should be calculated for road crossing areas (Stratum I segments) and for areas not affected by the crossings (Stratum II and Stratum III). The Stratum I expansion factor is the total length of all Stratum I segments, divided by the length of all the sampled Stratum I segments. The Stratum II/III expansion factor is the total length of all Stratum II and III segments, divided by the total length of the sampled Stratum II segments. The final standard procedural count can then be determined by multiplying the Stratum I count by the Stratum I expansion factor, multiplying the Stratum II/III count by the Stratum II/III expansion factor, and summing these two results.

1. If exclusion zones were designated, add the fish counted in these zones to the final standard procedural calculation to obtain a total fish kill count.
2. Section 4.12 provides further details calculating the number of dead fish from the samples.

For most fish kills, a count of dead fish in an approximately one 100-yd segment per ½ mile of stream is acceptable. However, the larger the percentage of affected stream counted, the more accurate the computed determination. Therefore, the investigator may decide to count a segment larger than the approximate 100 yd within each ½-mile sample area. On the other hand, the distance between sample segments may have to be adjusted to accommodate the total length of the kill and the investigative resources available. A two-person counting team can usually count three to four 100-yd segments in one day. The investigator must judge his or her segment length and distance carefully since it is desirable to make the count within a single day to avoid duplicate counts of drifting fish.

For example, if only two people are available for counting dead fish and the fish kill is determined to be 10 river miles long, it may take a two-person crew approximately five to ten days to count the approximate 40-100 yd sample segments spaced ½ mile apart as recommended by protocol. In this case, the investigator could call for assistance from other regional personnel. If additional assistance is not available, an alternative would be to make the sample segments about 2 or 2½ miles apart. For large fish kills, as many as six to twelve field staff may be involved in counts if the ½ mile segment guideline is followed. As needed, the Central Office WQM Fish Kill Coordinator will attempt to assist with determining how to select sample areas, allocate effort to an investigation, or seek assistance from other Central Office or regional staff.

* + 1. **Lakes and large river fish kill counts**

1. For fish kills in lakes or large rivers, obtain a good map of the water to measure surface area if possible (digital maps and electronic measurements and calculations are preferred).
2. Establish a baseline for the water body along the long axis of the lake or affected large river section (Figure 4). For example, the lake in Figure 5 below has a 1500 yd baseline.
3. Determine how many transects will be counted based on available staff and resources. Sampling transects extend perpendicular to the baseline, across the entire width of the water body. For example, assume that 4 transects can be counted. Divide the water body base line into equal size sample areas based on the number of transects that will be sampled (4 in this example). Each sample area is 375 yd wide along the baseline (1500 yd / 4 = 375 yd).
4. Determine the width of the sample transects, which is the width over which all fish can be collected, based on the width of boat and net length. In this example, transects are 5 yd wide.
5. Randomly select the transect location for the first sample area. This case has 75 possible transects in each section (375 yd/ 5 yd =75). Using a random numbers table or random number generator select a number from 0 to 74. Suppose 38 was chosen, the first transect location will be 38 \* 5 yd = 190 yd from the end of the baseline (y-axis = 0 in Figure 4). The next transects will be in the same position in each of the other segments and 375 yd away from each other along the baseline. In the example, transect 1 =190 yd, transect 2 = 565 yd, transect 3= 940 yd, and transect 4 = 1315 yd along the baseline.

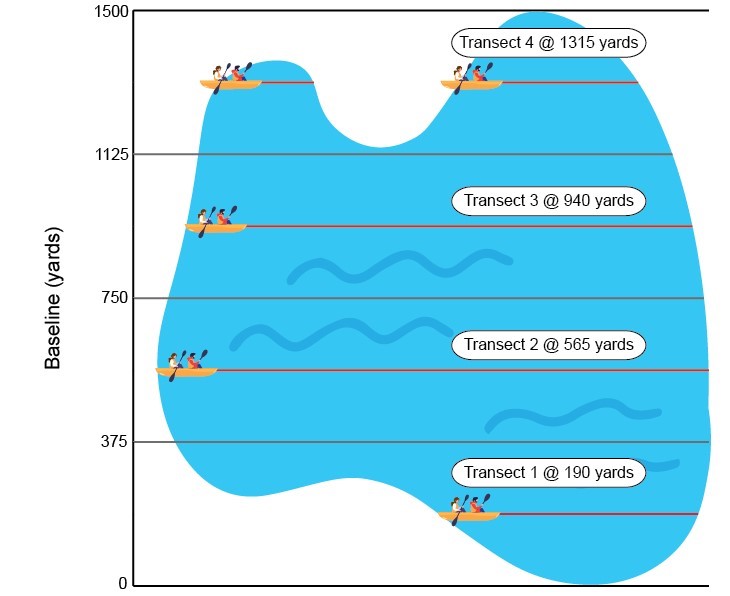


Figure 4. Fish Kill in an impoundment with a baseline = 1500 yards. Four sample area are shown (grey line and 4 sample transects 5 yards wide are shown (red line). Transects are sampled by boat.

1. Boats follow a transect and investigators pick up fish in the transect boundary (5 yd), but none outside of it. Measure the length of each transect (a laser range finder may be used, or field teams may obtain GPS points of the transect ends and calculate distances using a mapping application).
2. Exclusion zones: Fish in lakes or large rivers will often be clumped along a shoreline due to wind and/or water currents. If fish are clumped along a shoreline, an exclusion zone can be made (Figure 5). Fish in the shoreline exclusion zone can be counted using the same technique for narrow streams. Keep shoreline counts separate from transect counts until the two are combined to get the total count of the kill.
3. See section 4.12 for methods of calculating the number of fish killed over the entire lake or river system. In some cases, a fish kill may be clearly limited to one portion of a lake. In such cases, it may be most efficient to confine the entire baseline, and all transects to the affected area. In many cases, however, it is difficult to determine the extents of a kill over large water bodies. If doubt exists as to the extent, the entire lake should be surveyed.

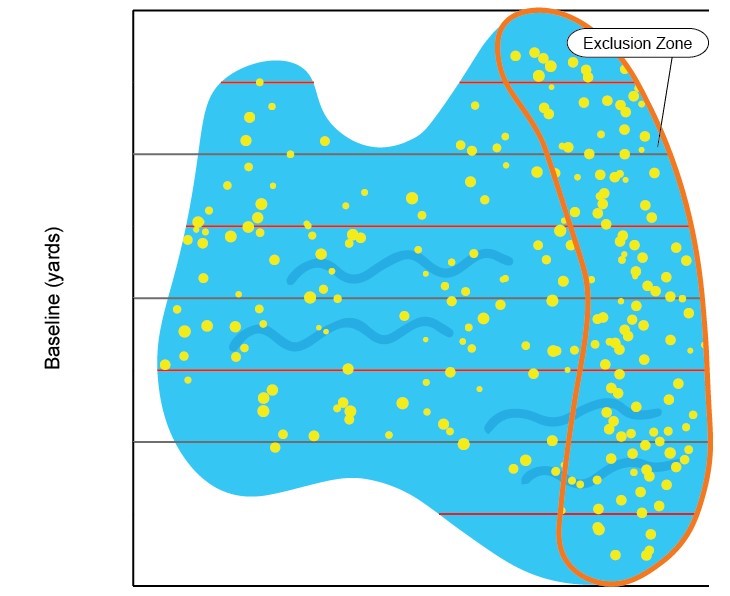


Figure 5. Exclusion zone established in an impoundment due to clumping of fish carcasses (yellow dots) on shoreline due to wind and/or water currents.

* + 1. **Estimate**

An estimate, where no systematic sampling techniques are employed, should be made if neither a total count nor a procedural count can be made. Estimates may be used for very large fish kills, such as a large menhaden kills, where several hundred thousand fish may be a foot deep in a small channel or bay, making a count very difficult. Estimates provide an idea of the magnitude of a fish kill but should be avoided for cost recovery purposes, because the number of dead fish is not calculated using an accepted sampling method and may contain an unknown amount of error. Investigators making an estimate of the number of dead fish should still use a systematic approach to make the estimate and record the methods and assumptions used.

* + 1. **Multiple Day Counts**

Multiple day counts are problematic and should be avoided if possible. Multiple day counts are more expensive than single day counts, and it is often unclear how to sum counts made over several days. While single day counts could underestimate the number of dead fish, multiple day counts can have the opposite problem of overestimating the count due to counting the same fish over. However, multiple day counts may not be avoided in cases where fish in a river with a toxic plume are killed for days as the plume progresses downstream. AFS recommends basing each day count on a separate survey. The investigator can then offer their professional opinion on how to provide a grand total for the surveys, although the estimate cannot be supported statistically (Southwick and Loftus, ed. 2018). One option to address counts done over multiple days is to base the rate of turnover of visible dead fish in a lake on the fraction of fish that appear fresh (red gills, clear eyes, fresh odor. etc.) each day.

* 1. **Mollusk Kills**

The recent version of the AFS Fish Kill Guidelines (Southwick and Loftus, ed. 2018) also have procedures to conduct mollusk kill counts. Investigators should contact the DWR [Aquatic Wildlife Conservation Center](https://dwr.virginia.gov/awcc/) located at the Department's Buller Fish Cultural Station near Marion, Virginia for assistance with mollusk kill counts since they can be more complicated and time consuming compared to fish kill counts. In addition, mussel identifications can be problematic. Mollusk kill investigations may also require specialized equipment and typically employ wading, SCUBA, and/or snorkeling. Notify the [US Fish and Wildlife Service Virginia Field Offices in Gloucester and Abingdon](https://www.fws.gov/office/virginia-ecological-services/contact-us) of mussel kills, particularly if federal threatened or endangered species may have been impacted.

* 1. **Determining the total number of fish killed**

Record final fish counts for the stream segments on the Fish Kill Count Form (Appendix B). For narrow, completely accessible streams, the number of fish killed for each species size class may be calculated using either the mean number of fish counted per segment or an expansion factor for the total number of fish counted in the sample segments (both methods should yield the same result). Examples of each method are provided below. For narrow, incompletely accessible streams, expansion factors must be used (Example b below). For lakes and large rivers, investigators should consult AFS guidelines for applying the expansion factors. AFS recommends using appropriate significant digits during calculations (Southwick and Loftus, ed. 2018).

Example calculation for a narrow stream:

In a 2-mile long fish kill, a total of 240 2-inch Bluegills were counted in 4 segments of 110 yd, spaced 1/2 mile apart.

(a) Using the Mean number of fish counted per segment for a narrow stream:

Mean number = 240 fish (total count) / 4 segments = 60 fish per segment

In 2 miles or 3520 yd, there are 32 - 110 yd segments (3520 yd/110 yd = 32 yd)

thus:

Total fish killed = 60 fish per segment x 32 segments

= 1920 2-inch Bluegills estimated killed

(b) Using an Expansion factor for narrow stream:

Expansion Factor = Total Length of fish kill/Sum of the Lengths of transects

= (1,760 yd/mile\*2.0 miles) / (4 segments \* 110 yd/segment) = 8

thus:

Total fish killed = 240 fish (total count) \* 8 (expansion factor)

= 1920 2-inch Bluegills killed.

Example calculation for a narrow, incompletely accessible stream:

For narrow, incompletely accessible streams, separate expansion factors may need to be calculated for stream segments affected by road crossings (Stratum I) and segments not affected by crossings, which include both Stratum II and Stratum III segments. The example in Figure 4 includes 6 road crossings, 6 100-yd Stratum I segments (600 yd) and 12 100-yd Stratum II segments (1200 yd). Assume an additional 1200 yd is located in inaccessible areas (Stratum III) and that fish counts occurred in all Stratum I segments and in 3 of 12 Stratum II segments. Assume a total of 500 two-inch Bluegills were counted across all Stratum I segments and a total of 350 two-inch Bluegills were counted across all Stratum II segments.

Expansion Factor for Stratum I (EStratum I)=

Total length of stream in Stratum I / Total Length of Stratum I counted

= 600 yd / 600 yd = 1

Note: in this case the E Stratum I was not strictly necessary, as all Stratum I segments were surveyed. This expansion factor is necessary when road crossings are too numerous to survey all in one day.

Expansion Factor for Strata II and III (E Stratum II+III)=

Total length of stream in Stratum II or Stratum III / Total Length of Stratum II counted

= 2400 yd / 300 yd = 8

Total fish killed (standard procedural count) =

E Stratum I \* Total fish in Stratum I segments + Estratum II+III \* Total fish in Stratum II segments

= 1 \* 500 + 8 \* 350

= 500 (total fish at road crossings) + 2800 (total fish in segments not affected by road crossings)

= 3300 total two-inch Bluegills killed.

Example calculation for a lake:

The total number of fish killed may be determined using an expansion factor, which is the total surface area as a proportion of the area sampled.

Method 1: Expansion factor calculation with a good map

Method 1 is preferred. Investigators should consult with DEQ GIS staff to ensure that a digital coverage with sufficient resolution is not available to calculate area before using Method 2.

Expansion Factor = Area of Water Body (yd2)/ Sum of transect Lengths (yd) \* transect width (yd)

Method 2: Expansion factor calculation without a good map

Expansion Factor = Baseline length (yd)/transect width(yd) \* number of transects

Number of killed = (Expansion factor \*number of dead fish counted) + number of dead fish in exclusion zone

* 1. **AFS Fish Kill Investigation Guidelines**

Investigators should refer to the AFS Guidelines (Southwick and Loftus, ed. 2018) for more detailed information regarding standard procedural counts, fish and mollusk kills, and fish kill investigative techniques. Detailed procedures and counting examples are presented in AFS Guidelines. AFS has modified procedural counts for fish kills in narrow completely and incompletely accessible streams, narrow streams with drifting fish, wide streams, large meandering streams, lakes, and multiple day counts. Additional modifications are needed for calculations involving an exclusion zone.

* 1. **Additional Observations**

There are several other important points to remember when conducting fish kill investigations.

* The investigator should take good field notes and photographs to document everything.
* Investigators should also look closely at the fish species involved in the kill even if not assigning replacement cost. Remember that threatened or endangered species or rare endemic populations may be impacted. In addition, aquatic or semi-aquatic animals other than fish may have been killed.
* Impacts to benthic invertebrates, amphibians, reptiles, birds, and mammals should be documented. The final report of the incident should include information about the number of non-fish wildlife losses to show the total environmental impact of the pollution incident.
* Counting procedures typically underestimate the number of fish killed for a variety of reasons. For example, fish may not be counted that are too deep or too small to be seen, or they may have been scavenged by predators. In addition, time is against investigators of fish kill events. For example, estimates for the number of fish killed may decrease by approximately 50% after 24 hours of a fish kill (Nielson and Johnson, 1983).
* The underestimation may be reduced by more complex and more costly counting techniques (Southwick and Loftus, ed. 2018). Such techniques are not reviewed here as they are not typically employed; however, when a kill is suspected to involve high value, threatened or endangered species, the field team should consult with DWR and the Central Office WQM Fish Kill Coordinator to discuss the case and the most appropriate investigation procedures. In such cases, DWR may also apply correction factors to adjust the fish counts to account for underestimation when determining the cost of the killed fish.

# CHAPTER 5. COSTS

## 5.1 Fish Cost Analysis

DEQ’s policy is to have DWR determine the replacement costs of the fish killed. For DWR to assign a cost to the fish, the fish must be correctly identified, and the length measured because the restocking values DWR uses to calculate costs differ among species and size classes. The American Fisheries Society Guidelines (Southwick and Loftus, ed. 2018) can assist with determining which information to obtain. This publication represents the results of the AFS Pollution Committee work on the value of fish species and the cost of their replacement. The Southeastern Division of AFS now accepts these costs. Familiarization with this document will assist the investigator in determining what information to gather on fish counted in the field.

For the DWR to assign costs, record information on a "Replacement Cost of Fish" form (Appendix C). To assist in the evaluation of replacement costs, note the body of water and location of the kill. The completed form contains an itemized listing of fish killed separated by species, size, and the number counted. In cases where only weight is recorded, use the # fish per pound value and extrapolate for the total number of fish killed. Continuation sheets may be used if needed.

DEQ regional management or staff transmits this form to the DWR Chief of Fisheries (<https://dwr.virginia.gov/contact/senior-staff-directory>). DWR assigns the costs, signs and returns the original copy to the regional investigator. For DWR to accurately assign a cost to the kill, it is essential that investigators include all the information referenced in Appendix B, and that all information needed to derive a total count from any standard procedural counts employed is provided. This typically includes the methodology used, any expansion factors to be applied, the location of sample segments, strata, and apparent limits of the kill. The original form becomes part of the case file for each fish kill investigation and is stored in ECM. DWR will use the counts, notes on methodology and expansion factors provided to derive a total count of each species and ultimately to provide a total cost for the kill. In addition, in cases involving high value, threatened, or endangered species, DWR may apply additional expansion factors to account for uncertainty in extrapolating count data to total fish killed or for inflation that has occurred since published values were last updated.

## 5.2 Cost of Investigation

According to state law, DEQ may recover the costs incurred because of a fish kill investigation, as well as any cost incurred by DWR. Hence, investigators should keep records of investigative expenses as a basis for cost recovery.

Complete a [DEQ Cost of Investigation Form](https://covgov.sharepoint.com/sites/deqnet/_layouts/15/download.aspx?UniqueId=08d3b7ea-7304-4c9c-b78d-883a0e67998d) (Appendix D) to document investigation costs. The form is used to record the actual costs incurred per individual during the investigation and the costs of any follow-up investigation, report writing, or other work on the case. The form has several major parts, including:

* Follow the steps on the Instructions tab of the form, which is located on the Finance DEQNet page: <https://covgov.sharepoint.com/sites/deqnet/_layouts/15/download.aspx?UniqueId=08d3b7ea-7304-4c9c-b78d-883a0e67998d>
* Detail the hourly labor costs. Select the appropriate fiscal year tab at the bottom of the form. The pay band is that of the employee’s position at the time of the investigation. Multiply the standard pay band hourly rate by the number of hours spent on the investigation to arrive at a total labor cost.
* Itemizing vehicle mileage and other travel expenses, such as food, lodging, and parking fees. These expenses are reported exactly as claimed for reimbursement on travel vouchers. Staff should consult with office management and the Office of Financial Management travel policies when itemizing expenses (<https://covgov.sharepoint.com/sites/DEQ-Finance/SitePages/Travel-Forms.aspx>).
* Itemize materials and equipment. Items may be included such as costs of ice, tolls, sample containers, laboratory costs (obtained from the catalog of laboratory services if conducted by DCLS), and any other expenses needed to complete the investigation.

The expenses are totaled, and the form is signed and dated by the staff involved in the investigation. If DWR personnel assist with an investigation, they should also complete an expense form. If a responsible party is identified, this is the amount that will be requested for reimbursement.

## 5.3 Cost Recovery

The cost recovery process begins when regional office staff refer an incident to DEQ’s Cost Recovery Manager in Central Office (typically by email to costrecoveryofrwp@deq.virginia.gov). Ideally, staff should refer a case for cost recovery no later than 120 days after the investigation is complete, cost information is received from DWR, and DEQ has identified a responsible person. If a responsible party is not identified, then cost recovery cannot proceed.

The table below shows the legal citations applicable to the cost recovery process based on the type of fish kill incident (oil or chemical).

|  |  |  |  |
| --- | --- | --- | --- |
| **Task** | **Oil Incident** | **Chemical Incident** | **Notes** |
| Fish Kill investigation | § 62.1-44.15 (11)(a) | § 62.1-44.15 (11)(a) | This could also include PREP staff time to identify the source of pollution |
| Fish Replacement Costs | § 62.1-44.15 (11)(a) | § 62.1-44.15 (11)(a) |  |
| Pollution Investigation/Response | § 62.1-44.34:18 9VAC25-590-210 | § 10.1-2502 | Recovery of staff time for the on-going investigation/response could be subject to OFM having to credit the funding source for staff salaries and debiting the VEERF |
| Contractual Costs | § 62.1-44.34:18 9VAC25-590-210 | § 10.1-2502 |  |

To prepare the cost recovery file, regional staff must provide certain documents to the Cost Recovery Manager when making a referral:

* DEQ Cost of Investigation Form (Appendix D).
* Fish Kill Cost Recovery Referral Form (Appendix E).
* Invoice from DWR with the fish replacement costs.
* Final Fish Kill Investigation Report

Once the Cost Recovery Manager receives the referral package from the regional office, they will review the package to verify that all the necessary information to pursue a cost recovery action is present and the responsible party named in the referral is the appropriate one. If the Cost Recovery Manager is satisfied that the package is complete and correct, they will prepare the demand letter. The draft demand letter and supporting documentation is sent to the Office of Financial Management (OFM) for signature and to create the accounts receivable for the cost recover amount. OFM mails or emails the final demand letter to the Responsible Party and notifies the Cost Recovery Manager, the regional office. and DWR by email that the letter has been mailed.

Assembling the appropriate documentation to support a cost recovery action before issuing the demand letter is a critical part of the cost recovery process. In the case of recalcitrant Responsible Party, the Office of the Attorney General (OAG) may have to file a civil action to obtain payment. The elements the OAG will need to prove to obtain a judgment are:

1. The presence of environmental pollution.

2. The pollution was caused by or contributed to by the Responsible Party; and

3. The pollution caused the Commonwealth to incur response costs.

For additional information regarding fish kill cost recovery cases, please contact Suzanne Taylor in the Office of Financial Responsibility and Waste Programs at (804) 659-1533 or suzanne.taylor@deq.virginia.gov.

# CHAPTER 6. CAUSES OF FISH KILLS

Fish kills may have many different causes, most of which can be grouped under seven general categories:

1. Industrial Operations
2. Municipal Operations (domestic sewage systems)
3. Agriculture and related activities
4. Construction/other causes
5. Transportation operations/storage
6. Natural causes
7. Fish dumping from commercial operations

## 6.1 Industrial Operations

Once the outfall of a suspected industrial waste discharge has been located, an attempt should be made to identify the owner of the facility from which the outfall originates and encourage the owner or operator to halt the suspected toxic discharge. If an in-plant inspection is warranted, contact the plant manager or person in charge and request a brief tour of the facility. If denied entry, contact regional office management or the Central Office Division of Enforcement. During a tour, the investigator can obtain general information concerning the products manufactured; raw materials used in the manufacturing process; quantities, sources, and characteristics of wastes generated; and waste treatment units if any. The plant manager may be able to supply a flow diagram of plant operations. The investigator should also request specific information concerning facility operations (i.e., accidental spills, etc.) immediately prior to the beginning of the fish kill.

## 6.2 Municipal Operations

Waste discharges from municipal or domestic sewage treatment plants may contain domestic sewage or industrial wastes combined with domestic sewage. These wastes may have been partially treated at the treatment plant or discharged untreated directly into a stream. Since the municipality, owner, or operator of the sewage system is generally held responsible for any discharge from such a system, the owner/operator, or their representative (i.e., city engineer, public works supervisor, a subdivision developer, etc.) should be contacted if samples of a suspected wastewater discharge are collected. The investigator should obtain information about plant operations. If the cause of the fish kill is determined to be the result of industrial waste discharging to a municipal treatment facility and then to a stream, data about the industry and its discharge should be obtained from municipal officials.

## 6.3 Agriculture and Related Activities

Fish kills can occur as a result of pollution from agricultural practices such as crop dusting, fertilizer application, and manure or other organic material discharges to a stream. Fish kills resulting from these agricultural operations are usually associated with runoff due to rainfall. The source or type of pollution may be difficult to identify and may involve a large nonpoint source area. Talking to residents may help pinpoint the problem area. Runoff from fields, drainage ditches, and small streams leading to the kill area may provide good sampling sites to trace the cause. Noting changes in turbidity in the stream may help to locate possible sources of runoff. As is the case with several types of potential pollutants (e.g., metals and complex organic compounds), analyses of sediment or fish tissue samples may be more reliable than water samples where pesticides or herbicides are suspected as a cause of the kill. This is because contaminant concentration may be transient in the water column, and be exported or diluted by current, whereas the same contaminants may bond tightly to sediment particles or biomagnification in animal tissue.

## 6.4 Construction and Other Causes

Fish kills may result from construction or mining activities as well as from such temporary or intermittent activities as; mosquito spraying; construction activities involving chemicals, concrete, and oils; and weed spraying with herbicides or other toxic substances. As with agricultural activities, tracing the cause of these kills is difficult and may require extensive investigation Discharges of swimming pool chemicals and chlorinated water by homeowners and community pool operators have caused several fish kills in Virginia. These are often accompanied by increased water clarity and/or elevated chlorine levels within the fish kill area.

## 6.5 Transportation Operations

Fish kills occurring from transportation accidents/incidents are usually readily identifiable. Highway accidents releasing toxic substances have resulted in chemicals spilling into nearby waterbodies causing fish kills. The investigation should include specifics of the accident such as the vehicle license number, vehicle owners, cargo type, and DOT placard number.

DEQ does not expect employees to risk their personal safety in responding to oil, flammable, or hazardous substance releases or spills or any pollution complaint. In instances where oil, flammable, or hazardous susbstances are involved, (particularly if one is not familiar with the material), stand clear and contact the Virginia Emergency Operations Center (VEOC) (Call: 1-800-468-8892) and request a callback from the Regional HazMat Officer (RHMO) for assistance.

## 6.6 Natural Causes

There are several possible natural causes for fish kills:

1. Oxygen depletion due to ice and snow cover on surface waters.
2. Oxygen depletion due to excessive respiration.
3. Abrupt temperature changes.
4. Epidemic and endemic diseases, parasites, and other naturally occurring biological causes.
5. Lake water inversion during vernal or autumnal turnover that results in toxic material or anoxic water rising to the surface.
6. Poor lake management resulting in overcrowding, degraded water quality or introduction of harmful species.
7. Fish spawning stress.
8. Toxins released by algae or microbes that directly kill fish*.*

There are few truly natural kills. Almost all kills, including disease outbreaks, occur due to external stresses. Pollution events may exacerbate factors such as those listed above. The investigator should aim to identify the environmental stresses. Data about environmental stresses may rule out pollution events and allow for a better characterization of natural stressors that may be exacerbated by pollution and give a more complete understanding of causes of a fish kill.

## 6.7 Fish Dumping from Commercial Operations

Commercial fishing operations may release dead fish into waterways that can be mistaken for fish kills. This problem occurs predominantly in the piedmont and tidewater regions in marine and estuarine waters but can occur anywhere there are commercial fishing operations. Most reported kills result from the emptying of nets as a “fish dump” or “bycatch discards”, or wash-down operations. In addition, commercial nets that break may release large numbers of dead or dying fish. VMRC requires commercial fisherman to report such incidents in Virginia coastal waters.

# CHAPTER 7. ADDITIONAL METHODS TO ASSESS ENVIRONMENTAL IMPACTS

The following are additional methods of obtaining information that may assist in determining the cause(s) of fish kills and assessing environmental impacts. The information below is intended to provide general guidelines for these specialized procedures and to convey a basic understanding of their purposes. In many cases, field teams may lack the expertise, equipment, and access to funding to execute these procedures. In these cases, the investigators should consult with their regional management and the Central Office Fish Kill Coordinator for guidance on how to include these procedures in an initial or follow-up investigation. In cases where time does not permit extensive consultation before an investigation, field teams should consider retaining samples that may be used as described below, and consulting with other staff to decide if they should be used or discarded after the investigation.

## 7.1 Water Samples

The analysis of water by collecting water samples is a standard procedure for investigating fish kills. Substances in solution or suspension largely determine the quality of water and affect fish directly and indirectly. The addition of dissolved or suspended material to water or the altering of amounts of substances naturally found in the environment can be harmful to fish and thereby cause a fish kill. Since fish kill investigations can lead to enforcement actions, the collection methods are of considerable importance. Water samples must be collected in accordance with the procedures set out in the most current DEQ Water Quality Standard Operating Procedures and Quality Assurance Project Plan (see <https://www.deq.virginia.gov/water/water-quality/monitoring>). Staff must identify and handle all samples properly as described in the SOP and QAPP. Not all fish kill investigations involve sampling that requires strict adherence to Chain of Custody procedures. In cases where sampling data may be used as admissible evidence to enforce the Commonwealth’s environmental laws and regulations, staff must follow the enhanced Chain of Custody procedures as outlined in [DEQ Guidance Memo 00-2016](https://townhall.virginia.gov/l/ViewGDoc.cfm?gdid=2922) and DEQ’s [Ambient Water Quality Monitoring Project Plan.](https://www.deq.virginia.gov/home/showpublisheddocument/4824/637891713480170000) Fill out all sample tags and lab sheets completely. DCLS will reject and may discard any samples with improper documentation. Whenever Chain of Custody samples are necessary, the field team should notify the Central Office WMA Laboratory Liaison, or other WMA staff if the liaison is not available. Central Office WMA staff will assist in making arrangements for sample receipt and timely analysis by DCLS or the laboratory engaged on the case. The field team should consult with regional management, and the regional office may consult with the Central Office Division of Enforcement, as needed, to determine whether Chain of Custody procedures are necessary (see further details in Section 4.8).

The most difficult part of collecting water samples may be knowing what parameters to sample for. Broad guidelines are provided here, but most cases require detailed knowledge of the affected system, the potential pollutants and natural stressors, and the careful preparation and planning of experienced investigation teams. If a fish kill occurs:

* Whenever possible, water samples and field measurements (e.g. temperature, pH, DO, specific conductivity) should be taken upstream of the fish kill or discharge (or in an adjacent, comparable water body if an upstream sample is not practical), within the fish kill area, and downstream of the fish kill (or otherwise outside of the affected area). In many cases, identifying the causes of a fish kill requires a carefully planned and thoroughly executed investigation (see further details on sampling effort in Chapter 4).
* Downstream of a sewage treatment plant: Collect samples for BOD, COD, bacteria nutrients, metals, organic priority pollutants and pesticides analysis if the area is industrialized.
* Downstream of certain industrial discharges the nature of the industrial activity should determine the type of sample collection needed. For example, below a pulp or paper mill, investigators may need to collect samples for BOD, COD, pH, and phenols for analysis. In agricultural areas, the investigator may need to collect samples for pesticides, metals, and nutrients from fertilizers for analysis.
* Conduct a harmful algal bloom investigation (see section 7.9 below) if water quality measurements, appearance or odor indicate that an algal bloom may be present, or if there have been reports of such at a site.

## 7.2 Sediment Samples

In some cases, the collection of water samples is not sufficient. The investigator may have arrived after the toxic discharge has stopped, the pollutant may have passed downstream or been diluted, or it may have been bound quickly to the bottom substrate. In such cases, investigators may need to collect sediment samples to identify pollutants no longer in the water, or to find a source that may no longer be discharging.

The sediment sample may retain pollutants that have settled to the bottom and out of the water column. Analysis of some parameters measured in water may not be appropriate in sediment due to differing chemical or physical properties between the two media.

Sediment samples are useful for identifying metals, pesticides, and some organic materials that may be the cause of the fish kill. Typically, investigators need to collect a minimum sediment sample of one pint (551 ml), but the volume of sediment required, and collection locations may vary. In the case of fish kills, where very recent contamination is involved, one is interested in only the top few millimeters of sediment. A grab or scoop of sediment that includes deeper layers may dilute the recent surface contaminant and prevent detection. The sediment type or texture is important to specify on the lab sheet and other documentation provided to the laboratory and should be recorded in the field notes. The absorption of some pollutants in silt or mud may differ greatly from that of sand. As always, a good control, collected outside the fish kill area, is essential. Analysis of sediment samples is time consuming and expensive. Sediment samples should be collected to identify a specific pollutant and the analysis cancelled if found unnecessary to verify the pollutant type. Detailed procedures for sampling sediments may be found in the [VADEQ Water Quality Monitoring Standard Operating Procedures Manual](https://www.deq.virginia.gov/water/water-quality/monitoring/fish-tissue-monitoring). Preserve and protect the samples until they are analyzed and use proper sample [chain of custody procedures](https://townhall.virginia.gov/l/ViewGDoc.cfm?gdid=2922) as needed.

## 7.3 Benthic Macroinvertebrate Surveys

The term "Benthic Macroinvertebrate" refers to invertebrate animals such as larval and adult insects, mollusks, aquatic worms, and crustaceans that live on the bottoms of lakes, streams, estuaries and ocean floors. Although susceptibility to toxic compounds varies among the invertebrate groups, a concentration of a toxic substance that will kill fish may also kill benthic organisms.

Benthic investigations at fish kills should only be conducted under the supervision of experienced DEQ biologists. Caution must be taken to ensure that specimens collected during a benthic investigation are living and have not been killed by the disturbance that caused the fish kill. This issue may be avoided by waiting several days after the incident to collect samples or by observing benthic materials in the field to determine whether benthic animals are alive on them. In many instances, it is beneficial to collect benthic samples upstream of the fish kill, or at other comparable sites outside of the kill area (e.g. those on a nearby stream) to serve as a basis for comparison.

After a toxic compound enters the water, the abundance of benthic macroinvertebrates in the affected area may be reduced, compared to upstream control areas. In other cases, the abundance of pollution-tolerant species may increase rapidly, whereas other species decline or are extirpated, and overall diversity is reduced. The boundary between the affected and unaffected areas is often distinct. This difference in the concentrations of bottom dwelling organisms above and below a spill or discharge point makes benthic surveys useful in pinpointing the source of a toxic pollutant when its origin is unknown. After or even during a fish kill, by examining the benthic life as you proceed to the head of a kill area, you may discover the sharp boundary between the affected and unaffected zones. This boundary may point to a particular discharge in an area with many discharges close together. It may also be possible to trace the path of the toxicant into tributaries too small to contain permanent fish life, which might have otherwise been overlooked without a cursory benthic inspection.

Even when the source of pollution is known, information on how the benthic life was affected provides evidence on the magnitude of the impact, as the benthic assemblage is typically reflective of the overall ecological integrity of the system. In addition, most benthic animals are sources of food for fish. Severe reduction in their numbers over a lengthy stretch of stream may, therefore, inhibit re-colonization of the stream by fishes. Surveys of benthic macroinvertebrates and other aquatic communities are also useful for many follow-up investigations, as aquatic biota often recover relatively quickly (within months) when a stressor is removed and there are unaffected waters nearby that can serve as sources for recolonization.

Benthic field investigations are conducted following the agency’s Biological Monitoring Quality Assurance Project Plan (<https://www.deq.virginia.gov/home/showpublisheddocument/6996/637520993335570000>). The methods in this QAPP are appropriate for conducting benthic surveys in wadeable, freshwater, free-flowing streams and rivers and only during the time periods between March 1 and May 31 (spring season) and between September 1 and November 30 (fall season). Surveys conducted in other systems (e.g., lakes, swamps, estuaries) may require special methods of sampling and data analysis not covered in the QAPP. Whenever possible benthic surveys should be conducted by, or in collaboration with, a WMA Program Regional Biologist in the appropriate DEQ region and, in the case of estuaries, the Central Office Estuarine Probabilistic Monitoring Coordinator. The resulting data are analyzed using one of several assessment indices, as chosen by a Regional Biologist or the Estuarine Probabilistic Monitoring Coordinator, based on the site location, condition and standard agency practices. Consultation with these staff is essential in the data analysis and interpretation phases.

For the most meaningful results, the benthic macroinvertebrate survey should be completed as soon as possible. Benthic macroinvertebrates typically require several months to recolonize after a disturbance has subsided (though this varies widely among streams). The differences between the affected and unaffected areas may remain distinct for several weeks, after which time, re-colonization by benthic organisms from less affected areas makes the differences continually less apparent. If effects on the benthic assemblage are observed in initial samples, one or more follow-up investigations may be conducted to evaluate recovery. For most cases, these investigations should begin approximately two months after the initial benthic study, as substantial recovery of the benthic assemblage would typically be expected over this time period in the absence of disturbances or persistent pollutants. The time between the initial sampling and follow-up may vary, however, based on timing and study objectives. For example, seasonal variations and natural disturbances may inhibit the collection of meaningful data from benthic studies during certain times.

## 7.4 Bioassay Toxicity Testing

Bioassay toxicity tests are not typically recommended to investigate potential toxicants during fish kill investigations. Rather, if the specific chemical can be identified, the manufacturer or the Material Safety Data Sheet (MSDS) should be consulted to determine the toxicity of the chemical. Many chemicals are required to be tested on aquatic organisms before they are marketed, and the information is available from the manufacturer or MSDS. Another option is to search the EPA on-line AQUIRE Database at <https://cfpub.epa.gov/ecotox/>. This database provides information regarding the toxicity of chemicals to aquatic organisms.

Toxicity tests may be conducted under special circumstances, particularly in follow-up cases, where evaluating the long-term effects of a residual pollutant in the sediment or other part of the waterbody has been chosen as an important objective. If conducted, they must be performed by a contract laboratory (e.g., private consultant or university) since DEQ does not have the capability to conduct bioassay toxicity testing. Section 7.4.1 presents general guidelines for conducting toxicity tests, however the DEQ Central Office WMA Manager and the Quality Assurance Officer should be consulted before tests are ordered and the resulting data are used. Central office staff will help ensure that the study design is adequate and that all appropriate quality assurance and quality control measures are followed.

## 7.4.1 Arranging a Toxicity Test for Fish Kill Investigations

1. Contact a contract laboratory to determine if a test can be performed, establish a contractual agreement in accordance with DEQ contract procedures, and arrange sample delivery.
2. Collect at least 3 gallons of the material to be tested and immediately store on ice (or follow alternate procedures as provided by the lab).
3. Include information as to the composition of the sample and an MSDS if available. (This protects the staff from known hazards).
4. Ship the sample overnight, or deliver the sample, to the laboratory selected for testing, using the procedures they provide.

## 7.5 Chemical Analyses of Fish Tissue

As with many investigative practices, the tissue of dying or dead fish may yield information as to the cause of death. Fish tissue analyses are an indirect method of identifying the pollutant involved but can provide valuable information on the effects of pollutants on the health of aquatic life and the health of humans, pets and wildlife that might consume the affected fish.

Important characteristics for choosing affected fish for tissue analysis include:

* Fish must be representative of the kill.
* Fish should be alive rather than dead if possible. Fresh dead fish may be acceptable, but during the summer months, significant deterioration of organs and muscle tissue may occur within hours. Such deterioration often causes evaluations of concentrations in specific tissues or organs to be inaccurate.
* Individual fish often make better samples than composites when the objective is to evaluate the effects of a specific pollution event, because composite samples of multiple individuals tend to average concentrations of pollutants and make range determinations difficult. When possible, replicate samples (i.e., multiple individuals of the same species and size class, each taken as an individual sample) should be taken. Increasing the number of replicate samples always increases the power, accuracy, and precision of the dataset for evaluating pollution effect. However, staff time demands, and analytical costs associated with fish tissue samples are high. Therefore, when it is possible to take replicate samples, the number is typically limited to 3-5 replicates.

The use and analysis of fish samples is far too complex to cover fully here. The "[DEQ Quality Assurance/Quality Control Project Plan for the Fish Tissue and Sediment Monitoring Program](https://www.deq.virginia.gov/home/showpublisheddocument/5313/637499493599870000)" provides an outline of the fish handling procedures. Further handling and analysis are best determined on a case-by-case basis.

## 7.6 Shellfish

Shellfish, when present, offer excellent opportunities for analysis of many metals, pesticides, and organic pollutants. Being filter feeders and unable to move about, oysters, clams, and even freshwater mussels, tend to accumulate and concentrate pollutants in their tissues. Sample variation is decreased if animals of the same size are taken from a single location.

Handling of shellfish samples is relatively easy once the animal is obtained from the bottom. Since oysters and clams can live for days out of water, simply keep them cool and bring them in for shipment to the laboratory.

Control or reference samples are often a serious problem. Since collection is normally done in tidal areas, one must collect control samples a considerable distance from the affected area to avoid contamination. Unfortunately, shellfish may be of different sizes or not present at all if salinity is different in the control area.

For specific instructions on the preparation of shellfish samples see EPA Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories [https://www.epa.gov/sites/default/files/2015-06/documents/volume2.pdf](https://www.epa.gov/sites/default/files/2015-06/documents/volume2.pdf%20Appendix%20G.)

## 7.7 Pathology

The causes behind fish disease may be divided into three categories:

1. Pathogenic organisms (bacterial, viral, fungal, or protozoan),
2. Toxins,
3. Physical stress or changes in water quality

One or a combination of the above factors may affect fish populations and cause a kill. Natural diseases may be exacerbated by the presence of toxins or by physical or chemical stressors. Investigation of fish kills and the diagnosis of diseases should be a systematic procedure. The investigator’s goal should be to gather as much information as possible to both identify diseases and determine their causes.

Consult the "Fish Kill Pathological Examination Report" form for the water quality data required to perform the pathological examination (Appendix F).

Notations on conditions at a kill site and the affected species may often be as helpful to the diagnostician as samples sent to the lab. Investigators should pay close attention to the behavior of ailing fish and accurately record any abnormalities. Provide information on the:

* Species,
* Size,
* Appearance,
* Distribution of affected fish on-site,
* Estimates of the number of sick and dying fish,
* Size of the waterbody,
* Measurements of water quality parameters (pH, DO, temperature, conductivity, etc.) may help in diagnosis.

Carefully select the diagnostic specimens sent in for analyses. Use affected fish that are near death or freshly dead whenever possible. Poor or grossly decomposed samples provide little information and may confuse or mislead the examiner. Clearly note any specimens that were dead upon collection for the diagnostician and package any dead fish separately from those collected alive to prevent contamination. Samples for analysis should be collected as gently as possible, minimizing damage to the fish tissue, clearly labeled, and shipped or transferred for examination as soon as possible.

Fish for pathological examination are normally shipped to one of the following laboratories:

* USGS Eastern Ecological Science Center, Contact Dr. Vicki Blazer (304) 724-4434, 11649 Leetown Road, Kearneysville, WV 25430.
* The College of William & Mary, Virginia Institute of Marine Sciences, Contact Dr. Wolfgang Volgelbein, (804) 684-7261, e-mail: [wolf@vims.edu](mailto:wolf@vims.edu), Chesapeake Bay Hall Room N107, Gloucester Point, VA 23062
* Virginia Tech, Contact Dr. Stephen Smith (540) 231-5131, e-mail: [stsmith7@vt.edu](mailto:stsmith7@vt.edu), Biomed. Sciences & Pathology, College of Veterinary Medicine, 205 Duck Pond Drive, Blacksburg, VA 24061

The WMA Fish Kill Coordinator should be contacted prior to shipping to coordinate the shipment of fish collected for pathological examination. Preserve specimens that cannot be examined immediately for future investigation. Most fish pathogens (especially ectoparasites) will quickly drop off or perish during the decay of host tissues. The investigator who selects diagnostic specimens must first make an assumption of the general cause of the disease in order to use the most suitable method of preservation. Common methods of preservation and probable uses are listed below.

CHILLING is generally used to preserve samples where the freezing or chemical fixation of host tissues would prevent the isolation or identification of disease agents. Whole fish or fish parts may be preserved in this manner if the time until examination is one day or less. Bacteria, fungi, protozoa, as well as histological and blood samples are generally preserved by this method. This process should also be used to in cases where the appearance of the fish tissues is important in diagnosis. The best procedure for chilling is to place the specimen in a waterproof container and transfer on wet ice or under refrigeration.

FREEZING is used in those cases where living organisms are thought to be present in the specimen and the destruction of tissue cells is not important. Tissues thought to be harboring larger parasites may be preserved in this manner. Tissue samples to be analyzed for the presence of chemicals or toxins should also be preserved by freezing. Specimens should be frozen as soon as possible to avoid overgrowth of contaminating organisms. The samples should be placed in a waterproof container on dry ice or in a mechanical freezer.

All samples should remain frozen until they are ready for examination.

CHEMICAL preservatives are generally used to preserve tissue samples and large animal parasites in cases when the viability of organisms is not necessary and contamination of tissues by fixatives does not hinder examination. Specimens selected for chemical preservation should be small enough to allow penetration of the preservative within a short time. Fishes up to five centimeters can be placed directly into the preservative while larger specimens should be opened along the abdomen before being placed in the chemical. Common chemical preservatives include a 70% isopropyl alcohol or a 10% solution of formalin.

Fish samples are collected for pathological examination whenever the investigator feels that the kill is the result of pathogenic organisms or under conditions where unusual symptoms are noted. For the purposes of pathological analysis, fish kills may be divided into two groups; those kills which are the result of pathogenic organisms (which may be viral, bacterial, fungal, or protozoan), and those that are the result of the introduction of a toxic material or materials.

In the first group, the goal of the investigator is to determine the specific pathogen involved so that a more concise analysis of the cause of the kill is possible and, in some instances, so that appropriate remedial action may be taken. Important field observations would be species involved; size class, behavior, and appearance of affected fish; distribution of affected fish; and the normal water quality parameters of DO, pH, specific conductance, and temperature.

In the second group, fish samples are usually taken with an eye to toxicity testing. For example, a fish kill occurs, and a given toxicant is suspected but the literature fails to support this hypothesis. Pathological examinations are performed on the affected fish and abnormalities are noted. Later, a toxicity test is conducted using similar circumstances and the fish from this test undergo pathological examination. If the abnormalities noted on the kill fish correspond to those noted on the test fish, then the investigator has an expanded database to support the original hypothesis. Also, examine control fish so that any abnormalities noted can be directly attributed to the toxicant and not to factors such as other toxicants, spawning stress, seasonal variation, and the like.

Fish samples submitted for pathological examination (concerning toxicant caused fish kills) do not replace necessary fish tissue, sediment or water samples collected for chemical analyses.

## 7.8 Algae

Under certain circumstances and in particular periods of high algal productivity, algae or their toxic products may cause illness or death in humans, fish, and other animals. Algae commonly causes fish kills by dissolved oxygen depletion from blooms or by direct toxicity effects on fish from algal toxins. Algal toxins that have been detected to date in Virginia’s fresh waters have been almost entirely produced by cyanobacteria (whose toxins are called cyanotoxins). Toxigenic algae in Virginia’s marine environments are predominantly dinoflagellates and diatoms. The detection of substantial concentrations of algal toxins in Virginia’s marine waters is relatively uncommon. However, fish kills caused by oxygen depletion following marine algal blooms are relatively common.

Staff conducting fish kill investigations should be trained in how to identify the potential signs of algae blooms. Ideally, at least one investigator present should be trained in how to conduct a harmful bloom investigation if needed. Signs of a bloom include mats of algal growth on the bottom (primarily in streams), discolored water, surface scums or mats, odors, elevated dissolved oxygen (above 100% saturation), depressed dissolved oxygen, elevated pH, and elevated chlorophyll or other algal pigments (e.g., phycocyanins or phycoerythrins if they can be measured). In addition, if dead or dying fish are noted with no other apparent cause, algal toxicity may be present.

When signs of an algal bloom are observed, a harmful algal bloom (HAB) investigation should be conducted, following agency protocols (<https://www.deq.virginia.gov/home/showapublisheddocument/16202/637995398321570000>. Suspected algal blooms should be reported to the Virginia Department of Health (VDH) via the online portal (<https://www.vdh.virginia.gov/waterborne-hazards-control/harmful-algal-bloom-online-report-form/>). In addition, any time a suspected bloom is reported, it should be submitted to the VDH portal, regardless of whether the field investigation team decides to conduct a HAB investigation. In addition to the information requested on the form, staff should indicate if a harmful algal bloom investigation was conducted or the reasons for not conducting an investigation in the comments section of the form. If samples are taken for algal taxonomy or toxin analysis, the ODU Phytoplankton Laboratory or other supporting laboratory should be contacted to arrange for delivery of the samples, which generally must occur within 24 hours of collection (ODU lab: contact Leah Gibala-Smith: 757-683-4994, [lgibalas@odu.edu](mailto:lgibalas@odu.edu) or Margaret Mulholland: [mmulholl@odu.edu](mailto:mmulholl@odu.edu), 757-683-3972).

## 7.9 Special Studies/Surveys

Follow-up surveys can be conducted if needed. Investigators may perform special studies/surveys to determine if the kill area is still receiving pollutants, or whether the problem has been corrected. The survey can aid in determining the source of a pollutant and will provide data on the effectiveness of a facility's waste treatment process. A regional biologist can conduct a benthic macroinvertebrate survey of the stream, lake, or estuary. The benthic survey can ascertain the effect of the discharge on the body of water that receives the wastes. The survey may also judge the severity of the effect of the discharge and the extent of the area affected. Some kills may involve only a few dead fish because the stream supported only a small number of fish, yet a benthic survey may reveal that the stream has been impacted for miles.

# CHAPTER 8. DATA INTERPRETATION AND REPORT PREPARATION

After completing the fieldwork and any additional research, the chemical and biological laboratory samples have been analyzed, and the results returned to the investigator, the data are ready to be organized and interpreted into a final written report. The investigator should consider all phases of the investigation when determining the cause(s) of the kill. The data should be arranged in a logical sequence to make interpretation as easy as possible. Begin the report preparation by clearly writing the report’s objectives. These objectives may not be directly stated in the report but writing them out will provide an outline for the investigator that can be used to focus their narrative and data presentation. These objectives may vary among cases, but for most cases, the general objectives are to:

1. Quantify the number, locations, sizes, conditions, and taxonomic identities of the killed fish.
2. Identify the cause(s) of the kill and the evidence or information used to make this determination, when causal factors can be established.
3. If the cause is related to a pollution event, determine the extent and magnitude of the event, and, where possible, work with the PREP response team to stop the pollution event.
4. Determine if there is a responsible party for the event, and if so, their identity.
5. Document the findings of the investigations and all costs associated with it in a manner that may be used for enforcement actions, cost recovery or other necessary corrective actions.
6. Major steps in the report preparation process, and the order in which they are generally approached are: Present the fish kill data in a clear manner that allows for their monetary value to be easily conveyed and provides clear understanding of the extent and magnitude of the kill. Highlight the key findings of the fish survey, for example, species most prominent in the kill, their numbers and conditions, species of conservation concern (e.g., threatened or endangered species), and species of high economic value.
7. Review all water quality, biological and other environmental data collected at the site (See chapters 4 and 7) Where appropriate, compare the data to the Virginia Water Quality Standards (9 VAC 25-260-10), and other thresholds used by the agency, noting any values that may not comply with the WQS. Compare the data to those collected from control stations and to published data from similar systems when such data are available and accessible. These comparisons may involve statistical analysis to determine the probability that conditions at the kill site are different than would be expected under natural conditions. Conduct literature research to determine if the water quality conditions indicated by the dataset may have caused the kill. Contact the Water Quality Standards Program or WMA to assist with literature searches and to determine the appropriate thresholds and analytical methodology for interpreting the data.
8. Review the field observations made during the investigation (e.g., fish behavior, appearance and condition of fish, benthic macroinvertebrates, algae, and other wildlife). Do the fish behavior or other observations relate to the water quality data as the potential cause of the kill or is another cause suspected? Review any pathologists’ reports associated with the case for any evidence that a disease caused the kill. If there is such evidence, investigate the cause for the pathogen outbreak by reviewing the available data and conducting follow-up site visits if appropriate to determine the extent of the water quality degradation.
9. Determine what additional data sets or investigative activities are needed. Ideally the report should provide all the data needed to provide the best possible account of the kill and the most likely causative factors. Additional data needed may include toxicity tests for a source of comparison to chemical data (see section 7.4 Bioassay Toxicity Assay). This may require customized testing in order to be representative of the natural conditions in the water body. Datasets that include extensive taxonomic identifications of benthic macroinvertebrates or algae, algal toxin assays or other non-standard parameters may need to be added to provide important evidence to supplement the initial dataset. The investigator should seek the guidance of their management team to consult with all DEQ staff and outside-agency staff engaged in the case on how to proceed. Based on this consultation, the report may be delayed until any added datasets are complete, or the report should be issued along with recommendations for further follow-up.

A report that addresses the items above is the minimum information to be complete. Staff may need to add information to address unique aspects of a fish kill to make the final report comprehensive. For instance, note any special characteristics of the stream section impacted that would indicate the overall impact to the environment from the incident (e.g., Exceptional state water, trout waters, 303(d) listing, approved TMDL or Implementation Plan). Fish kills may require enforcement action to collect penalties, recover replacement costs of the dead fish and investigation costs, as well as to secure injunctive relief to prevent future kills. Keeping the legal possibilities in mind, the investigation report should include as many of the steps above, as well as other information, necessary to support a legal case.

A critical error that an investigator can make is to assume that they can easily demonstrate field conditions in a final report and fail to gather the necessary information. Data, sample analyses, photos, detailed notes in a waterproof field logbook, and other evidence must be satisfactory to convince someone with less knowledge of the subject than the investigator. The cost and time loss of obtaining extra samples that are discarded, or extra data that are not used, are minimal compared to the loss of quality that results from not collecting crucial information because too few measurements or samples were taken.

# CHAPTER 9. REFERENCES

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# APPENDIX A. DEQ FISH KILL REPORT/NOTIFICATION

VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY   
FISH KILL REPORT/NOTIFICATION

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| IR# |  | | | | | | Stream | |  | | | | | | Basin | |  |
| City/County | |  | | | | | | | | Region | | |  | | | | |
| Date Investigated | | | | |  | | | | | Investigator | | |  | | | | |
| Reported by: | | | | Name | |  | | | | | | | | | | | |
|  | | | | Address | |  | | | | | | | | | | | |
|  | | | | Phone | | home | |  | | | cell | | |  | | | |
| Reported to: | | | Name | | |  | | | | | | | | | | | |
|  | | | Office | | |  | | | | | | Date | | | |  | |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Date Kill Started | | |  | | | Date Kill Ended |  | | Total # Killed | | |  |
| Fish Killed (Common name) | | | | |  | | | | | | | |
|  | | | | | | | | | | | | |
| Location of Kill | |  | | | | | | | | | | |
| Cause of Kill | |  | | | | | | | | | | |
| Staff Hours |  | | | | | Total Cost of Investigation | | | |  | | |
| Investigators Signature | | | |  | | | | Date | | |  | |

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| --- | --- | --- | --- |
|  | Investigator’s Expense |  | Map |
|  | Summary Memo |  | Fish Count |
|  | Lab Analyses |  | Bioassay Results |
|  | Benthic/Algal Results |  | Pathology Results |
|  | Bioassay Costs |  | Lab Cost |
|  | Pathology Costs |  | Total Cost |

# APPENDIX B: DEQ FISH KILL COUNT FORM

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| DEQ FISH KILL COUNT FORM | |  |  |  |  | |  |
|  |  |  |  |  |  | |  |
| Incident Report Number: | |  |  | Page\_\_\_\_\_\_\_\_\_\_ of \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | |  |
| Fish Kill Location: |  |  |  | Date: | | Investigators: |  |
|  |  |  |  | Time: |  | |  |
| Methodology (please attach pages or files to fully describe count methodology) | | | |  |  | |  |
| |  | | --- | |  | | Direct Count | |  | | --- | |  | | Standard Procedural Count | | |  | | --- | |  | | | Other (please describe) |
| Sample Segment Identification | Species | Total Length (in) | Weight (lb) | Condition | Number of Individuals | | Notes |
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| Sample Segment Identification | Species | Total Length (in) | Weight (lb) | Condition | Number of Individuals | | Notes |
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# APPENDIX C: REPLACEMENT COST OF FISH FORM

**Virginia Department of Wildlife resources**

**REplacement cost of fish**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Incident Report (ir)#** | |  |  |  | **pAGE** |  | **OF** |  |
| **Stream** |  |  | **City/county** | |  |  |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species (Common Name)** | **Size (inches)** | **Number** | **Individual Costs** | **Total Cost** |
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|  |  | **Grand Total** | |  |  |
|  |  | **Signed** |  | | |
|  |  |  | (Chief, Fish Division DWR) | | |
|  |  | **Date** |  | | |

# APPENDIX D: DEQ COST OF INVESTIGATION FORM

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Pollution Incident Investigation** | | | | | | | | |
| **Time, Travel and Materials Cost Recovery Schedule** | | | | | | | | |
| **Expenses incurred by the Department of Environmental Quality** | | | | | | | | |
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|  |  |  |  |  |  |  |  |  |
| **Incident Report (IR)** | |  |  |  |  | Date |  |  |
| **IR Site Location** | |  |  |  |  |  |  |  |
| **Region** |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  | **Name** |  | **Signature** |  |  |  |  |  |
| Emp #1 |  |  |  |  |  | Date |  |  |
| Emp #2 |  |  |  |  |  | Date |  |  |
| Emp #3 |  |  |  |  |  | Date |  |  |
| Emp #4 |  |  |  |  |  | Date |  |  |
| Emp #5 |  |  |  |  |  | Date |  |  |
| **Supervisor Signature** | |  |  |  |  | Date |  |  |
|  |  |  |  |  |  |  |  |  |
| **Time** |  |  | **Avg hourly rate** |  | **Extended** | **Benefits** | **Indirect** | **Total** |
| **Spent** | **Time Code** | **Pay Band** | **\*See below** | **Hours** | **Cost** | **(see below)** | **@28.5%** | **Time Costs** |
|  |  |  |  |  |  |  |  |  |
| Emp #1 |  |  |  |  | 0.00 | 0.00 | 0.00 | 0.00 |
| Emp #2 |  |  |  |  | 0.00 | 0.00 | 0.00 | 0.00 |
| Emp #3 |  |  |  |  | 0.00 | 0.00 | 0.00 | 0.00 |
| Emp #4 |  |  |  |  | 0.00 | 0.00 | 0.00 | 0.00 |
| Emp #5 |  |  |  |  | 0.00 | 0.00 | 0.00 | 0.00 |
| Sub-Total Employee Time | | | 0 |  | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | **No of Miles** | |  |
| **Travel-Vehicle** | |  | **Date(s)** | | **Pool** | **Agency** | **Private** | **Cost** |
|  |  |  | **From** | **To** | **0.246** | **0.246** | **0.56** |  |
| Emp #1 |  |  |  |  |  |  |  | 0.00 |
| Emp #2 |  |  |  |  |  |  |  | 0.00 |
| Emp #3 |  |  |  |  |  |  |  | 0.00 |
| Emp #4 |  |  |  |  |  |  |  | 0.00 |
| Emp #5 |  |  |  |  |  |  |  | 0.00 |
| Sub-Total Employee Travel | | |  |  | 0.00 | 0.00 | 0.00 | 0.00 |

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|  | | **Travel - Meal and Lodging** | | |  |  |  |  |  |  |
|  |  | |  |  |  |  | **Meals** | **Lodging** | **Other** | **Costs** |
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| Emp #1 |  | |  |  |  |  |  |  |  | 0.00 |
| Emp #2 |  | |  |  |  |  |  |  |  | 0.00 |
| Emp #3 |  | |  |  |  |  |  |  |  | 0.00 |
| Emp #4 |  | |  |  |  |  |  |  |  | 0.00 |
| Emp #5 |  | |  |  |  |  |  |  |  | 0.00 |
|  | | Sub-Total Employee Meals and Lodging | | |  |  | 0.00 | 0.00 | 0.00 | 0.00 |
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|  | | **Materials and Equipment Used** | | |  |  |  | **Hours/** | **Rate/** |  |
|  |  | |  | **Activity** | **Date** | **Vendor** |  | **Units** | **Costs** | **Costs** |
|  | | Boat Usage | |  |  |  |  |  |  | 0.00 |
|  | | Laboratory Analysis | |  |  |  |  |  |  | 0.00 |
|  | | Laboratory Analysis | |  |  |  |  |  |  | 0.00 |
|  | | Other- Specify | |  |  |  |  |  |  | 0.00 |
|  | | Other- Specify | |  |  |  |  |  |  | 0.00 |
|  | | Other- Specify | |  |  |  |  |  |  | 0.00 |
|  | | Sub-total Equipment and Supplies | | |  |  |  |  |  | 0.00 |
|  |  | |  |  |  |  |  |  |  |  |
|  | | **Total Time, Travel and Materials Cost Recovery Schedule** | | | | |  |  |  | 0.00 |
|  |  | |  |  |  |  |  |  |  |  |
| **Pay Band 4** |  | | 29.65/hr |  |  |  |  |  |  |  |
| **Pay Band 5** |  | | 39.53/hr |  |  |  |  |  |  |  |
| **Pay Band 6** |  | | 52.90/hr |  |  |  |  |  |  |  |
|  | | **Pay Rates will be updated when there are salary adjustments approved by General Assembly.** | | | | | | | |  |
|  | | **Hourly rates include fringe benefits** | | |  |  |  |  |  |  |
|  | |  | | | |  |  |  |  |  |

# APPENDIX E FISH KILL COST RECOVER REFERAL FORM

FISH KILL COST RECOVERY REFERRAL FORM

I. Site Name/PC#:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Address:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Region:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

II. Responsible Party: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Responsible Party SSN or EIN: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Responsible Party Address:

HOME:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Telephone:

WORK:

Telephone:

Responsible Party is: Individual Corporation

Partnership Limited Liability Co.

Other (Please specify: )

Summary of Responsible Party Determination: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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# APPENDIX F: DEQ FISH KILL PATHOLOGICAL EXAMINATION REPORT

VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY

FISH KILL PATHOLOGICAL EXAMINATION REPORT

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| POLLUTION COMPLAINT NO. | | | | | |  | | | | | SUBMITTED BY | | | | | |  | | |
| REGION |  | | | | COUNTY | | | |  | | | | | | | | | | |
| OFFICE/DIVISION | | |  | | | | | | | | | PHONE | | | |  | | | |
| SITE DESCRIPTION | | | |  | | | | | | | | | | | | | | | |
|  | | | |  | | | | | | | | | | | | | | | |
| DATE SHIPPED | |  | | | | | SHIPMENT METHOD | | | | | | | |  | | | | |
| DATE RECEIVED | | |  | | | | | | | | | | | | | |
| TYPE OF SAMPLES SUBMITTED: | | | | | | | Fish |  | | Water | | |  | Other | | | |  |

Collection Data

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| COLLECTED BY | | | |  | | | | | | | | | | | | | | | | | | | | | | OFFICE/DIVISION | | | | | | | | | | | |  | | | | | | | | | | | | | | | |
| PHONE |  | | | |  | | | | | | | | | | | | |
| FISH SIZE (INCHES) | | | | | | Fry | | | |  | | | | | 1-6 | | | | |  | | | | | 7-12 | | | | |  | | | | 13-18 | | | | | |  | | | | >18 | |  | | |
| FISH SPECIES | | |  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SAMPLE CAPTURED: | | | | | | | | Alive | | | |  | | | | Dead | | | | | |  | | | | | Sick | | | |  | | | | Feeding | | | | | |  | | | | Unknown | | | | |  | | |
|  | | Hook | | | | |  | | | | Cast Net | | | | | | | |  | | | | | Seine | | | | |  | | | | Hand | | | |  | | | | | Dip Net | | | | |  | | | |
|  | | Shot | | | | |  | | | | Unknown | | | | | | | |  | | | | |
| NUMBER OF FISH IN SAMPLE | | | | | | | | | | | | | |  | | | | | | |
| SAMPLE CONDITION: | | | | | | | | | Alive | | | |  | | | | Dead | | | | | |  | | | | | Iced | | | |  | | | | Frozen | | |  | | | | Preserved | | | | |  | | | |

Pond/Stream Data

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| DISSOLVED OXYGEN: | | | | | | | | | | | | A.M. | | | | | |  |  | | | P.M. | | | |  | | | pH | |  | | | | NO2 | | | |  | | CO2 | | |  | |
| HARDNESS | | | |  | | | |  | | | | | ALKALINITY | | | | | | | |  | | | | TEMPERATURE | | | | | | | | | |  | | | SALINITY | | | | |  | |
| AMMONIA | | | |  | | | | | |  | | | | | CONDUCTIVITY | | | | | | | | |  | | | OTHER | | | | |  | | | | | | | | | | | | | | |
| POND ACREAGE | | | | | | |  | | | | |  | | | | STREAM FLOW | | | | | | | | | |  | | | COLOR | | | | |  | | |
| OTHER | |  | | | |  | | | | |  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | |  | | |  | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Clear |  | | Cloudy | | | | | |  | | | | |  | | | Calm | | |  | | | Windy | | | | |  | | Rain | | |  | | | Temp | | | |  | |

Mortality Data

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| TOTAL NUMBER OF DEAD | |  | DURATION OF KILL |  |
| REMARKS: |  | | | |
|  | |  | | |
|  | |  | | |
|  | |  | | |
|  | |  | | |
|  | |  | | |

# APPENDIX G. CLINICAL SIGNS

CLINICAL SIGNS\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | BEHAVIOR |  |  |  | HEMORRHAGES | Rate |  |  | GAS BUBBLES | Rate |
|  | Gasping |  |  |  | Fins | / |  |  | Gills | / |
|  | Flashing |  |  |  | Head | / |  |  | Fins | / |
|  | Lethargic |  |  |  | Mouth | / |  |  | Skin | / |
|  | Fin twitching |  |  |  | Eyes | / |  |  |  |  |
|  | Convulsions |  |  |  | Peduncle | / |  |  | PHYSICAL - Internal |  |
|  | In Shallow Water |  |  |  | Ventral | / |  |  | Normal | / |
|  | Around Inflow |  |  |  | Dorsal | / |  |  | Postmortem Change | / |
|  | Around Drain |  |  |  | Lateral | / |  |  |  |  |
|  | Around Aeration |  |  |  | Vent | / |  |  | INTESTINE |  |
|  | Head up-tail down |  |  |  | Cranial Foramen | / |  |  | Normal | / |
|  | Head-tail whirling |  |  |  |  |  |  |  | Hemorrhagic | / |
|  | Pect fins folded forward |  |  |  | HEMORRHAGES Sizes |  |  |  | Flaccid | / |
|  | Anorexia |  |  |  | Petechiae | / |  |  | Gas | / |
|  | Belly up |  |  |  | Eccymoses | / |  |  | Mucus | / |
|  | Loss of Balance |  |  |  | Suffusion | / |  |  | Feces | / |
|  | Other |  |  |  |  |  |  |  | Fluid | / |
|  |  |  |  |  | ULCER Location |  |  |  | Intussusception | / |
|  | PHYSICAL External | Rate |  |  | Fins | Rate |  |  |  |  |
|  | Normal | / |  |  | Head | / |  |  | STOMACH |  |
|  | Emaciated | / |  |  | Eyes | / |  |  | Normal | / |
|  | Depigmented | / |  |  | Mouth | / |  |  | Hemorrhagic | / |
|  | Hyperpigmented | / |  |  | Pedunclt | / |  |  | Gas | / |
|  | Exophthalmia | / |  |  | Ventral | / |  |  | Mucus | / |
|  | Endophthalmia | / |  |  | Dorsal | / |  |  | Food | / |
|  | Swollen Belly | / |  |  | Lateral | / |  |  | Fluid | / |
|  | Scoliosis & Lordosis | / |  |  |  |  |  |  |  |  |
|  |  |  |  |  | ULCER — Size |  |  |  | KIDNEY |  |
|  | FINS — Eroded |  |  |  | 1.5 mm | / |  |  | Normal | / |
|  | Dorsal | / |  |  | 5-10 mm | / |  |  | Pale | / |
|  | Pectoral | / |  |  | 1.25 cm | / |  |  | Hemorrhagic | / |
|  | Pelvic | / |  |  | 1.2.5 cm | / |  |  | Swollen | / |
|  | Anal | / |  |  | >2.5 cm | / |  |  | Brown | / |
|  | Adipose | / |  |  |  |  |  |  | Soft | / |
|  | Caudal | / |  |  | ULCER — Shape |  |  |  |  |  |
|  |  |  |  |  | Irregular | / |  |  | LIVER |  |
|  | LESION — Shape |  |  |  | Regular | / |  |  | Normal | / |
|  | Irregular | / |  |  |  |  |  |  | Pale | / |
|  | Regular | / |  |  | ULCER — Appearance |  |  |  | Hemorrhagic | / |
|  |  |  |  |  | Clean | / |  |  | Brown | / |
|  | LESION Appearance |  |  |  | Dirty | / |  |  | Black | / |
|  | Clean | / |  |  | Yellow | / |  |  | Mottled | / |
|  | Dirty | / |  |  | Red | / |  |  | Brown | / |
|  | Yellow | / |  |  |  |  |  |  | Hemorrhagic | / |
|  | Red | / |  |  | GILLS |  |  |  |  |  |
|  | White | / |  |  | Normal | / |  |  | SPLEEN |  |
|  |  |  |  |  | Pale | / |  |  | Congested | / |
|  | LESION — Location |  |  |  | Brown | / |  |  | Normal | / |
|  | Fins | / |  |  | Cherry Red | / |  |  | Mottled | / |
|  | Head | / |  |  | Necrotic | / |  |  | Brown | / |
|  | Cranial Foramen | / |  |  | Hyperplasis | / |  |  | Hemorrhagic | / |
|  | Eyes | / |  |  | Gas Bubbles | / |  |  |  |  |
|  | Mouth | / |  |  | Aneurisms | / |  |  | SWIM BLADDER |  |
|  | Peduncle | / |  |  | Hyperemia | / |  |  | Normal | / |
|  | Ventral | / |  |  | Cellular Edema | / |  |  | Hemorrhagic | / |
|  | Dorsal | / |  |  | Golden Spherules | / |  |  |  |  |
|  | Lateral | / |  |  | Clubbed | / |  |  | BLOOD |  |
|  |  |  |  |  | Swollen | / |  |  | Normal | / |
|  |  |  |  |  | Puffy | / |  |  | Anemic | / |
|  | LESION — Size |  |  |  | Other | / |  |  | Brown | / |
|  | 1.5 mm | / |  |  | Hamburger Gill | / |  |  | Black | / |
|  | 5-10 mm | / |  |  | Postmortem Change | / |  |  | Cherry Red | / |
|  | 1.25 cm | / |  |  |  |  |  |  | Methemoglobin | / |
|  | 1.2.5 cm | / |  |  |  |  |  |  | Hot | / |
|  | >2.5 cm | / |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  | COELOM |  |
|  |  |  |  |  |  |  |  |  | Ascites | / |
|  |  |  |  |  |  |  |  |  | Cloudy | / |
|  |  |  |  |  |  |  |  |  | Bloody | / |
|  |  |  |  |  |  |  |  |  | Clear | / |
|  |  |  |  |  |  |  |  |  | Gas | / |

|  |
| --- |
| Histology Samples Taken |
| Tissues Taken |

DIAGNOSIS\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | PARASITES |  |  | WATER QUALITY |
|  | Ichthyoboda |  |  | Ammonia |
|  | Ich |  |  | Nitrite |
|  | Chilodon |  |  | Gas Bubble |
|  | Trichodina |  |  | DO Depletion |
|  | Trichophrya |  |  | Thermal Shock |
|  | Ambiphrya |  |  | pH |
|  | Epistylis |  |  | Other\_ |
|  | Henneguya |  |  | Suspected DO Depletion |
|  | Monogenea (Gills) |  |  |  |
|  | Gyrodactytus |  |  | NUTRITIONAL |
|  | Yellow Grub |  |  | A |
|  | White Grub |  |  |  |
|  | Black Grub |  |  | TOXICITY |
|  | Lernaea |  |  | Bluegreen algae |
|  | Other |  |  | Overtreatment |
|  | Bodamonas |  |  | Pesticides |
|  | Apiosoma |  |  | Other |
|  |  |  |  |  |
|  | BACTERIA |  |  | MISCELLANEOUS |
|  | A. hydrophila |  |  | Handling |
|  | A. sobria |  |  | Genetic |
|  | Aeromonas sp. |  |  | Tumors |
|  | Plesiomonas shigelloides |  |  | Crowding |
|  | E. tarda |  |  | Moving |
|  | E. ictaluri |  |  | Inadequate Sample |
|  | Flexibacter external |  |  | Unknown |
|  | Other myxobacteria |  |  | Inspection |
|  | Pseudomonas f. |  |  | Routine Check |
|  | Pseudomonas sp. |  |  | Other\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |
|  | Klebsiella |  |  | Hamburger Gill |
|  | Enterobacter |  |  | Anemia |
|  | Proteus |  |  | Winter Kill |
|  | Unknown |  |  |  |
|  | Other |  |  |  |
|  | Flexibacter internal |  |  |  |
|  |  |  |  |  |
|  | FUNGI |  |  |  |
|  | External |  |  |  |
|  | Systemic |  |  |  |
|  | Brachiomyces |  |  |  |
|  | Other |  |  |  |
|  |  |  |  |  |
|  | VIRUSES |  |  |  |
|  | CCV |  |  |  |
|  | GSV |  |  |  |
|  | Lymphocystis |  |  |  |
|  | Other |  |  |  |

|  |
| --- |
| Histology Results |
| Remarks |