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MINNESOTA DEPARTMENT OF NATURAL RESOURCES

Division of Fish and Wildlife

Investigation of Fish Kills in Minnesota Waters

DEPARTMENT OF NATURAL RESOURCES

Investigation of Fish Kills in Minnesota Water

Prepared by

Marilyn Danks
Heidi M. Rantala
Ling Shen
Sean P. Sisler
Al G. Stevens

Minnesota Department of Natural Resources
Section of Fisheries
500 Lafayette Road
Saint Paul, MN 55155

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Introduction

The Department of Natural Resources is charged with conservation and management of the State's natural resources. The Department is interested in determining the cause of a fish kill, determining if there is a responsible party, and if necessary, we are authorized to collect restitution related to fish and wildlife kills. A fish kill could be caused by an environmental factor such as change in temperature, low oxygen, a disease, other type of pathogen, or more direct human causes such as contaminated water discharge or chemical spill. The role of a fish kill investigation is to determine the most likely cause of an observed fish kill and quantify the extent of the fish kill.

This Fish Kill Investigation Manual serves as a supplement to the AFS Special Publication #30 and the USFW "Field Manual for the Investigation of Fish Kills". It provides information that is specific to fish kill investigations in Minnesota including contacts, checklists, fish sampling protocols, water sampling protocols, and relevant forms. A checklist is set up as a reference with page numbers indicating where more detailed instructions can be found. It is important to follow the Fish Kill Investigation Manual so the State has defensible data for potential litigation. If the protocols are not followed, it may weaken the ability of the State to collect restitution. Any deviation from these protocols must be documented, including the reason for the deviation.

Collecting samples in a timely manner is KEY to determining the cause of a fish kill. Aquatic systems are dynamic, and the cause of the fish kill could quite literally be washing away before samples are collected. Dead fish begin to decay immediately and clinical signs of the cause of death rapidly disappear. Area Fishery Offices should be familiar with the Fish Kill Investigation Manual and the other resources mentioned above. Staff should know where the sample kits are so they can respond quickly and efficiently.

State agencies and individual DNR divisions have different regulatory authorities and responsibilities during a fish kill. The DNR may need to coordinate with other agencies for their expertise during a fish kill investigation. DNR Fish and Wildlife is responsible for managing game and some non-game animals in Minnesota. When a fish kill occurs, the Division of Fish and Wildlife has a responsibility to determine the cause of a fish kill, allowing us to better manage the species. If cause of the fish kill is natural, the DNR needs to determine how the natural system could be modified to prevent future fish kills. If a fish kill is determined to be related to human activities that portion of the investigation will be turned over to the Division of Enforcement to determine if any natural resource regulations have been violated (DNR Operation Order 21). The DNR will also coordinate with other governmental agencies when appropriate to facilitate a thorough investigation.

Check List:

Prior to Incident (p. 7)

- ☐ Determine when to conduct fish kill investigation
  - o In general, if reported dead fish over 50, multiple species
  - o Any number of valuable game fish such as adult Musky, Sturgeon etc.
  - o Use professional judgment (public concerns, history of the waterbody, etc.)
- ☐ Document all fish kills, regardless of doing a complete investigation
- ☐ Review this document before going out to the field
- ☐ Maintain Field Sampling Kits

What to take to the Field (p.7)

- ☐ Water and Fish Sample Cooler/Kit
- ☐ Forms located in this document (Fish Kill Field Data, Fish Count, Evidence Inventory and Transmittal, etc.)
- ☐ Field Gear, including Personal Protective Equipment (PPE)
- ☐ Dichotomous key (Included in this manual)
- ☐ Camera
- ☐ Optional: Bring a Rugged Tablet

Notifications when investigating a spill/fish kill (p. 8)

- ☐ If fish kill is reported by the public, contact the State Duty Officer **(1-800-422-0798)**.
- ☐ Notify the Statewide Fish Health Consultant (651-259-5213), the Regional Manager, and Ecological and Water Resources Kills/Spills Program Specialist.
- ☐ Notify Area Conservation Officer and copy Enforcement District Supervisor and Area Fisheries Supervisor.
- ☐ If you need an officer to accompany you, contact in this order: 1) Area Conservation Officer > 2) District Supervisor > 3) Regional Captain.
- ☐ Pathology Lab about collecting fish samples (651-259-5096).
- ☐ Water Quality Consultant about collecting water samples.

Field Reconnaissance and Data Collection (p. 8)

The primary function of reconnaissance is to determine the extent of the kill and collect samples. Gather evidence and samples as you conduct the investigation.

- ☐ Water samples should be collected before other activities in the water to avoid contamination. **Do not disturb the sediments while collecting water.**
- ☐ Continually assess the safety of the situation before entering the area.
- ☐ Determine upstream extent of the fish kill (locate the source of spill if applicable).
- ☐ Determine the downstream extent in rivers or the spatial extent in lakes of the fish kill.
- ☐ Collect water and fresh fish samples as soon as possible at each site while determining the extent of the fish kill (making sure to label).

Document Observations (p. 9)

- ☐ Use Fish Kill/ Pollutant Spill Field Data Sheet, Appendix 1.
- ☐ Names and affiliations of field crew.
- ☐ Time and date.
- ☐ Name of waterbody.
- ☐ Weather: current weather as well as previous weather.
- ☐ Water conditions: clarity, water color, water levels, size of stream.
- ☐ Location of Sample Sites: GPS coordinates (preferred), legal descriptions (township, range, and section), major landmarks. Indicate sites investigated on a map.
- ☐ Fish and macroinvertebrate appearance and behavior: signs of illness in animals, gross abnormalities, air gulping behavior, jumping out of water.
- ☐ Other observations: oily or strange colored appearance of water, unusual smells, location of kill relative to tributaries or human activity.

Take photographs and document for each photo (p. 9)

- ☐ Time and date
- ☐ Photographer
- ☐ Location of photo
- ☐ Direction of photo (e.g., facing east)
- ☐ Subject of photo
- ☐ Place something in the photo for scale
- ☐ Take photos of dead fish at multiple scales

Collect water samples and physical measurements (p. 10)

- ☐ Water samples should be collected before other activities in the water to prevent contamination. **Do not disturb the sediments while collecting water**.
- ☐ Use a separate Fish Kill/ Pollutant Spill Field Data sheet at each sampling site.
- ☐ Collect physical chemistry measurement at each sample site: DO, pH, water temperature, chlorine, ammonia, etc.
- ☐ Collect water samples in kill reach and beyond the extent of the kill (upstream of the apparent source works best for background comparisons).
- ☐ If you have an idea of the source of the spill, use appropriate containers for water samples (e.g., glass containers for pesticides or other organic compounds, bacteriological bottles for microbial tests, etc.).
- ☐ Put samples in a cooler on ice and ship to the lab as soon as possible. Some tests require the samples be analyzed within 24 hours.

Collect fish samples and count fish (pp 10-13)

- ☐ Collected fish should be as fresh as possible. Either dying fish or dead fish with reddish color in their gills.
- ☐ Place fish sample in clean plastic bag and into a cooler with ice.
- ☐ Do not freeze fish for disease testing. Frozen fish have limited utility.
- ☐ Deliver to Pathology Lab as soon as possible within 24 hours. Always use overnight delivery. Notify Pathology Lab before sending samples.
- ☐ Use Fish Counting Record to tally the number of dead fish at each site.
- ☐ Start downstream, working upstream, when counting fish. Be careful not to double count floating fish.

Deliver samples to appropriate laboratory(s)

- ☐ Deliver Water Samples to MDA (include Water Sample Submittal Form).
  MDA Lab Services/Sample Receiving 601 N. Robert St. St. Paul, MN 55155-2331 (651-201-6669).
- ☐ Deliver Fish Samples to Lab (include Evidence Inventory and Chain of Custody Form).
  DNR Pathology Lab 500 Lafayette Rd. St. Paul, MN 55155-4025 (651-259-5096).
- ☐ Use a carrier that will ensure the overnight delivery if you ship the samples.

Public Communications Guidance (p. 14)

Reporting (p.15)

- ☐ Draft a Fish Kill Report describing the steps taken to investigate the fish kill including the suspected cause.
- ☐ Send a copy of the Fish Kill Report to the appropriate Regional Office and the Statewide Fish Health Consultant.

Field Data and Sample Collection Protocols

Purpose. The purpose of this guidance document is to outline a set of standard operating procedures for individuals responding to suspected fish kill or other pollution incidents.

It provides:

- a list of suggested field data to be gathered during the investigation
- possible sources for obtaining these data
- proper techniques for the collection and preservation of water and fish samples

Different circumstances that may be encountered in the field may require a departure from these procedures. If this is the case, make note of the fact and document what was done in lieu of the prescribed procedure.

Safety Note: The first question that must be addressed is whether it is **safe** to enter the affected area before proceeding with the investigation. If you notice any noxious odors or see evidence of potentially toxic chemicals present, it may not be safe to enter the area. Examples include empty chemical containers, unusual odors, or discolored water. If there is any question about whether it is safe to proceed, report this information to the State Duty Officer and your supervisor, and await further instructions. Don't trespass on private property; contact a conservation officer for assistance with access.

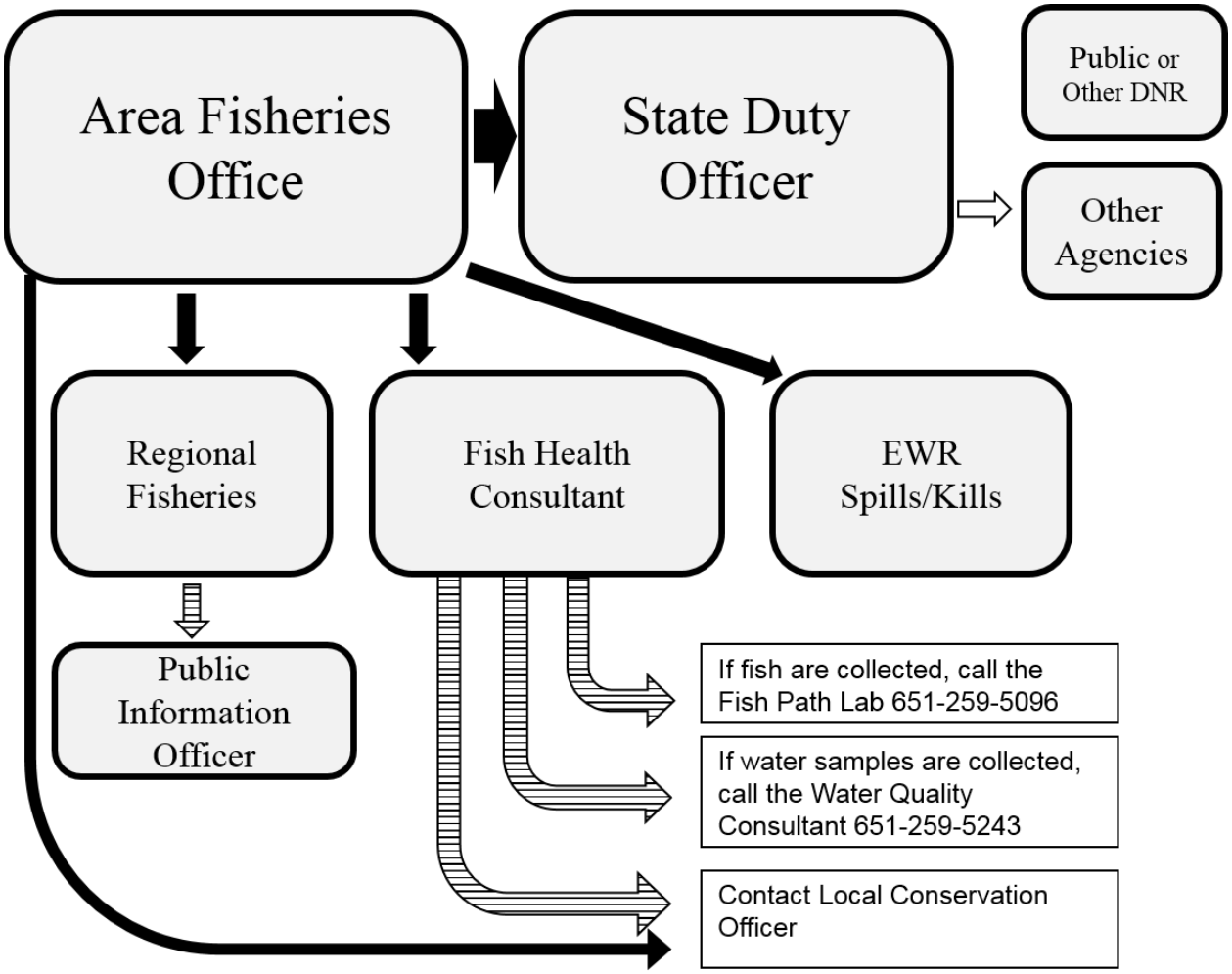
Use personal protective equipment (PPE) in the field. This includes waders or boots, hats, gloves, personal floatation devices, sunscreen, and insect repellant. Take precautions to stay hydrated, and be aware of weather changes.

Notification: The area office is often the first point of contact when a fish kill occurs. Contact the State Duty Officer to report the kill, allowing for notification of other agencies. Contact the Statewide Fish Health Consultant to notify Central Office staff. The Fish Health Consultant will make sure the area has been contacted (if they have not already) and facilitate logistics and communications with other DNR staff and cooperating agencies.

State Duty Officer:
1-800-422-0798 (Minnesota only)
651-649-5451 (Twin Cities metro area and outside Minnesota)

Statewide Fish Health Consultant:
651-259-5213

Initial Notification of a Fish Kill
when the Area Fisheries receives the first information

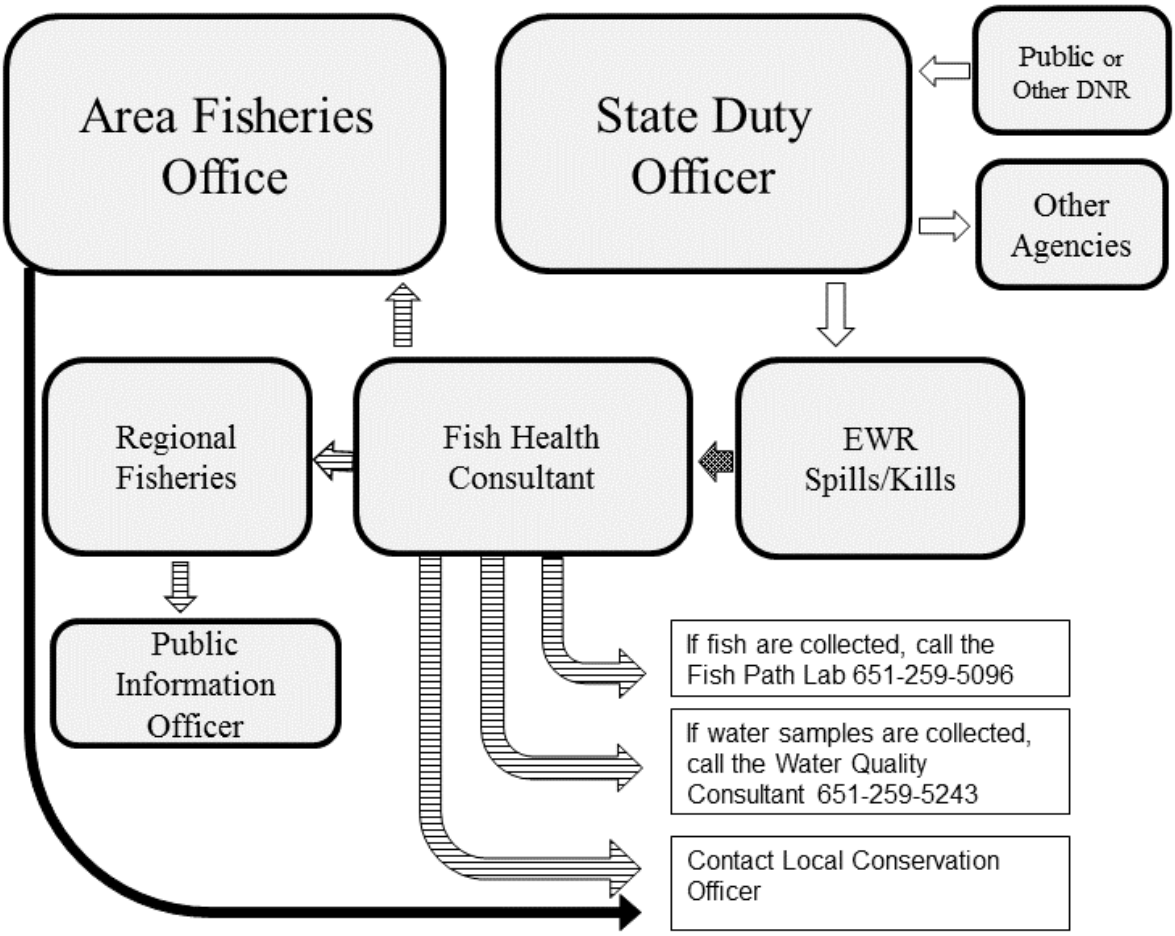


This flow diagram shows the movements of information immediately following a fish kill.

- Fish kills get reported to DNR Fisheries staff.
- Area fisheries staff call the State Duty Officer (if not already informed).
- Area fisheries inform their Region, the Fish Health Consultant, and the EWR Spills/Natural Resource Damage Assessments Program Specialist
- Fish Health Consultant coordinates with Pathology Lab and Water Quality Consultant to prepare for incoming samples

If the Fish Health Consultant is not available, the Area Fisheries Office resumes the role of the Consultant.

Initial Notification of a Fish Kill
when the Duty Officer receives the first information



This flow diagram shows the movements of information immediately following a fish kill.

- Fish kills get reported to the State Duty Officer
- The EWR Spills/Natural Resource Damage Assessments Program Specialist is notified by the DO
- The EWR Spills/Natural Resource Damage Assessments Program Specialist informs the Fish Health Consultant
- Fish Health Consultant informs the area and regional fisheries offices and coordinates with the Pathology Lab and Water Quality Consultant to prepare for incoming samples

If the Fish Health Consultant is not available, the Area Fisheries Office resumes the role of the Consultant.

Monitoring Objectives. If the area can be safely entered, the monitoring objectives include:

- Documentation of the impacts to the affected waterbody.
- Documentation of the extent of the fish kill or pollution.
- Sampling of identified and suspected pollution sources.
- Sampling of an upstream, un-impacted reach, preferably along the same watercourse, for background comparison.

Preparation prior to an incident

Water Sampling Kit. The water sampling kit consists of an insulated cooler which contains:

A) Sample Containers The insulated cooler contains three sets of bottles for the collection of water samples for submission for laboratory analysis. Each bottle set includes the following types of sample containers:
   - Sterile bacteriological bottle – 2 x 100-ml, containing sodium thiosulfate to neutralize chlorine if present
   - General chemistry bottle – 2-liter plastic
   - Pesticide bottle – 2 x 1-liter amber glass

B) Water Chemistry Field Kit For on-site water-quality screening purposes, the kit contains the following items:
   - pH test strips
   - Chlorine color field kit
   - Ammonia field kit

C) Fish Sampling Kit
   - Clean cooler
   - Clean, large plastic bags
   - Ice pack
   - Labels

D) Other Supplies: Evidence Inventory and Transmittal forms, field data sheets, fish count sheets, labels, custody seals, pens, permanent marker, disposable gloves, plastic bags, safety glasses, paper towels, scissors, strapping tape, cooler, ice, field DO, temperature, and pH meters, if available.

Responding to an incident

Communications. Communication with other DNR staff is important so field staff have the support they need and preparations for samples to be received by the laboratories. The diagrams on pages 5 and 6 outline communications.

The Ecological and Water Resources Kills/Spills Program Specialist receives State Duty Officer Reports from the Department of Public Safety and forwards fish kill reports to the Fish Health and Water Quality/Limnology consultants. Duty Officer Reports received by the DNR are stored in the DutyOfficerReports.dnr@state.mn.us email inbox.

Access to Waterbody. You must have legal access to the waterbody. Do not trespass on private property; contact a conservation officer for assistance with access. Legal access points include road right-of-ways, bridge crossings, public lands, and locations where landowner permission has been obtained.

Field Reconnaissance and Data Collection. The following is a list of equipment, resource materials, and informational items which are relevant to fish kill/pollution spill investigations. The list of informational items is not intended to be inclusive, but serves as a reminder of the pertinent field data that should be collected and recorded in your field log books. All data collected in the field should be recorded in pen, as they may become legal evidence.

Use the procedures outlines in DNR Operations Order 113: Invasive Species to prevent the introduction, establishment, and spread of invasive species and to limit their impact. Additionally, label all samples from infested waters as such, to ensure proper disposal.

Equipment/Resource Materials

- Site map(s) – copies of county highway maps, USGS quad maps, Satellite images, or hand-drawn site maps
- GPS unit
- Camera
- Watch
- Polarized sunglasses
- Flashlight or headlamp
- Fish collection equipment. Dip nets, block nets, seines, buckets
- Measuring tape
- Fish measuring board/meter stick
- Plastic bags
- Personal Protective Equipment: waders, gloves, boots, goggles, PFD, etc.
- Cooler and ice

Documentation of background information (Fish Kill/Pollution Spill Field Data sheet)

1) Field staff information-names, affiliations, phone numbers, names of staff from other agencies that respond
2) Location information-coordinates, legal description of site (Township, Range, Section), boundaries of area of kill
3) Current weather conditions – ambient temperature, wind direction and speed, cloud cover, precipitation
4) Recent weather conditions – unusual weather extremes, rainfall events. Potential data sources: local residents, nearby weather recording station, local wastewater treatment facility
5) Water quality characteristics – temperature, pH, DO, residual chlorine, water color, water clarity, algal blooms, and odors associated with the water, visible sheens, scums, or floating material on or in the water column, floating material caught in backwater areas, on dead-fall branches in the water, or on vegetation along the banks. Stream or lake bed characteristics – composition of bed (muck, sand, gravel, stones), “sewage fungus” attached to submerged logs or rocks, odors associated with the sediments, color of the sediment
6) Check for the presence of dead or moribund fish and for aquatic invertebrates under rocks or on submerged logs, branches, or other debris
7) Check for live fish displaying unusual behaviors, such as erratic swimming, air gasping, lethargy, and convulsive behavior
8) Stream flow estimates
9) Aquatic vegetation – emergent, submerged, duckweed
10) Predominant land use in the area
11) Field tiles/discharge pipes – note whether they are flowing, if any odors, if any
12) Identify tributaries to the impacted watercourse, record observations, any water quality tests performed, and estimate flows
13) Identify the number of residences and farms near the impacted watercourse being assessed
14) Keep records of photo documentation, including direction and location of photo. Place an object in photos for scale when deemed appropriate. Take pictures of dead fish, both close up and from a distance with landmarks. Include photos of DNR staff.
15) If you talk to anglers or local residents, collect their contact information.

Notification for the Delivery of Samples

1) Notify Water Quality Consultant of anticipated delivery of spill/kill water samples to the MDA lab. Notify Pathology lab of anticipated delivery of fish samples. (See Checklist with general instructions)
2) Within 24 hours, deliver water samples to the MDA lab and fish samples to the Pathology Lab.
3) When relinquishing samples, have recipient sign (unless sent via courier) and date Evidence Inventory and Transmittal Form. Retain a copy.
4) Contact the Water Quality Consultant for replacement of sample containers and other supplies (all containers are one-time use only).

Water Sample Collection

Select the sample sites that allow you to document the impact and possibly determine the cause of the fish kill. Collect samples first at the sites suspected of greatest impact, as these are of highest priority and should be sampled as early in the process as possible. Un-impacted reference sites should then also be sampled. Background samples can often be collected later in a repeat visit to the site if necessary.

1) Label the sample containers and note the location of sampling point on a site map.
2) Use care to avoid stirring up sediments. It is best to stay out of the stream until you are at the desired sampling site.
3) Collect bacteriological sample first, being careful not to lose sodium thiosulfate from the bottle. Do not rinse bottle before filling. Avoid touching inside of the bottle or cap. If in a stream, point bottle upstream as it fills, only fill to the line. After filling, invert bottle several times to disperse sodium thiosulfate.
4) Nutrient, general chemistry, and pesticide bottles should be rinsed with sample water 3x before collecting water sample. Fill bottles only to the "shoulders".
5) Using the Field Screening Kit, measure water temperature, pH, DO, ammonia, and residual chlorine. Record field data and the time of collection.
6) When sampling has been completed at each site, the set of bottles for that site should be placed in a single bag, sealed, and have an evidence seal placed over the bag. Each site should have a separate Evidence Inventory and Transmittal Form.
7) At completion of all sampling, samples should be carefully packed in cooler and filled with ice. All necessary sample submittal forms should be filled out and placed in cooler in water-tight sealable bags. Seal the cooler with evidence tape. Be sure to sign and date the Evidence Inventory and Transmittal form.
8) Deliver Water Samples to MDA (**including Evidence Inventory and Chain of Custody Form**):

    MDA, Lab Services/Sample Receiving
    601 N. Robert St.
    St. Paul, MN 55155-2331
    Tel. 651-201-6669

Fish Sampling

1) Place ice or freezer packs in a separate cooler prior to collecting any samples. Fish and water samples should be collected during reconnaissance of the incident area.
2) Record the spatial extent of the fish kill.
3) Note the appearance of fish and behavior. Note deformities (lesions, fungi, abnormal color, parasites, etc.) to fish. Be sure to take pictures, as the fish can change over time.

4) Collect fish samples according to references on page 9 of this document.
5) Use a separate datasheet for each sampling site/reach.
6) Fish collected for analysis should be as fresh as possible. Dying/sick fish are preferred. Fish gills should be a dark red to a dark pink in color. The flesh should be firm, not mushy.
7) Fish samples should be handled with gloves to reduce contamination. Fish should be placed in a clean plastic bag with a label and the bag sealed.
8) Collect 5 – 10 fish for analysis by the Pathology Lab. If the fish kill includes multiple species, collect a few of each species.
9) If toxic substances are suspected, a representative series of dying or recently dead fish of affected species should be collected and frozen. If possible, healthy fish of the same species and sizes from the unaffected area should also be collected. Once collected, the whole fish should be placed in plastic bags and frozen as soon as possible. Do not freeze fish if you are uncertain whether the cause is due to toxic substances. **Do not freeze fish samples for disease testing.** To keep samples fresh they should be placed on ice as soon as possible. However, if it is not possible to deliver or mail the fish immediately to the pathology lab due to a weekend, freeze until delivery is possible.
10) Deliver Fish Samples to Path Lab (**Include a signed Evidence Inventory and Chain of Custody form with shipment**)

    DNR Pathology Lab
    500 Lafayette Rd
    St. Paul, MN 55155-4025
    Tel. 651-259-5096

Fish Counts

1) Select the sampling method that is appropriate for the waterbody and number of fish killed.
2) *Methods to count dead fish* (Reference AFS special publication #30 for more details)
   a. Narrow streams, completely accessible
      - Determine the length of stream affected
      - Determine number and location of sampling segments where dead fish will be counted, using at least 3 sample segments.
      - If feasible, sample a 100-yard segment every 0.5 miles in the kill area.
      - Identify (to species) and count all the fish in each segment. Record length, weight, or both for each fish. When there are large numbers of fish to count, you may subsample either the sample segment or subsample the collected fish.
      - Use a separate field Fish Counting Talley Form for each sample segment.
      - Extrapolate the number of fish counted in each segment to the whole length of affected stream (see AFS #30 page 40).

b. Narrow streams, incompletely accessible
   - Determine the length of stream affected.
   - Accesses to these streams are typically at bridge crossings (Stratum I) and include upstream and downstream extensions of this stratum, or include portions of the stream beyond the influence of the bridge (Stratum II). Kill areas can be described by three strata:
     - Stratum I. Reaches of a stream under the immediate influence of road-crossing structures.
     - Stratum II. Accessible portions of the stream beyond the immediate influence of road-crossing structures.
     - Stratum III. The inaccessible remainder of the stream.
   - Set up a stratified sampling plan, sampling at least 3 stream segments of Stratum I type, and Stratum II if available. Measure the length of each segment.
   - Some streams may only have Strata I and III. For those streams where all three strata exist, more of the stream that can be included in Strata II the better the total counts of dead fish throughout the affected reach of stream.
   - Use AFS Special Publication #30 (pages 20-24) to guide your selection of sampling segments (Appendix 7). Narrow streams with drifting fish
   - Fish drift into and out of segments while counts are being made
   - If there are few floating fish, they can be ignored or they can be added to shoreline and stream-bottom counts
   - If floating fish are abundant, keep a separate count.
     - Count a) fish on the bank or stream bottom and b) floating fish.
     - Record time it takes to count and measure fish and whether you are moving upstream or downstream.
     - Then, for an equivalent period of time count all fish drifting past a stationary point.
   - Record information of water clarity to document how visible the drifting fish are.
   - Attempt to use a trap net lead or seine to collect drifting fish that are not visible. Record how long the lead or seine is “fished” for each collection made. Keep data separate for each collection of drifting fish.

c. Lakes
   - As most visible dead fish accumulate along the shore, set up segments of uniform width in the shoreline zone and select a systematic sample of segments, starting at a random point.
   - In open water zone, establish transects of known width from the shoreline to the opposite shoreline.
   - Identify and count all fish in selected segments/ transects.
   - Refer to pages 25-28 in AFS Special Publication 30 for details.

d. Large streams
   - Large rivers may be samples similarly to lakes using segments along shorelines and transects from shoreline to the opposite shoreline. Large meandering rivers may be divided into smaller successive sections and a baseline is established for each. Each section is then sampled independently.
   - Review AFS Special Publication 30 for details (pages 28-31).

e. Multiple day fish counts
   - There are several ways to perform multiple day counts, and often the method needs to be decided at the end of the first day. Multiple day counts can be made at the same location each day or progress downstream depending on the situation.
   - Review AFS Special Publication 30 for details (pages 31-32).

3) Document which sampling regime was used and why. For example, if a stream is only accessed at road crossings, give an explanation for that decision.
4) Start at a downstream segment and move upstream to avoid counting the same fish more than once.
5) Record the species and sizes of fish affected. Lengths of game fish are used to determine restitution, but restitution for non-games species of fish is dependent on lengths for smaller fish and weight for larger individuals. Make sure to measure lengths in order to use the length/weight conversion table

Cessation of the Field Investigation

While the legal components of an individual investigation may continue for months or years, field staff should cease their investigation one week after the last dead fish has been observed in the field, using their professional judgement, or per direction of management.

Subsequent fish kills in the same waterbody may happen shortly after the field portion a fish kill investigation is done. Those events warrant additional investigation and collection of more data.

Weekend Response

For investigations of weekend incidents, the Area Conservation Officer is often the best contact for carrying out inspections in their work areas. If an incident requires an immediate response, State Patrol Dispatch can be contacted to reach the nearest CO. Depending on the resources affected, the various disciplines (Fish & Wildlife, Ecological & Water Resources, Parks & Trails, Lands & Minerals) staff/supervisors would be emailed a copy of the State Duty Officer report and supervisors of various disciplines would be contacted. For fish kill incident reports, the Fisheries Fish Health/Aquaculture and Water Quality/Limnology Consultants would be included on the distribution.

For incidents that do not rise to the level of an emergency, the inspection of the incident can often wait until the next business day. The Area Conservation Officer may not be on call or otherwise available during the weekend so an inspection

might not occur until the following Monday when resource staff return to work. Conservation Officers are not obligated to carry out a request for assistance and should engage on the bases of their schedule and duties as other priorities may take precedent over some incidents.

Public Communications

Fish Kills: Guidance to Fisheries Staff

This document provides guidance to DNR Fisheries staff on releasing information regarding fish kills. Guidelines for releasing general information are given below. Underlined phrases should be customized for individual incidents.

General Statement

The DNR responded to a fish kill on the Waterbody name on Date. Fisheries staff collected Insert fish and/or water samples, which are being analyzed to determine the cause of the fish kill. The incident affected Fish species and other animal species. Biologists estimated that Number fish were killed. The DNR is continuing to collect information about the fish kill.

Example

The DNR responded to a fish kill on the South Branch of the Whitewater River, near Altura on June 30. Fisheries staff collected fish and water samples, which are being analyzed to determine the cause of the fish kill. The incident affected trout and white sucker. Biologists estimated that about 500 fish were killed. The DNR is continuing to collect information about the fish kill.

Example (disease specific)

The DNR responded to a fish kill on Sandy Lake on June 30. Fisheries staff collected fish samples, which are being analyzed to determine the cause of the fish kill. Fish kills related to disease are common this time of the year, as temperature changes and spawning cause stress to the fish. The incident affected yellow perch. Biologists estimated that about 500 fish were killed. The DNR is continuing to collect information about the fish kill.

Responding Further to Open Investigations

During an open investigation (as assigned/determined by general counsel), almost all data are protected nonpublic or confidential until the investigation is closed. Work with DNR Data Practices Officer and the Division's liaison for data practices for releasing additional information to the public.

DNR Data Practices Officer: Sheila Deyo, 651-259-5345
Fish and Wildlife: Grant Wilson, 651-259-5186

Reports

Report forms and requirements are being developed. Refer to attached references for in-depth discussion of fish kill investigations.

Original copies of field information should stay with the area office. A copy of the Fish Kill Report should be sent to the Regional Office and the Fish Health Consultant. Additional copies may be made for other DNR staff upon request. These data are not to made public until the case is officially closed.

Files related to fish kills will be archived on the Fisheries shared drive and will be archived by the Fish Health Consultant.

Revision of this Manual

Revision of this manual should be scheduled to occur on a five-year basis or more often if deemed necessary by DNR Management.

References

All area offices should have the following references on hand:

Fisheries Management Planning Guide for Streams and Rivers. 1993. MN DNR Division Fish and Wildlife, Special Publication No. 148. Appendix A1 provides a general overview of methods for investigating fish kills, a checklist of standard equipment needed for field work, and outlines procedures for estimating numbers of dead fish on narrow or wide streams. Techniques for estimating dead fish in lakes are not covered.

Meyer, F.P., and L.A. Barclay, editors. 1990. Field Manual for the Investigation of Fish Kills. U.S. Dept. of Interior, Fish and Wildlife Service, Resource Publication 177. An excellent source of information on field observations made when investigating fish kills. In particular, it provides tables and a dichotomous key (see Chapters 3 and 4) that can be used to diagnose the scene and aid in identifying likely causes of fish kills.

Minnesota Department of Natural Resources. 2013. Operational Order 113: Invasive Species. This operational order outlines the policy and procedures for MNDNR staff to limit the introduction, spread, and impact of invasive species on Minnesota's resources.

Southwick, R.I., and A.J. Loftus, editors. 2003. Investigation and Monetary Values of Fish and Freshwater Mussel Kills. American Fisheries Society, Special Publication 30, Bethesda, Maryland. This publication provides detailed information on investigating fish kills and pollution incidents and Chapters 1,2,3, and 5 should be reviewed by staff likely to serve as crew leaders on fish kill investigations. In addition to providing methods and procedures for estimating statistically valid numbers of dead aquatic animals in streams and rivers, it also provides methods for fish kills in lakes.

Additional Resources

Knowles, S.J., S. Massarani, and N. Phelps. 2015. Minnesota Fish Kill Investigation Manual. University of Minnesota, College of Veterinary Medicine, Saint Paul, MN.

List of Appendices

DEPARTMENT OF NATURAL RESOURCES

FISH KILL/POLLUTANT SPILL FIELD DATA

Case Title:	**ICR/Case No:**
Data collected by:	**Date &Time:**
Name and Location of water body:	
GPS Coordinates:	
Attached information: ☐ Site map ☐ Photographs ☐ Additional Field Notes ☐ Other (describe):	

BACKGROUND INFORMATION

Weather:

Temperature:	Has there been any notable weather in the past 24-48 hours? If so, describe.
Cloud Cover:	
Precipitation:	

Watershed:

Landuse: ☐ Row Crops ☐Feedlot/CAFO* ☐ Wastewater Plant *Confined Animal Feeding Operation ☐ Pasture ☐ Industry ☐ Construction ☐ Urban ☐ _____	Recent activity in the watershed (pesticide application, manure spreading, accidents, etc.):

FIELD DATA

Water Quality:

Water Temperature:	Other water quality observations (odor, color, algae, turbidity, etc.):
pH:	
DO (ppm):	
Chlorine (ppm):	

Extent of Incident:

Describe the spatial extent of the incident (including GPS coordinates). For streams, include the upstream and downstream extent of the incident. For lakes, include information about locations in the lake dead fish were observed:

Fish Observations:

Describe any observations regarding the number and fish species affected and the behavior or condition of fish (include air gulping, lesions, hemorrhaging, discoloration, etc.):

Other Observations or Information:

Any other info including oily or strange colored appearance of water, unusual smells, location of kill relative to tributaries or human activity:

DEPARTMENT OF NATURAL RESOURCES

FISH COUNTING RECORD

Case Title:	**ICR/Case No:**
Data collected by:	**Date &Time:**
Name and Location of water body:	
Method used to sample fish (From AFS Special Pub #30):	

Species	**Size Class (inches):**																			***for fish >21", specify length of each individual**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	***21+**

Other Observations or Information:

EVIDENCE INVENTORY AND CHAIN OF CUSTODY

Case Title:	**ICR/Case No:**
Evidence/Property Seized by:	**Date &Time of Seizure:**
Source of Evidence/Property (Person and/or Location)	☐ Seized from: ☐ Received from: ☐ Found at:

LIST OF EVIDENCE		
Item No.	**Quantity**	**Description of Evidence/Property (Include sample numbers and model or serial numbers)**
1		Bacterial sample, 250-ml plastic bottle, preserved
2		Pesticide sample, 1-L amber glass bottle
3		Nutrient sample, 500-ml plastic bottle, preserved
4		General chemistry sample, 2-L plastic bottle
5		Fish (provide detail):

LABORATORY SERVICES
Analyses Requested:
Comments (include observations that may help identify unknown causes of fish kill):
Case Officer Name and Phone Number:

CHAIN OF CUSTODY					
Item No.	**Released By**	**Date/Time**	**Delivery Via: (USPS, in person, other)**	**Received By**	**Date/Time**
Final Disposition of Property:					

DICHOTOMOUS KEY FOR FISH KILL INVESTIGATIONS

Source: F.P. Meyer, and L. A. Barclay Field Manual for the Investigation of Fish Kills. U.S. Dept. of Interior, Fish and Wildlife Service, Resource Publication 177.

After the initial visual inspection of the scene, an investigator can sometimes make preliminary assumptions about the cause of a fish kill. By using a process of elimination based on the evidence at hand, certain types of causes may be highly unlikely. A dichotomous key is provided below as an example of how the thought process might proceed. This key is offered as a tool-not as a definitive reference-for assessing fish kills. Opportunities to use the key to help reach a presumptive conclusion concerning the cause of a fish kill are provided in Chapter 13. Seven case histories are described to help potential investigators test their skill in evaluating the information that became available during the on-site investigation. Although the thought process would be the same for ponds, lakes, streams, and estuaries, most of the examples used in preparing the key were taken from data on fish kills in ponds. In streams, where evidence at the site may be transitory because of the flow, the investigator may have to check downstream to attempt to reconstruct the scene.

1. Kill occurred in less than 24 hours 2
1. Not known when kill occurred, or kill continued for longer than 24 hours 16

2. Kill occurred between midnight and sunrise..... 3
2. Kill occurred at times other than between midnight and sunrise 8

3. Water dark in color, musty odor, or odor of sour cabbage 4
3. Water conditions normal in color and odor 6

4. Some fish alive..... 5
4. All fish dead 16

5. Large fish dead, some small fish alive 6
5. Small fish dead, some large fish alive..... 18

6. Dissolved oxygen less than 2 ppm 7
6. Dissolved oxygen 2 ppm or more 9

7. Algal cells absent or dead if present..... 8
7. Algal cells present and alive 10

8. Dead algal cells abundant.....
.....Oxygen depletion due to enrichment
8. Algal cells absent.....
..... Oxygen depletion due to algicidal substance

9. Kill occurred between 9:00 a.m. and 5:00 p.m. ... 10
9. Kill occurred at other times as well 23

10. pH above 9.0..... 11
10. pH not above 9.0 14

11. Dissolved oxygen high, often saturated, or near saturation 12
11. Dissolved oxygen low or near normal for water temperature recorded 13

12. Heavy bloom of one or more species of blue-green algae .....Toxic algal bloom
12. Heavy bloom of dinoflagellate algae.....
.....Toxic algal bloom

13. Vegetation dead (appears burned) 14
13. Vegetation normal 15

14. Ammonia levels not high, near zero..... 15
14. Ammonia levels high.....
.....Anhydrous ammonia spill

15. pH 6.0 to 7.0 .....Oxygen depletion
15. pH below 6.0 .....Possible lethal low pH or heavy metal poisoning; possible mine drainage

16. Some fish still alive..... 17
16. All fish dead 23

17. Kill size selective..... 18
17. Kill not size selective 25

18. Some small fish alive, large fish dead.....6
18. Small fish dead, some large fish alive..... 19

19. Zooplankton and insects alive..... 7
19. Zooplankton and insects dead 20

20. Algal cells alive 21
20. Algal cells dead or absent.....
..... Toxic herbicidal substance

21. Fish showing convulsive or aberrant behavior 22
21. Fish seemingly normal 24

22. Fins in normal position.....23
22. Pectoral fins of fish thrust to extreme forward position .....Organophosphate pesticide

23. Kill occurred throughout day .....
..... Pesticide poisoning
23. Kill occurred between 9:00 a.m. and 5:00 p.m. .....
..... Toxic algal bloom (see also 11)

Appendix 4: Dichotomous Key for Fish Kill Investigations (continued).

24. Recent temporary major change in water temperature .... Temperature kill (as from shut·down of thermal power generating plant or plant exceeding the allowed ΔT in discharge)
24. Normal seasonal change in water temperature ..... Temperature falls below or exceeds thermal tolerance-e.g., die-off of threadfin shad in cold weather; kill usually restricted to one species

25. Species selectivity evident 26
25. No species selectivity evident .... Very high level of a toxic substance

26. Lesions evident on fish 27
26. No lesions on fish ....Low toxicity or low concentration of toxic substance (see also 23)

27. Organisms in lesions visible to naked eye 28
27. No organisms visible 29

28. Organisms wormlike, attached to external surface of fish .... Leeches (not a cause of death)
28. Organisms resemble copepods or have jointed body parts ....Parasitic copepods or isopods (known to kill fish)

29. Lesions not hemorrhagic 30
29. Lesions hemorrhagic .... Possible bacterial or viral cause

30. Lesions as small discrete bodies or masses in tissues..... 31
30. Lesions appear as gray, yellow, or white areas on body .....Bacterial or fungal cause

31. Lesion or mass filled with cellular material ....Cysts caused by sporozoans, protozoans (such as lchthyophthirius) , or helminths
31. Lesion or mass filled with gas..... 32

32. Bubbles of gas present in gills, fins, and behind eyes .... Gas bubble disease, due to supersaturation with a gas
32. Odorous gas in large bubbles in necrotic lesions..... .....Bacterial disease caused by *Edwardsiella tarda*

Appendix 5: Diagram of Sampling Strata from AFS Special Publication 30, page 23.

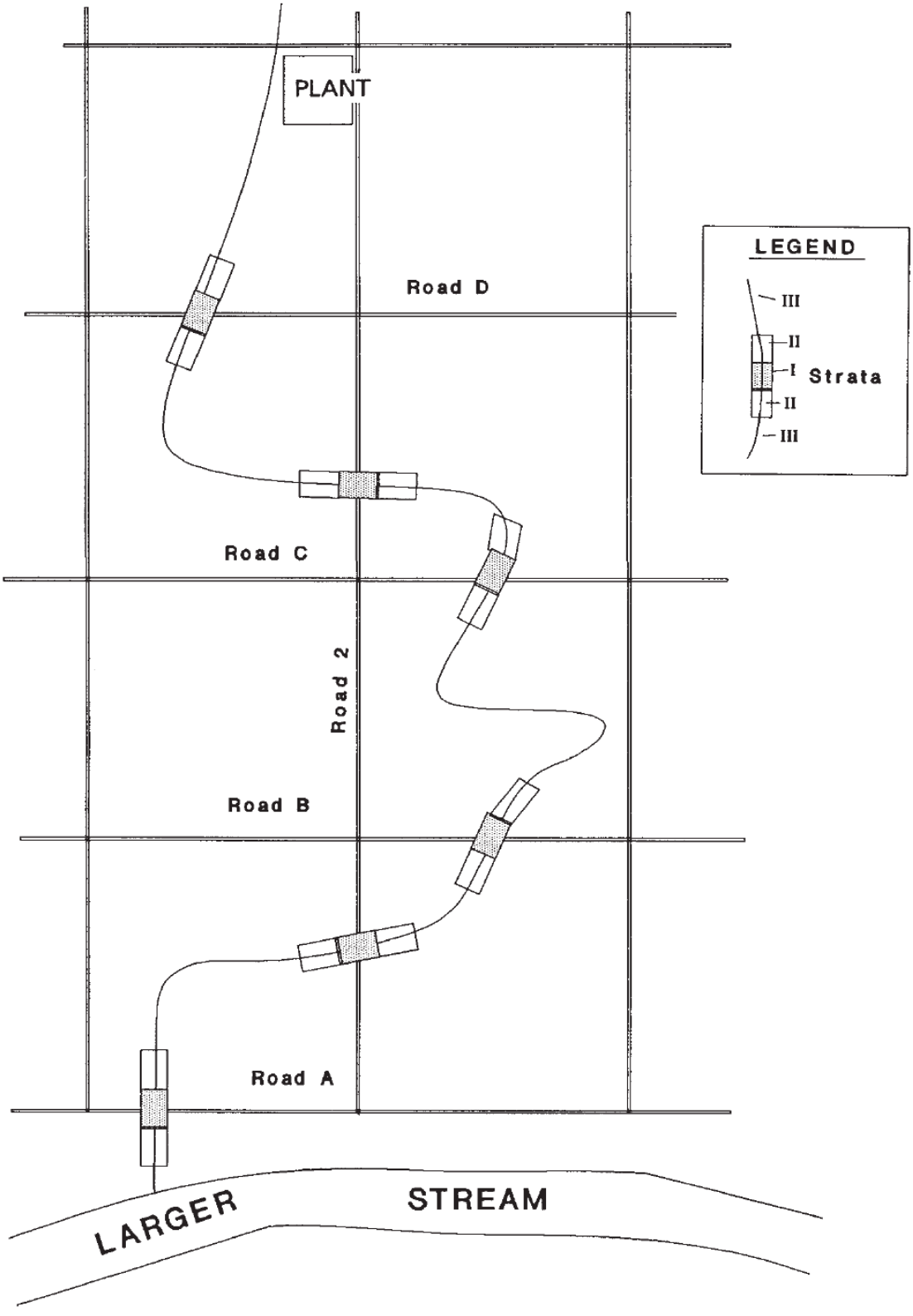


FIGURE 2.1. Sampling strata for an incompletely accessible stream.

Chapter 4. Causes of Fish Kills in Minnesota

Fish kill events are typically caused by a variety of factors working in conjunction. Often, stressful environmental conditions or biological life events can reduce the immune function of fish leaving them more susceptible to secondary infectious pathogens and death. However, one stressful environmental event or an infectious disease outbreak may be the primary cause of the fish kill. Detailed information on environmental, chemical, infectious and biological causes of fish kills in Minnesota and confirmatory testing methods are provided in the following sections (4.A.-4.D.), respectively.

4.A. Environmental Causes

GENERAL DIFFERENTIALS:

- Environmental changes within a body of water induce stress upon fish. These stressors can increase the susceptibility of fish to secondary pathogens or they can be great enough to be the primary cause of death.
- **Generally it is not specific to a single species of fish**.

GENERAL TESTS:

- **Ideally, visual observation and collection of environmental data and samples should be conducted during or right after death of the fish.**
- **Weather conditions** at the time of the kill should be recorded: temperature, cloud cover, precipitation and wind.
- The **location should be surveyed for land use and potential runoff** (i.e. agricultural, industrial, municipal and transportation use).
- **Water quality** must be collected on-site: temperature, pH, dissolved oxygen (DO), ammonia, turbidity, color, and odor. Measurements should be taken at the site of the kill and another site lacking dead fish. Water samples can be collected for further lab testing.
- **Fish should be observed and physically examined on-site**. Behavior, lesions and other abnormalities should be recorded.
- **If the fish cannot be examined on-site**, wrap each fish individually in plastic bags and store them on wet ice. Do not freeze the fish or put them on dry ice. Any collected tissue samples should be placed into sterile, sealed containers with 10% buffered formalin. Transport the fish and any other samples to the lab as soon as possible.

Appendix 6: Chapter 4 from Knowles et al. (2015).

Table 4.A.: Environmental events that are known to cause or are associated with fish kill events.

4.A.1. Algae bloom	
Environmental Signs	*NON-TOXIC OR TOXIC:*
	Single species of alga present in large numbers
	Low DO levels and corresponding signs post-event
	Season: Summer-fall; periods of warm, sunny weather
	Heavy precipitation resulting in runoff from developed areas
	TOXIC ONLY:
	Diatoms and/or dinoflagellates may also be present in water
	Dead insects, zooplankton, birds and terrestrial animals may also be present
	Event occurs during the day due to photosynthetic activity and toxin production
Fish Affected	Non-specific
Clinical Signs	Clogged or irritated gills
	Increased respiration rate
Tests	*NON-TOXIC or TOXIC:* Algal cell counts from water samples
	TOXIC ONLY: Presence of toxins in collected carcasses

4.A.2. Gas supersaturation, "Gas bubble disease"	
Environmental Signs	Rising water temperatures and/or atmospheric changes in a body of water heavily concentrated with aquatic plants
	Can occur downstream from dams, infrastructure or natural barriers
	Fish move away from cold water into warm: may occur naturally (i.e. cross a thermocline) or may move into warm discharges from power plants, dams and other infrastructures
Fish Affected	Non-specific
Clinical Signs	Bubbles ("gas bubbles") visible around eyes, under skin, and in the fins and capillaries of gills
	Exophthalmia ("pop-eye") can occur without visible bubbles
Tests	Physical examination and microscopy of affected tissues and gills

Appendix 6: Chapter 4 from Knowles et al. (2015).

4.A.3. Low dissolved oxygen (DO)	
Environmental Signs	Fish are dead early in the morning
	DO concentration is low (< 2 ppm)
	Water is "pea-soup" green, brown, gray or black in color
	Temp: High water temperature
	Season: Summer + calm, cloudy, hot weather; Winter + ice and snow cover present, mortalities occur at any time of day
	Prior period of drought
	Low water level
	High mass of aquatic plants, algae or decaying organic material
	Body of water is highly eutrophic from high amount of organic matter
	Heavy rainfall may have increased runoff
	Turnover event brought anoxic water and decaying organic material up from the bottom of the water column
	Zooplankton and insects are dead or dying
Fish Affected	Larger fish and species with high oxygen requirements are affected first
	Fish that normally gulp air at surface or have a superior-oriented mouth are NOT affected
Clinical Signs	Increased respiration rate
	"Gulping" air at surface
Tests	DO probe measurement (in ppm)
***NOTE: A low oxygen event can be easily confused with other events (e.g. chemical spill, algae bloom, etc.)**	

4.A.4. Temperature change		
Environmental Signs	Temp: Prolonged or rapidly elevated or depressed seasonal water temperatures	
	Rapid changes in solar heat, cloud cover and atmospheric temperature	
	Abnormal water movements	
	Rapid precipitation events	
	Changes in thermal discharges from power generation and production industries	
	Alterations in water control structure(s)	
	Commercial fish harvesting and aquaculture presence	
Fish Affected	Specific species may be affected according to their range of temperature tolerance and which specific pathogens are virulent during the event	
Clinical Signs	Decreased respiration rate	Sporadic periods of hyperactivity
	Lethargy	Hyper or reduced response to stimuli
	Loss of equilibrium	Reduced predator evasion
	Uncoordinated swimming	Secondary infection or infestation
Tests	Temperature measurement with probe or thermometer	

4.B. CHEMICAL CAUSES

GENERAL DIFFERENTIALS:

- Chemical agents (e.g. toxins, pollutants) can cause a variety of effects on a body of water and the organisms it supports. The chemical may or may not be visible to the eye. Effects are typically non-characteristic to the specific chemical. As well, organisms can be acutely or chronically affected making it difficult but important to thoroughly investigate these kill events.
- **Small fish are most susceptible but all size classes may be affected**.
- **Multiple species** will be affected.
- **Acute effects are severe and cause rapid, large-scale mortality events** due to the short-term exposure of a highly concentrated or toxic chemical.
- **Chronic, sublethal chemicals or concentrations** are usually seen as bioaccumulation and bio-magnification in fish. Signs of toxicosis or death are generally **seen during periods of cold water temperatures or stressful events** due to the toxins being released into the bloodstream from the fish's fat stores. **Mortality rates are slower but may be continuous.**

GENERAL TESTS:

- **Water quality** must be collected on-site: temperature, pH, dissolved oxygen (DO), ammonia, turbidity, color, and odor. Measurements should be taken at the site of the kill and another site lacking dead fish. Water samples can be collected for further lab testing.
- The **location should be surveyed for land use and potential runoff** (i.e. agricultural, industrial, municipal and transportation use).
- **Physical examination and observation** of freshly dead or dying fish should be conducted on-site. Behavior, lesions and other abnormalities should be recorded.
- **Skin scrapes, fin clips and gill clips** of these fish should be collected, examined on wet mounts and compared against those from healthy appearing fish.
- **Necropsy** can be conducted on-site or in the lab to investigate for internal lesions and pathogens. **Tissues of significance to collect and examine include gills, blood and brain**.
- **If the fish cannot be examined on-site**, wrap each fish individually in plastic bags and store them on wet ice. Do not freeze the fish or put them on dry ice. Collected tissue samples should be placed into sterile, sealed containers with 10% buffered formalin. Transport the fish and any other samples to the lab as soon as possible.
- **Sediment and animal carcasses** can also be collected from the kill site and a reference site to be tested in lab for chemical residues. Samples should be transported in sterile, sealed containers and carcasses should be individually wrapped in plastic bags and stored on ice. Do not freeze.

Table 4.B.1.: Clinical signs seen in fish affected by chemical toxins and pollutants.

Target	*Clinical Signs*
Behavior	Lethargy, weakness
	Increased respiration rate
	Erratic, uncoordinated swimming
	Rapid, jerky movements and tremors
	Loss of equilibrium
	Increased sensitivity to stimuli
Gills	Clogged
	Covered in white film
	Sloughing of epithelium
	Bright red or dark in color
	Hemorrhage
	Distended opercula
Mouth	Covered in white film
Skin	Covered in white film
Internal Viscera	Hemorrhage
	Blood clots
	Blue stomach
Blood	Bright red in color
	Brown in color

Table 4.B.2.: Characteristic effects of chemical agents on algae, plant, insect and invertebrate populations in a body of water.

Chemical Agent	*Organisms*	
	Dead or Dying	*Alive*
Herbicide	Algae	Insects
Insecticide	Insects, Invertebrates	Algae, Aquatic plants
Acid, Heavy metals, Other highly toxic substances	Algae, Aquatic plants, Insects, Invertebrates	None

4.C. INFECTIOUS CAUSES

GENERAL DIFFERENTIALS:

- Fish kills involving bacteria, viruses, fungi, or parasites usually occur when a fish population is or has been under some other environmental or biological stressor(s). The spread of the disease is typically a secondary effect.
- A **single fish species or family** is generally affected.
- **Small fish** are most susceptible.

GENERAL TESTS:

- **Physical examination and observation** of freshly dead or dying fish should be conducted. Behavior, lesions and other abnormalities should be recorded.
- **Skin scrapes, fin clips and gill clips** of these fish should be collected, examined on wet mounts and compared against those from healthy appearing fish.
- **Necropsy** can be conducted on-site or in the lab to investigate for internal lesions and pathogens. Tissues suspected of or displaying infection or infestation should be collected for further examination and testing.
- **For transport to lab**, wrap each fish individually in plastic bags and store them on wet ice. Do not freeze the fish or put them on dry ice. Any collected tissues should be placed into sterile, sealed containers with 10% buffered formalin. Transport the fish and any other samples to the lab as soon as possible.

4.C.1. Bacteria

GENERAL DIFFERENTIALS:

- Most bacterial diseases are due to secondary opportunistic infections following environmental stressors, parasitic infestations, other pathogens, and trauma induced injuries. Typically, the stressful event occurred 10 to 14 days prior to the start of the fish kill.
- Many bacterial infections cause similar clinical signs including skin erosion or ulceration (Figure 4.C.1.a.). A key characteristic of ulcers is hemorrhagic or bloody edges of the lesion (Figure 4.C.1.b.).

Figure 4.C.1.a.: Skin ulceration on the dorsal caudal peduncle.

Figure 4.C.1.b.: Skin ulceration on the left lateral side of the body. Note the hemorrhagic edges.

ADDITIONAL TESTS:

- **Skin scrapes** should be taken from the edge of external lesions. Examine by wet mount observation and gram staining.
- **Material from the edge of lesions, infected gills or internal organs should be collected using separate sterile swabs and streaked onto appropriate culture plates**. An unaffected internal area, or control location, should be swabbed separately and streaked onto culture plates. Following lab culture and isolation, identification tests should be conducted.

Table 4.C.1.: Specific bacterial agents that are known to cause or are associated with fish kill events.

4.C.1.a. *Aeromonas hydrophila* and complex, "Bacterial hemorrhagic septicemia"		
Environmental Signs	Temp: ≥ 10°C	
	Season: Winter-Spring	
	Current or previous stressful conditions, especially low but rapidly increasing water temperature, reduced winter feeding, nutritional deficiencies	
	High concentrations of organic material in water	
Fish Affected	Species: All freshwater species, some more susceptible	
Clinical Signs	Hemorrhage on body, fins, gills, vent, and internal organs	Distended abdomen
	Inflammation and erosion of mouth	Blood-tinged fluid in body cavity
	Exophthalmia ("pop-eye")	Soft and swollen kidney
	Ulcers	Enlarged spleen
	Abscesses	Lethargy
Samples to Collect	Kidney + other affected internal organs	
	Fin clips and skin scrapes	
Tests	Kidney: Bacterial culture and isolation + identification via biochemical tests	
***CAUTION: Bacterium is pathogenic to other cold-blooded vertebrates (i.e. frogs, turtles, reptiles) and mammals including immunocompromised or wounded humans.**		

Appendix 6: Chapter 4 from Knowles et al. (2015).

4.C.1.b. *Aeromonas salmonicida*, "Furunculosis"		
Environmental Signs	Temp: Cold water 8-20°C; may see with higher water temperature + low DO	
	Season: Follows seasonal temperature patterns	
Fish Affected	Species: Freshwater including trout, salmon, minnows, carp, goldfish, perch, chub, pike, bullheads and catfish	
Clinical Signs	Darkened skin color	Soft kidney
	Hemorrhage at base of fins	Enlarged spleen
	Ulcerative skin lesions	Pale and mottled liver
	Raised lesions on muscle that resemble boils ("furuncles")	Lethargy
	Hemorrhage of internal organs	
Samples to Collect	Kidney, intestinal material + other affected internal organs	
Tests	Kidney: Bacterial culture and isolation + identification via biochemical or serological tests	

4.C.1.c. *Flavobacterium branchiophila*, "Bacterial gill disease"		
Environmental Signs	Temp: Increasing water temperatures	
	Season: Spring-summer	
	Previous or current stressful conditions	
Fish Affected	Size: Small most susceptible	
	Age: All	
	Species: Includes trout, salmon, walleye, silver carp, rohu, and catla	
Clinical Signs	Pale, swollen and necrotic gills	Lethargy
	Flared opercula	"Gulping" air at surface
	Darkened skin color	Swimming high in water column
	Congested eyes	Slow response to stimuli
Samples to Collect	Gill clips	
Tests	Wet mounts and staining (i.e. gram-stain, simple-stain, etc.)	
	Histology	

Appendix 6: Chapter 4 from Knowles et al. (2015).

4.C.1.d. *Flavobacterium* columnaris, "Columnaris disease"		
Environmental Signs	Temp: Warm water >20°C, may occur at lower temps	
	Season: Seasonal temperature patterns in spring	
	High concentrations of organic matter in body of water	
Fish Affected	Species: Most freshwater fish	
	Age: All, young more susceptible	
	Cold water fish that spawn in springtime	
Clinical Signs	Grayish colored lesions on body or fins	Shallow ulcers
	Brown or yellowish colored gill tissue	Lethargy
	Necrotic skin, fins and/or gills	Swimming near the surface
	Skin lesions, especially those that cross over the back ("saddleback lesions")	
	FISH MAY DIE WITHOUT ANY GROSS CLINCIAL SIGNS	
Samples to Collect	Gill clips and skin scrapes from edges of lesions	
	Kidney and spleen	
Tests	Wet mounts show bacteria that collect into columns ("haystacks")	
	Internal organs: Bacterial culture and isolation + identification via Griffin screen	

4.C.1.e. *Pseudomonas fluorescens*, "Pseudomonas septicemia"		
Environmental Signs	Temp: ≥ 10°C	
	High concentration of organic matter in body of water	
Fish Affected	Species: All freshwater species	
Clinical Signs	Hemorrhage on body, fins and internal organs	Fluid in body cavity
	Inflammation and erosion of mouth	Soft and swollen kidney
	Occasional external skin lesions	Enlarged spleen
	Exophthalmia ("pop-eye")	Lethargy
	Distended abdomen	
Samples to Collect	Kidney + other affected internal organs	
	Fin clips and skin scrapes	
Tests	Kidney: Bacterial culture and isolation + identification via biochemical tests	
***CAUTION: Bacterium is pathogenic to other cold-blooded vertebrates (i.e. frogs, turtles, reptiles) and mammals including immunocompromised or wounded humans.**		

4.C.2. Fungi and Oomycetes

GENERAL DIFFERENTIALS:

- Most of these diseases in fish are secondary opportunistic infections following trauma, disease or death. Fungi and oomycetes can be seen around lesions caused by injuries, bacteria or parasites. However, some species are primary pathogens.
- Many fungi and oomycetes can be **seen by the naked eye as tufts, nodules or other characteristic epithelial lesions** occurring on the external surface of infected fish.

ADDITIONAL TESTS:

- **Gills, brain, internal organs, and kidney** should be examined microscopically on wet mount, squash-prep and/or histology.
- If fungi or oomycetes are observed or suspected a sample can be **cultured in lab then identified** down to species level. Identification can be very difficult and requires advanced taxonomic knowledge

Table 4.C.2.: Specific fungi and oomycetes that are known to cause or are associated with fish kill events.

4.C.2.a. *Branchiomyces* sp., "Branchiomycosis," "Gill rot"		
Environmental Signs	Temp: Highest mortalities at ≥25°C.	
	Body of water is often highly eutrophic with high levels of organic matter	
	Strong association with poor water quality	
Fish Affected	Species: Various freshwater fish	
	May infect a single species or multiple	
Clinical Signs	*DISEASE IS SPECIFIC TO THE GILLS:* Infected areas necrotic and pale or brownish-grey in color	May show signs consistent with oxygen deprivation
	May swim listlessly	
Samples to Collect	Gill clips	
Tests	Wet mounts	
	Histology and staining for species identification	

4.C.2.b. *Saprolegnia* sp. and other Water molds, "Saprolegniasis," "Cotton wool disease"		
Environmental Signs	Temp: Cold water	
	Injuries, malnutrition, temperature shock, external parasitism, and spawning increase susceptibility	
	Also infects warm and cold water insect and amphibian species	
Fish Affected	Species: All freshwater fish	
Clinical Signs	Organism appears as focal white to brownish "cottony tufts" on skin and/or gills	SYSTEMIC INFECTIONS: Masses found in gut and surrounding viscera with hemorrhage, necrosis and adhesions
	Pale foci of lifted scales surrounded by areas of redness, may become ulcerated	SMALLER FISH: Distended abdomen
Samples to Collect	Skin scrapes of infected external surfaces	
	Gill clips	
Tests	Wet mounts	
	Isolation by culture	

4.C.3. Parasites

GENERAL DIFFERENTIALS:

- Many parasitic infestations are not the primary cause of major fish kills but create wounds or act as stressors that render the fish more susceptible to secondary infections or environmental stress.

ADDITIONAL TESTS:

- **Parasites are easiest to detect in freshly dead fish, thus any collected fish or tissues should be examined promptly**. At this time, they will be alive, moving and still attached to their host.
- **Gill clips, fin clips and skin scrapes**, especially taken from behind the fins, should be observed on wet mounts.
- **Histological preparations** of infested tissues can verify presence of the parasite(s).
- Parasites can be generally identified down to the levels of class and order easily, but special techniques and expert knowledge is necessary to classify the genus and species.

4.C.4. Viruses

GENERAL DIFFERENTIALS:

- **Small sized and fish in early life stages** (i.e. fry and fingerlings) are the most susceptible to viral infections.
- **Lesions and signs are not typically characteristic to a specific virus or consistent across host species. Laboratory tests must confirm the virus.**

ADDITIONAL TESTS:

- **Samples to collect vary according to fish size and life stage**. The entire body of small fish (<3 cm) should be collected. All internal organs, including the gills and kidney, of fish between 3 and 6 cm should be taken. Kidney, spleen, heart, brain, and gills should be collected from large fish (>6 cm). Ovaries or ovarian fluid should be collected from sexually mature fish (at least 1 mL).
- Suspected infected tissues can only be **confirmed in the lab by virus isolation in cell cultures followed by identification** using PCR, virus neutralization, molecular techniques, or immunological techniques.

Table 4.C.4.: Specific viral agents that are known to cause fish kill events.

4.C.4.a. Infectious Hematopoietic Necrosis virus		
Environmental Signs	Temp: ≤ 25°C	
Fish Affected	Species: Most of the Salmonidae family (i.e. salmon and trout)	
	Fry and fish in poor health are most susceptible	
Clinical Signs	Darkened skin color	Anorexia
	Pale gills and internal organs	Spinal deformities
	Exophthalmia (“pop-eye”)	Milky white fluid in stomach
	External and internal hemorrhaging, especially at base of the fins	Lethargy with bouts of erratic activity (e.g. spiral swimming, flashing)
	Distended abdomen	Swimming high in water column
	Fluid buildup (i.e. edema), especially in body cavity	Blood shows reduced hematocrit, leukopenia, degeneration of leukocytes and thrombocytes, large amounts of cellular debris
Samples to Collect	Proper tissue samples (according to fish size), especially spleen, kidney, heart and brain	
	Sexually mature fish: Ovaries or ovarian fluid (at least 1mL)	
	Blood	
Tests	Virus isolation in cell culture + identification via PCR, other molecular techniques or immunological techniques.	

Appendix 6: Chapter 4 from Knowles et al. (2015).

4.C.4.b. Infectious Pancreatic Necrosis virus		
Environmental Signs	Temp: Highest mortality 10-14°C	
	Low DO and changes in water temperature	
Fish Affected	Species: Salmonidae family (especially brook, rainbow and brown trout) + many other freshwater fish	
	Age: Fry and fingerlings most susceptible, adults may be affected in stressful conditions	
Clinical Signs	Darkened skin color	Hemorrhage on skin, at the base of and in the fins, and on internal viscera
	Exophthalmia ("pop-eye")	Stomach and intestines filled with mucoid material that may extrude from the vent
	Pale gills, liver and spleen	Erratic swimming (e.g. spiraling or "corkscrew" swimming)
	Distended abdomen	
	SIGNS VARY BY VIRUS STRAIN	
Samples to Collect	Proper tissue samples (according to fish size)	
	Sexually mature fish: Ovaries or ovarian fluid (at least 1mL)	
Tests	Virus isolation in cell culture + identification via PCR, other molecular techniques, or serological techniques	

4.C.4.c. Koi Herpesvirus (KHV)		
Environmental Signs	Temp: Warm water 16-28°C	
	Season: Spring and summer with rapid water temperature shifts from cold to very warm	
Fish Affected	Species: Common carp and varieties (especially koi and ghost carp)	
	Age: All age groups, young most susceptible	
Clinical Signs	Discoloration of skin	Swollen kidney and spleen
	Skin lesions	Lethargy
	Swollen, necrotic, pale and patchy gills	Loss of equilibrium
	Sunken looking eyes	Erratic swimming prior to death
Samples to Collect	Proper tissue samples (according to fish size), especially gill, kidney, spleen, brain and gut	
Tests	Virus isolation in cell culture + identification via PCR	
Pictures		
REPORTABLE DISEASE		

Appendix 6: Chapter 4 from Knowles et al. (2015).

<table>
<tr><td colspan="3">4.C.4.d. Spring Viremia of Carp virus (SVC)</td></tr>
<tr><td rowspan="2">Environmental Signs</td><td colspan="2">Temp: 10-17°C, fry may be affected at temperatures as high as 22-23°C</td></tr>
<tr><td colspan="2">Season: Spring; may occur in fall if temperatures are in appropriate range</td></tr>
<tr><td>Fish Affected</td><td colspan="2">Species: Cyprinidae family (especially carp) + young fish of various species including pike, perch and largemouth bass</td></tr>
<tr><td rowspan="5">Clinical Signs</td><td>Darkened skin color</td><td>Inflammation or fluid buildup of the vent often with mucoid fecal casts</td></tr>
<tr><td>Exophthalmia (“pop-eye”)</td><td>Lethargy and slow reaction to stimuli</td></tr>
<tr><td>Pale gills</td><td>Decreased respiratory and swimming rates</td></tr>
<tr><td>Hemorrhage on the eyes, gills and skin, muscles, fat, and internal organs</td><td>Loss of equilibrium</td></tr>
<tr><td>Distended abdomen</td><td>Fish gather at water inlet or sides of body of water</td></tr>
<tr><td>Samples to Collect</td><td colspan="2">Proper tissue samples (according to fish size), especially kidney and liver</td></tr>
<tr><td>Tests</td><td colspan="2">Virus isolation in cell culture + identification via RT-PCR, virus neutralization, or immunological techniques</td></tr>
<tr><td>Pictures</td><td colspan="2"> 
</td></tr>
<tr><td colspan="3">**REPORTABLE DISEASE**</td></tr>
</table>

4.C.4.e. Viral Hemorrhagic Septicemia virus (VHSV)		
Environmental Signs	Temp: Cold water with highest mortality at 9-12°C, upper limit of 18-20°C	
	Season: Spring	
Fish Affected	Species: Many including muskellunge, walleye, yellow perch, smallmouth bass, rock bass, white bass, black crappie, bluegill, lake whitefish, round goby, gizzard shad, freshwater drum, and common carp	
Clinical Signs	Hemorrhage on the eyes, skin, fins, muscle, and internal organs (kidney, intestines, swim bladder)	Lethargy
	Pale gills and liver	Abnormal and erratic swimming behavior (e.g. flashing, swimming in circles)
	Exophthalmia ("pop-eye")	Swimming near surface
	Distended abdomen due to fluid filled body cavity	ACUTE PHASE: Blood shows anemia and appears light red and transparent
Samples to Collect	Proper tissue samples (according to fish size), especially kidney	
	Sexually mature fish: Ovaries or ovarian fluid (at least 1mL)	
	Blood	
Tests	Virus isolation in cell culture + identification via RT-PCR or real-time RT-PCR, virus neutralization, or immunological techniques	
REPORTABLE DISEASE		

4.D. Biological Causes

GENERAL DIFFERENTIALS:

- Naturally occurring biological events can induce stress upon fish populations. This can cause **weakness, lethargy and poor body condition** in the fish exhausting their immune system and increasing their susceptibility to secondary pathogens. These events often lead to normally occurring mass fish kill events.
- **A single species or age group of fish** will be affected depending on the event.
- **Water quality will be normal**.

GENERAL TESTS:

- **Water quality must be collected on-site**: temperature, pH, dissolved oxygen (DO), ammonia, turbidity, color, and odor. Measurements should be taken at the site of the kill and another site lacking dead fish. Water samples can be collected for further lab testing.
- **Physical examination and observation** of freshly dead or dying fish should be conducted. Behavior, lesions and other abnormalities should be recorded. Tissues or carcasses suspected of having pathogen infections or infestations should be collected and tested accordingly.
- **If the fish cannot be examined on-site**, wrap each fish individually in plastic bags and store them on wet ice. Do not freeze or put them on dry ice. Collected tissue samples should be placed into sterile, sealed containers with 10% buffered formalin. Transport the fish and any other samples to the lab as soon as possible.

Appendix 6: Chapter 4 from Knowles et al. (2015).

Table 4.D.: Common biological events that are known to cause or are associated with fish kill events.

Event	***Spawning***	***Migration***	***Unusual Population Structure and Density***
Environmental Signs	Mass die-offs during fall or spring Water quality is normal, may be cloudy due to presence of eggs and sperm	Mass movement of fish May occur within or outside of normal migration periods or patterns Water quality is normal	High density of fish creating competition can lead to lack of prey or habitat, poor body condition and decreased immunity Water quality is normal
Fish Affected	Sexually mature fish of a specific species (according to natural spawning time and pattern) Multiple species may be affected depending on chronology of fish spawning in a body of water	Specific species	Specific size or age group of a specific species
Clinical Signs	Lethargy, weakness		
	Poor body condition		
	Secondary infections or infestations of pathogens		
Tests	Physical examination of fish		
	Water quality		

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