


COMMONWEALTH OF VIRGINIA

Department of Environmental Quality

Subject: Guidance Memo No. **GM23-2006**
Fish Kill Investigation Guidance

To: Water Planning Program Managers, Water Planning Division Director, Water Permitting Division Director, Regional Directors

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Copies: Deputy Regional Directors, Central Operations Director, Regional Operations Director, Policy Director, Director of Regulatory Affairs and Outreach

Summary:

This document provides guidance to conduct investigations of fish kills in support of § 62.1-44.15(11) which empowers the Department of Environmental Quality to investigate any large scale killing of fish and recover the costs of investigations and the replacement costs of dead fish. The document serves as a guide for fish kill investigations and employs procedures that are consistent with the American Fisheries Society published guidelines. The document provides guidance regarding field equipment required for an investigation, field observations and sample collections for locating the source and extent of the kill, fish counting and fish cost assessment guidelines, documentation of expenses for cost recovery. This guidance memo replaces Guidance Memo No. GM02-2002.

Electronic Copy:

Once effective, an electronic copy of this guidance will be available on the Virginia Regulatory Town Hall under the Department of Environmental Quality
<https://townhall.virginia.gov/L/gdocs.cfm?agencynumber=440>

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Certification:

As required by Subsection B of [§ 2.2-4002.1](#) of the APA, the agency certifies that this guidance document conforms to the definition of a guidance document in [§ 2.2-4101](#) of the Code of Virginia.

Disclaimer:

This document is provided as guidance and, as such, sets forth standard operating procedures for the agency. However, it does not mandate or prohibit any particular action not otherwise required or prohibited by law or regulation. If alternative proposals are made, such proposals will be reviewed and accepted or denied based on their technical adequacy and compliance with appropriate laws and regulations.

FISH KILL INVESTIGATION GUIDANCE
VIRGINIA DEPARTMENT OF ENVIRONMENTAL QUALITY
DIVISION OF WATER PLANNING

2024

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Introduction

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](#) (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 6, 7 and 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 8 and 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 Prevention & 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/>), 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.

2. 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](#) 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).

3. 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.

4. 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.

5. 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](#) (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 \(February 2023\)](#) 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.

6. 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 A: 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 A: Clinical Signs may be useful when identifying abnormalities.
- Collect field measurements of water quality (Section 7), water samples (Section 8) and conduct other tests as needed (Section 7).
- Count the number of dead fish using the techniques outlined in Section 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 A). 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](#) 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 11.

7. 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.

8. 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 Water Quality Monitoring Standard Operating Procedures (<https://www.deq.virginia.gov/our-programs/water/water-quality/monitoring>). When necessary, Chain of Custody procedures should follow those outlined in [DEQ Guidance Memo 00-2016](#). 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.

9. 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.

10. 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 \(11\)](#), 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.

10.1. 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.

10.2. 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.

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.

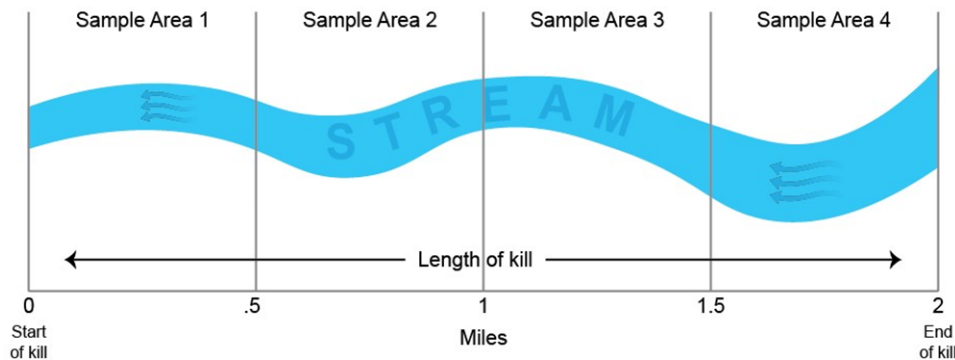


Figure 1. A two-mile fish kill in a stream (represented by blue line) has been divided into four (4) – 1/2-mile sample areas.

3. 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 ($7 \times 110 = 770$). 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).

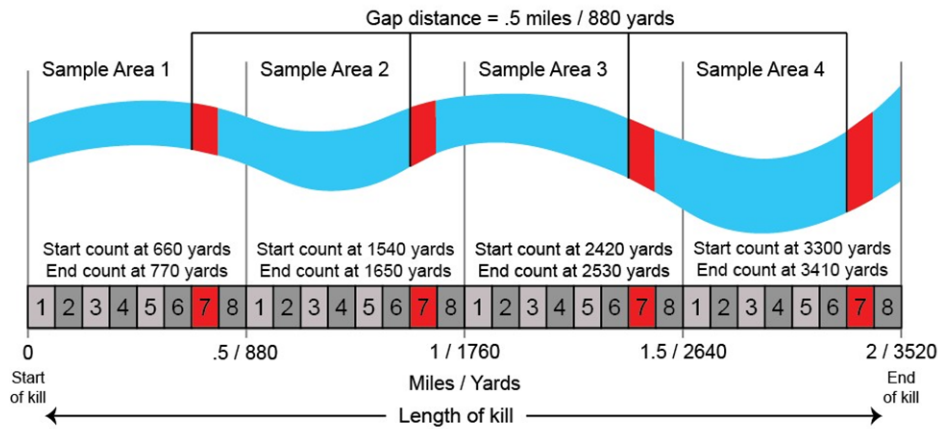


Figure 2. A 2-mile fish kill in a stream (blue line) marked into four 110-yard sample segments in red in each sample area using a random start and the same gap width (0.5 miles or 880 yards).

Identify and count killed fish in each sample segment (red areas in Figure 2) as described in Section 10.

4. **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.

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.
 - a. 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).

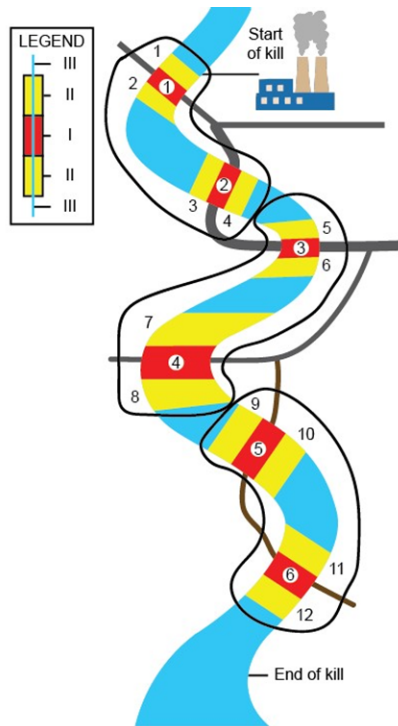


Figure 3. Twelve Stratum II sample segments (yellow) grouped into 3 possible systematic samples of four items each based on assumption that available staff can sample 3 Stratum II segments.

- b. 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.
- c. 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.
- d. 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.

$$\text{Expansion factor } E = L_{\text{total}} / L$$

Where:

L_{total} = 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.

- e. 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 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 $\frac{1}{2}$ 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 $\frac{1}{2}$ -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 $\frac{1}{2}$ 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 $2\frac{1}{2}$ miles apart. For large fish kills, as many as six to twelve field staff may be involved in counts if the $\frac{1}{2}$ 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.

10.3. 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 ($1500 \text{ yd} / 4 = 375 \text{ yd}$).
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 ($375 \text{ yd} / 5 \text{ yd} = 75$). 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 $38 * 5 \text{ yd} = 190 \text{ yd}$ 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.

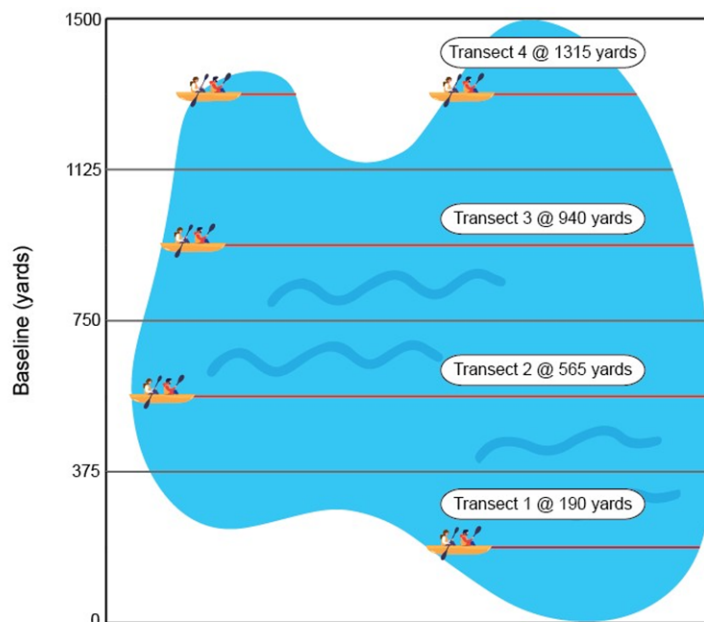


Figure 4. Fish Kill in an impoundment with a baseline = 1500 yards. Four sample area are shown (grey line and 4 sample transects 5 yards wide are shown (red line). Transects are sampled by boat.

6. 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).
7. 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.
8. See section 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.

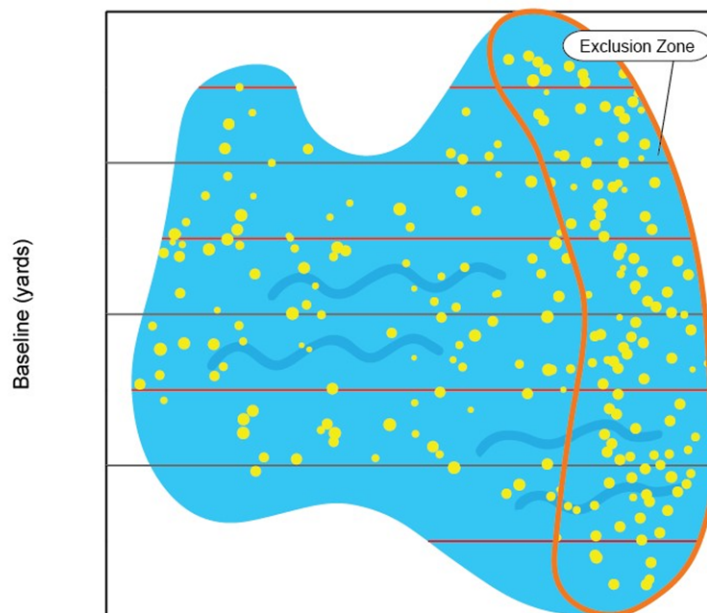


Figure 5. Exclusion zone established in an impoundment due to clumping of fish carcasses (yellow dots) on shoreline due to wind and/or water currents.

10.4. 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.

10.5. 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.

11. 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](#) 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](#) of mussel kills, particularly if federal threatened or endangered species may have been impacted.

12. 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:

$$\text{Mean number} = 240 \text{ fish (total count)} / 4 \text{ segments} = 60 \text{ fish per segment}$$

$$\text{In 2 miles or 3520 yd, there are } 32 - 110 \text{ yd segments } (3520 \text{ yd}/110 \text{ yd} = 32 \text{ yd})$$

thus:

$$\begin{aligned} \text{Total fish killed} &= 60 \text{ fish per segment} \times 32 \text{ segments} \\ &= \underline{1920} \text{ 2-inch Bluegills estimated killed} \end{aligned}$$

- (b) Using an Expansion factor for narrow stream:

$$\text{Expansion Factor} = \text{Total Length of fish kill} / \text{Sum of the Lengths of transects}$$

$$= (1,760 \text{ yd/mile} \times 2.0 \text{ miles}) / (4 \text{ segments} * 110 \text{ yd/segment}) = 8$$

thus:

$$\begin{aligned} \text{Total fish killed} &= 240 \text{ fish (total count)} * 8 \text{ (expansion factor)} \\ &= \underline{1920} \text{ 2-inch Bluegills killed.} \end{aligned}$$

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 ($E_{\text{Stratum I}}$) =
Total length of stream in Stratum I / Total Length of Stratum I counted
= 600 yd / 600 yd = 1

Note: in this case the $E_{\text{Stratum I}}$ 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 ($E_{\text{Stratum II+III}}$) =
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) =
 $E_{\text{Stratum I}} * \text{Total fish in Stratum I segments} + E_{\text{Stratum II+III}} * \text{Total fish in Stratum II segments}$
= 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

13. 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.

14. 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.

15. References

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APPENDIX A. CLINICAL SIGNS

CLINICAL SIGNS

BEHAVIOR		HEMORRHAGES		GAS BUBBLES	
<input type="checkbox"/>	Gasping	<input type="checkbox"/>	Rate /	<input type="checkbox"/>	Rate /
<input type="checkbox"/>	Flashing	<input type="checkbox"/>	Head /	<input type="checkbox"/>	Gills /
<input type="checkbox"/>	Lethargic	<input type="checkbox"/>	Mouth /	<input type="checkbox"/>	Fins /
<input type="checkbox"/>	Fin twitching	<input type="checkbox"/>	Eyes /	<input type="checkbox"/>	Skin /
<input type="checkbox"/>	Convulsions	<input type="checkbox"/>	Peduncle /	PHYSICAL - Internal	
<input type="checkbox"/>	In Shallow Water	<input type="checkbox"/>	Ventral /	<input type="checkbox"/>	Normal /
<input type="checkbox"/>	Around Inflow	<input type="checkbox"/>	Dorsal /	<input type="checkbox"/>	Postmortem Change /
<input type="checkbox"/>	Around Drain	<input type="checkbox"/>	Lateral /	INTESTINE	
<input type="checkbox"/>	Around Aeration	<input type="checkbox"/>	Vent /	<input type="checkbox"/>	Normal /
<input type="checkbox"/>	Head up-tail down	<input type="checkbox"/>	Cranial Foramen /	<input type="checkbox"/>	Hemorrhagic /
<input type="checkbox"/>	Head-tail whirling	HEMORRHAGES Size		<input type="checkbox"/>	Flaccid /
<input type="checkbox"/>	Pect fins folded forward	<input type="checkbox"/>	Petechiae /	<input type="checkbox"/>	Gas /
<input type="checkbox"/>	Anorexia	<input type="checkbox"/>	Eccymoses /	<input type="checkbox"/>	Mucus /
<input type="checkbox"/>	Belly up	<input type="checkbox"/>	Suffusion /	<input type="checkbox"/>	Feces /
<input type="checkbox"/>	Loss of Balance	ULCER Location		<input type="checkbox"/>	Fluid /
<input type="checkbox"/>	Other	<input type="checkbox"/>	Rate	<input type="checkbox"/>	Intussusception /
PHYSICAL External		<input type="checkbox"/>	Fins	STOMACH	
<input type="checkbox"/>	Normal	<input type="checkbox"/>	Rate /	<input type="checkbox"/>	Normal /
<input type="checkbox"/>	Emaciated	<input type="checkbox"/>	Head /	<input type="checkbox"/>	Hemorrhagic /
<input type="checkbox"/>	Depigmented	<input type="checkbox"/>	Eyes /	<input type="checkbox"/>	Gas /
<input type="checkbox"/>	Hyperpigmented	<input type="checkbox"/>	Mouth /	<input type="checkbox"/>	Mucus /
<input type="checkbox"/>	Exophthalmia	<input type="checkbox"/>	Peduncle /	<input type="checkbox"/>	Food /
<input type="checkbox"/>	Endophthalmia	<input type="checkbox"/>	Ventral /	<input type="checkbox"/>	Fluid /
<input type="checkbox"/>	Swollen Belly	<input type="checkbox"/>	Dorsal /	KIDNEY	
<input type="checkbox"/>	Scoliosis & Lordosis	<input type="checkbox"/>	Lateral /	<input type="checkbox"/>	Normal /
FINS — Eroded		ULCER — Size		<input type="checkbox"/>	Pale /
<input type="checkbox"/>	Dorsal	<input type="checkbox"/>	1.5 mm /	<input type="checkbox"/>	Hemorrhagic /
<input type="checkbox"/>	Pectoral	<input type="checkbox"/>	5-10 mm /	<input type="checkbox"/>	Swollen /
<input type="checkbox"/>	Pelvic	<input type="checkbox"/>	1.25 cm /	<input type="checkbox"/>	Brown /
<input type="checkbox"/>	Anal	<input type="checkbox"/>	1.25 cm /	<input type="checkbox"/>	Soft /
<input type="checkbox"/>	Adipose	<input type="checkbox"/>	>2.5 cm /	LIVER	
<input type="checkbox"/>	Caudal	ULCER — Shape		<input type="checkbox"/>	Normal /
LESION — Shape		<input type="checkbox"/>	Irregular /	<input type="checkbox"/>	Pale /
<input type="checkbox"/>	Irregular	<input type="checkbox"/>	Regular /	<input type="checkbox"/>	Hemorrhagic /
<input type="checkbox"/>	Regular	ULCER — Appearance		<input type="checkbox"/>	Brown /
LESION Appearance		<input type="checkbox"/>	Clean /	<input type="checkbox"/>	Black /
<input type="checkbox"/>	Clean	<input type="checkbox"/>	Dirty /	<input type="checkbox"/>	Mottled /
<input type="checkbox"/>	Dirty	<input type="checkbox"/>	Yellow /	<input type="checkbox"/>	Brown /
<input type="checkbox"/>	Yellow	<input type="checkbox"/>	Red /	<input type="checkbox"/>	Hemorrhagic /
<input type="checkbox"/>	Red	GILLS			

<input type="checkbox"/> White	<u> / </u>
LESION — Location	
<input type="checkbox"/> Fins	<u> / </u>
<input type="checkbox"/> Head	<u> / </u>
<input type="checkbox"/> Cranial Foramen	<u> / </u>
<input type="checkbox"/> Eyes	<u> / </u>
<input type="checkbox"/> Mouth	<u> / </u>
<input type="checkbox"/> Peduncle	<u> / </u>
<input type="checkbox"/> Ventral	<u> / </u>
<input type="checkbox"/> Dorsal	<u> / </u>
<input type="checkbox"/> Lateral	<u> / </u>

LESION — Size	
<input type="checkbox"/> 1.5 mm	<u> / </u>
<input type="checkbox"/> 5-10 mm	<u> / </u>
<input type="checkbox"/> 1.25 cm	<u> / </u>
<input type="checkbox"/> 1.2.5 cm	<u> / </u>
<input type="checkbox"/> >2.5 cm	<u> / </u>

<input type="checkbox"/> Normal	<u> / </u>
<input type="checkbox"/> Pale	<u> / </u>
<input type="checkbox"/> Brown	<u> / </u>
<input type="checkbox"/> Cherry Red	<u> / </u>
<input type="checkbox"/> Necrotic	<u> / </u>
<input type="checkbox"/> Hyperplasia	<u> / </u>
<input type="checkbox"/> Gas Bubbles	<u> / </u>
<input type="checkbox"/> Aneurisms	<u> / </u>
<input type="checkbox"/> Hyperemia	<u> / </u>
<input type="checkbox"/> Cellular Edema	<u> / </u>
<input type="checkbox"/> Golden Spherules	<u> / </u>
<input type="checkbox"/> Clubbed	<u> / </u>
<input type="checkbox"/> Swollen	<u> / </u>
<input type="checkbox"/> Puffy	<u> / </u>
<input type="checkbox"/> Other	<u> / </u>
<input type="checkbox"/> Hamburger Gill	<u> / </u>
<input type="checkbox"/> Postmortem Change	<u> / </u>

SPLEEN	
<input type="checkbox"/> Congested	<u> / </u>
<input type="checkbox"/> Normal	<u> / </u>
<input type="checkbox"/> Mottled	<u> / </u>
<input type="checkbox"/> Brown	<u> / </u>
<input type="checkbox"/> Hemorrhagic	<u> / </u>

SWIM BLADDER	
<input type="checkbox"/> Normal	<u> / </u>
<input type="checkbox"/> Hemorrhagic	<u> / </u>

BLOOD	
<input type="checkbox"/> Normal	<u> / </u>
<input type="checkbox"/> Anemic	<u> / </u>
<input type="checkbox"/> Brown	<u> / </u>
<input type="checkbox"/> Black	<u> / </u>
<input type="checkbox"/> Cherry Red	<u> / </u>
<input type="checkbox"/> Methemoglobin	<u> / </u>
<input type="checkbox"/> Hot	<u> / </u>

COELOM	
<input type="checkbox"/> Ascites	<u> / </u>
<input type="checkbox"/> Cloudy	<u> / </u>
<input type="checkbox"/> Bloody	<u> / </u>
<input type="checkbox"/> Clear	<u> / </u>
<input type="checkbox"/> Gas	<u> / </u>

Histology Samples Taken
Tissues Taken

DIAGNOSIS

PARASITES

<input type="checkbox"/> Ichthyoboda
<input type="checkbox"/> Ich
<input type="checkbox"/> Chilodon
<input type="checkbox"/> Trichodina
<input type="checkbox"/> Trichophrya
<input type="checkbox"/> Ambiphrya
<input type="checkbox"/> Epistylis
<input type="checkbox"/> Henneguya

WATER QUALITY

<input type="checkbox"/> Ammonia
<input type="checkbox"/> Nitrite
<input type="checkbox"/> Gas Bubble
<input type="checkbox"/> DO Depletion
<input type="checkbox"/> Thermal Shock
<input type="checkbox"/> pH
<input type="checkbox"/> Other_
<input type="checkbox"/> Suspected DO Depletion

- Monogenea (Gills)
- Gyrodactylus
- Yellow Grub
- White Grub
- Black Grub
- Lernaea
- Other
- Bodamonas
- Apiosoma

- _____
- NUTRITIONAL
- A

- TOXICITY
- Bluegreen algae
 - Overtreatment
 - Pesticides
 - Other

- BACTERIA
- A. hydrophila
 - A. sobria
 - Aeromonas sp.
 - Plesiomonas shigelloides
 - E. tarda
 - E. ictaluri
 - Flexibacter external
 - Other myxobacteria
 - Pseudomonas f.
 - Pseudomonas sp.
 - Klebsiella
 - Enterobacter
 - Proteus
 - Unknown
 - Other
 - Flexibacter internal

- MISCELLANEOUS
- Handling
 - Genetic
 - Tumors
 - Crowding
 - Moving
 - Inadequate Sample
 - Unknown
 - Inspection
 - Routine Check
 - Other _____
 - Hamburger Gill
 - Anemia
 - Winter Kill

- FUNGI
- External
 - Systemic
 - Brachiomyces
 - Other

- VIRUSES
- CCV
 - GSV
 - Lymphocystis
 - Other

Histology Results
Remarks

APPENDIX B: DEQ FISH KILL COUNT FORM

