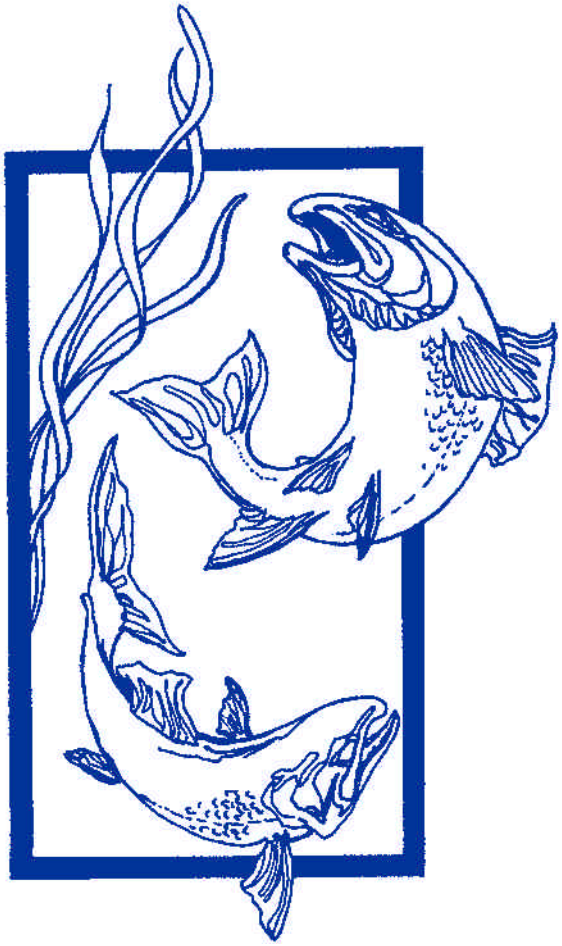
Field Manual

for the

Investigation of Fish Kills



UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service / Resource publication 177

Field Manual
for the
Investigation of Fish Kills

edited by

Fred P. Meyer
U.S. Fish and Wildlife Service
National Fisheries Research Center
P.O. Box 818
La Crosse, Wisconsin 54602

and

Lee A. Barclay
U.S. Fish and Wildlife Service
Division of Environmental Contaminants
18th and C Streets, N.W.
330 Arlington Square Building
Washington, D.C. 20240

1990

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Contributors

Georginia R. Ardinger, U.S. Department of the Interior, Fish and Wildlife Service, National Fisheries Research Center, P.O. Box 818, La Crosse, Wisconsin 54602

Lee A. Barclay, U.S. Department of the Interior, Fish and Wildlife Service, Fish and Wildlife Enhancement, P.O. Box 845, Cookville, Tennessee 38503 (present address)

Bernard L. Berger, U.S. Department of the Interior, Fish and Wildlife Service, Office of Research Support, Region 8, 18th and C Streets, N.W., Arlington Square Building, Washington, D.C. 20240

Susan D. Haseltine, U.S. Department of the Interior, Fish and Wildlife Service, Northern Prairie Wildlife Research Center, Jamestown, North Dakota 58402

Roger L. Herman, U.S. Department of the Interior, Fish and Wildlife Service, National Fish Health Research Laboratory, P.O. Box 700, Kearneysville, West Virginia 25430

Joseph B. Hunn, U.S. Department of the Interior, Fish and Wildlife Service, National Fisheries Contaminant Research Center, 4200 New Haven Road, Columbia, Missouri 65201

Fred P. Meyer, U.S. Department of the Interior, Fish and Wildlife Service, National Fisheries Research Center, P.O. Box 818, La Crosse, Wisconsin 54602

Rosalie A. Schnick, U.S. Department of the Interior, Fish and Wildlife Service, National Fisheries Research Center, P.O. Box 818, La Crosse, Wisconsin 54602

Preface

Fish kills are graphic evidence of serious problems in a lake or stream. If the kill is related to the presence of toxic chemicals, there may be human health concerns, in addition to the obvious damage to the ecosystem and the fisheries resources. Depending on the cause of a fish kill, legal and economic ramifications may be involved. If the kill is caused by human or corporate actions, litigation is likely to follow, with possible court-awarded damages and assessed costs for cleanup and restoration.

Federal and State agencies have expressed the need for a compendium of known and accepted methods and techniques that should be followed by anyone investigating a fish kill. This manual is an attempt to fill that need. It addresses the many facets involved in a fish kill investigation and provides instruction, guidance, examples, and sample forms that can be used.

The U.S. Fish and Wildlife Service is pleased to provide this manual to help fisheries biologists and others prepare for a fish kill investigation. Research and Development (Region 8) has cooperated with the Division of Environmental Contaminants in Fish and Wildlife Enhancement to provide expertise and funds. We hope that the manual proves to be useful for interpreting evidence at the site of a fish kill, gathering needed evidence and data, making the final determination of the cause and needed remedial and corrective actions, and preparing for appearance as a court witness.

John D. Buffington
Regional Director
for
Research and Development
Washington, D.C.

John A. Blankenship
Fish and Wildlife Enhancement
Division of Environmental Contaminants
Washington, D.C.

CHAPTER 1

Introduction

Fred P. Meyer

Angling is America's favorite outdoor recreation. An estimated 38 million persons fish in fresh water each year and an additional 12 million fish in salt water. Anglers spend more than $315 million annually in pursuing this popular sport. The number of persons who go fishing continues to increase each year. State and Federal fisheries resource managers actively strive to maintain adequate stocks of fish in the Nation's lakes and streams to meet the growing public demand.

In the public eye, any loss of fish, whether a result of natural or other causes, means that fewer fish are available for recreational use. Some regard fish as sentinel species and interpret a fish kill as a potential early warning of an impending environmental problem. Consequently, fish kills often receive seemingly disproportionate attention from the news media. If the kills are due to toxic substances, public concern usually extends beyond the losses of fish because of potential human hazards related to possible contamination of the water supply, harmful residues in fish flesh, or damage to the ecosystem. For these reasons, it is important that investigators be provided guidance in the techniques of how to investigate fish kills and how to interpret field and laboratory observations.

Environments that are free of pollution or toxic substances are pleasant, healthy, and inviting for all forms of life.

Thirty-eight million Americans go fishing each year. The competition for places to go fishing is becoming more intense and it is vital that waters be protected from contamination.

No fish kill is without a cause. The cause can usually be determined and corrective action taken to prevent future losses. However, determining the cause is often difficult, and a valid determination requires careful observation, accurate recording of data, and the proper use of sampling procedures. Furthermore, because many fish kills may lead to litigation or court action, the investigators must understand rules of evidence, custody of samples and data, valid record keeping, and other factors that may affect the admissibility of evidence.

Because fish kills can be caused by a wide array of factors, one must be careful not to reach premature conclusions. Although many people believe that toxic substances are the only causes of fish kills, many natural causes, including infectious disease agents, can sometimes lead to large-scale losses. Depletion of dissolved oxygen, excessive water temperature, toxic algal blooms, bacterial and viral infections, and parasitic infestations all have the potential for inducing widespread mortalities of fish in an ecosystem. However, each cause is usually accompanied by a distinctive set of characteristics that provides insight into the source of the fish kill. It becomes the responsibility of the investigator to make a complete assessment of the situation associated with a fish kill and to collect appropriate samples to ensure that the role of each potential factor can be identified or eliminated.

In the 10-year period 1970–79, an estimated 3.6 million fish died in 409 documented fish kills in the State of Missouri (Czarnezki 1983). The incidence of the types of causes is typical of that in many States, and the Missouri data thus provide useful insight into the most likely sources of fish kills (Fig. 1.1). In Missouri, municipal-related sources were the most common cause (26.4%) of fish kills, followed by agricultural operations (17.4%), and industrial operations (10.8%). Less important sources of fish kills were transportation accidents (7.6%), oxygen depletion (7.3%), other nonindustrial operations (6.8%), mining (6.6%), disease (3.7%), and others (2.7%); undetermined causes accounted for 10.7% of the kills. Although sewage-related causes

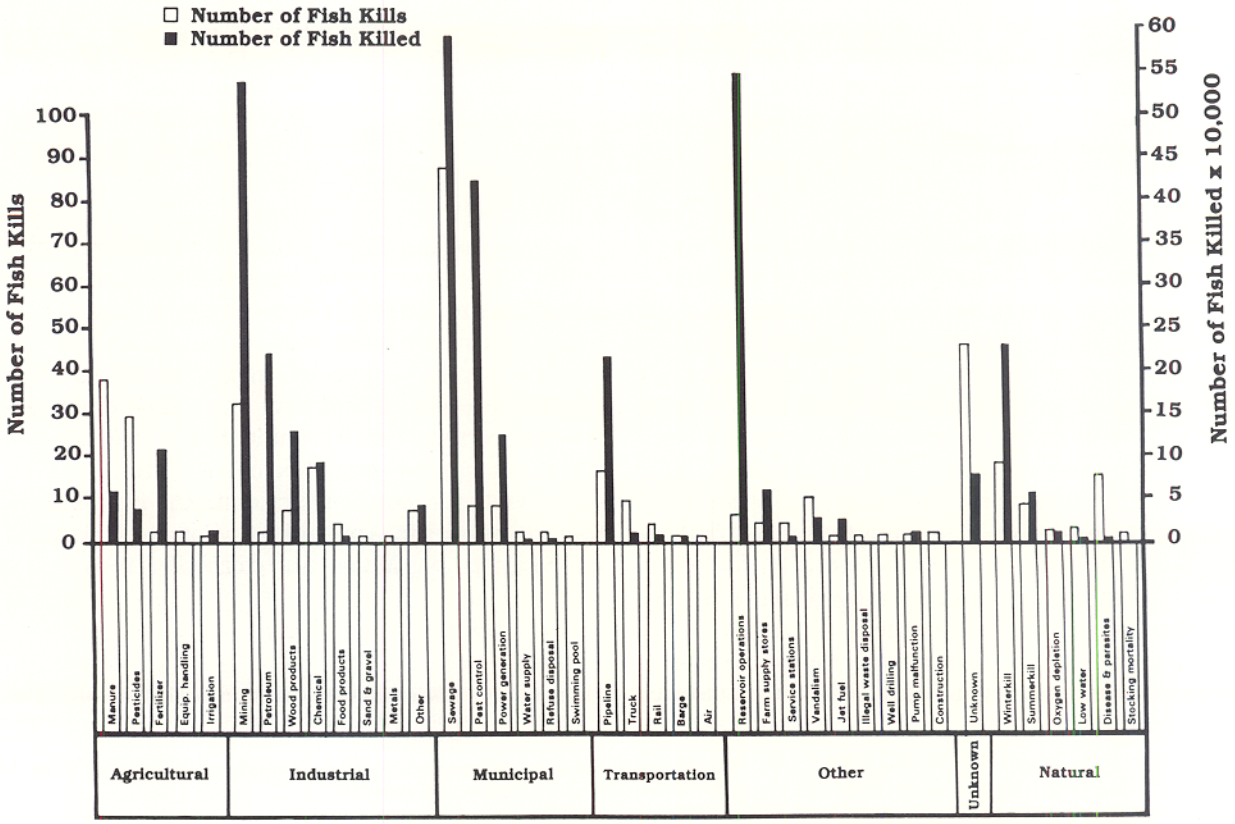


Fig. 1.1. Number of fish killed and number of fish kills, arranged by causes, in Missouri, 1970–1979 (modified from Czarnezki 1983).

were the most common and killed the most fish, mining, pest control, and reservoir operations also killed large numbers. Among natural causes, oxygen depletion during winter or summer was the most frequent problem.

Unfortunately, discerning what may be relevant information is often difficult. The evidence may point to several possible causes because the observed signs are common to more than one cause. Searching out the cause of a fish kill can be similar to a Sherlock Holmes investigation. Only careful observation, accurate recording, and complete laboratory analyses will enable an investigator to piece together the critical bits of information that eventually pinpoint the exact cause.

In the past, it has sometimes been difficult to prove the cause of a fish kill in court because of carelessness, lack of timely action, and a series of common failures: failures to record observations, conduct appropriate tests, collect the needed samples, maintain chain-of-custody procedures, or document evidence properly. The vital evidence on which a definitive decision must be based may be short-lived, especially in rivers or areas of tidal influence. It is critical that the investigator know and understand the need for prompt, precise action to record or preserve the relevant evidence.

Quality assurance to protect the validity of data, samples, and other evidence is a critical part of any fish kill investigation. Before beginning work at a fish kill site, every investigator should be thoroughly familiar with quality assurance requirements and rules of evidence (discussed in Chapter 7).

Before attempting to explain the cause of a fish kill, an investigator should carefully study the entire environmental picture. Seemingly insignificant factors of weather, water flow, vegetation, algal blooms, pollution, water chemistry, and other activities in the area may play important roles. The investigator should try to determine what factors in the environment suddenly changed and why. No evidence should be overlooked.

Frequently, the first indication that something is wrong is the presence of dead fish. Such evidence is after the fact and the investigator must mentally reconstruct the environmental situation that led to the kill. Unfortunately, dead fish often look alike, whether they were killed by a toxic substance or died of asphyxiation from an oxygen depletion. However, the site of the fish kill usually offers clues to the nature of the cause. It is the responsibility of the investigator to watch for and to recognize these clues.

This fish kill investigation manual is intended to serve as a guide to field fishery biologists to help them through the entire investigative process. It begins at the point of first notification of a fish kill, proceeds through the various stages, discusses the types of causes and the evidence associated with them, provides guidance at the various decision-making stages, and culminates in the preparation of a completion report.[1]

Additional information on the physiological requirements of fish, how changes in the environment affect fish, and why fish kills occur was given by Wedemeyer et al. (1976) in their book *Environmental Stress and Fish Diseases*. This useful reference discusses the causes and effects of most environmental changes, provides the optimal and stressful limits of environmental variables for a number of fish species, and discusses the activity and effects of a number of toxic substances. Other useful references have been published by the Aquatic-Life Advisory Committee (1956); U.S. Environmental Protection Agency (EPA; 1971, 1972); Bouwkamp (1980); American Fisheries Society (1982); and Tracy and Kittle (1982).

In the following chapters, each of the several types of fish kills is discussed in detail. Information is given about clues to watch for, data to collect, tests to run, equipment needed, the kinds of samples to collect, how to handle samples properly, where to have samples processed, and how to proceed when the results are received. All units of measure listed in this manual are presented in metric units; numerical equivalents for their conversion to the English system are shown in Appendix A. For more detailed information, see Moore and Mitchell (1987).

[1]The principal publications of interest in fish kill investigations that are referred to in this manual are listed in alphabetical order in the references that follow Chapter 13. In the text, the name of the author and year of publication (e.g., Hill 1983) identify the publication referred to.

The loss of catchable-sized sport fishes often attracts extensive media coverage and generates public alarm. Losses of single fish are usually natural occurrences that are no cause for concern.

Clean waters provide recreational fishing for people of all ages.

CHAPTER 2

Planning

Joseph B. Hunn

Introduction

Investigating a fish kill is like detective work; it requires the same keen observation and an inquisitive mind. In addition to inquisitiveness, a familiarity with literature on fish kill investigations and knowledge of the procedures involved are important. Likewise, you must know and understand the operations manual or other administrative directives that apply to your agency. Fish kill investigations commonly bring investigators into contact with personnel of other organizations, such as analytical and diagnostic laboratories, that may be involved in analyzing samples collected in the field. General knowledge about the sources of help (and the appropriate telephone numbers) should be maintained.

The possibility always exists that questions of legal liability will result from a fish kill, and that a judge or jury may scrutinize what was done, how it was done, and the record of the investigation. The need for a carefully planned, properly conducted, and legally defensible investigation is obvious.

Advance Preparations

Any fish kill investigation involves filling out a number of forms. The investigator should be familiar with the forms required and the types of information needed. Before the need arises to conduct an investigation, you should clear the forms through the legal staff of your agency to ensure that the types of information collected will adequately support the development of a legal case against the party responsible for the kill. In addition to having a supply of the required forms, it is strongly recommended that a bound field diary or logbook be used to record all information about a fish kill. A complete record should be developed of the date, site, and extent of the kill. The record should include photographic evidence, sample numbers, types and locations of sampling, and other pertinent information so that the chronology of the investigation can be reconstructed and documented (Davis 1986). Chain-of-custody procedures to be used to collect, record, and process samples should be reviewed frequently (see Chapter 7).

A checklist should be developed well before you leave for the field. The items should include (1) the forms required, (2) names and telephone numbers of persons to be contacted in the field, (3) names and telephone numbers of other elements of the organization to be contacted (e.g., analytical facility, diagnostic laboratory, and your law enforcement division), (4) maps of the kill area, (5) types of sampling gear needed, (6) sample bottles and chests to hold samples, (7) wet ice or "blue ice," (8) logbook, (9) camera and film, and (10) safety gear. A detailed list of the types of equipment and supplies that may be needed is given in Chapter 12.

Routine maintenance is required to keep the needed equipment and supplies in ready condition. A maintenance check sheet should be kept and periodic checks should be made in accordance with the manufacturer's recommendations. This is especially important when battery-operated gear is to be used. If possible, have available a backup system of analysis that does not require batteries. Culture media and solutions must be regularly replaced to ensure that these products are always fresh and ready to use. A maintenance and performance log should be maintained. Special gear or chemicals may require specific storage conditions to prevent deterioration or contamination.

If the fish kill is not on government property, you may need permission or a warrant to enter the property to make observations and to collect samples. A State collecting permit may be required to take samples of fish and other biota. Unless you have a warrant or permission to enter the area, the samples collected may be inadmissible as evidence in a court case. It is prudent to treat each investigation as though it will end up in court.

Data related to a fish kill should be accurately collected and logged in a permanent file.

The safety of participant investigators should always be a high priority when fish kill sites are investigated. This is especially true for kills that involve spills of unknown or hazardous materials. Individual and public safety must be a primary concern. If no guidance is available, consult the U.S. Coast Guard, EPA, or the designated lead State agency for advice (Hill 1983). The EPA uses four levels of hazards to human health and lists the following protective (safety) equipment required for dealing with the potential dangers associated with a particular site:

Level	Environmental conditions	Protective equipment required
D	Low probability of hazards—no known or suspected airborne pollution	Body and foot protection against possible noncorrosive hazards
C	Possible airborne hazards that can be specifically identified	Body and foot protection, plus gas mask with appropriate canisters
B	Possibility of a range of unknown airborne hazards	Level D body and foot protection plus scuba (self-contained underwater breathing apparatus)
A	High probability of range of unknown airborne hazards plus likelihood of contact with hazardous or corrosive materials	Special "moon suit" (nonpenetrable body and foot protection)

When safety is a concern, do not enter a hazardous spill site unless you have received clearance from the agency in charge of the response to the spill. The

U.S. Fish and Wildlife Service, for example, does not permit its employees to enter sites classified as level A or B. Human safety is more important than documenting the number of fish killed.

Coordinating a fish kill investigation starts even before you go into the field. A specific case number should be assigned to the investigation and used on all labels, tags, data sheets, photographs, and other records related to the incident. A number of flow charts (Fig. 2.1) have been published to help coordinate fish kill investigations (Hill 1983; Davis 1986), and each agency's procedures should always be followed, if they are available. Be sure to contact all agency officials who need to know about the kill. The names and telephone numbers of supervisors and other persons or agencies to be notified of a reported kill should also be on hand. If more than one agency is involved in the investigation, keep all other participants in the investigation fully informed so that the investigation can be done safely and effectively. However, as stressed below, it is important that only one spokesperson be designated to answer questions from the media.

It is imperative that a sample identification system be in place before samples are collected in the field. The same unique numbering system for each sample or subsample should be used by all parties dealing with sample collection and processing (for further information, see Chapter 7). It is important that the investigator communicate with the analytical agency or group before samples are collected, and that methods to be followed for sample preparation and analysis are agreed upon. Discussions between the analysts and the investigator will help determine the needed sample types, numbers, and sizes; the sample identification system; collection protocols; preservation methods; chain-of-custody requirements; the analyses to be made; when results can be expected; the format of the report; and how and by whom the results will be used. Selection of the appropriate analytical method is important because the method influences both the reliability and the cost of analysis (Keith et al. 1983).

Publicity and News Releases

In a fish kill investigation, one person should be designated by the agencies involved to be the contact person for the news media. This restriction helps avoid contradictions and embarrassment to investigators and their agencies. Publicity and news releases during the entire period of the investigation should be limited to factual accounting of the conditions observed. Conjecture as to the probable cause of the mortality or the persons or company that might be responsible must be avoided. Information that might be released would include a description of the fish mortality, its extent, when it was first observed, the duration of the kill, and the names of agencies and personnel involved in the investigation. The designated contact person should handle any later news interviews or releases.

Endangered Species

If a kill of an endangered fish species occurs, or if a fish kill occurs in an area known to contain an endangered species, it is critical that law enforcement personnel of the U.S. Fish and Wildlife Service be notified immediately (see Appendix H).

Fish Kill Investigation Flow Chart

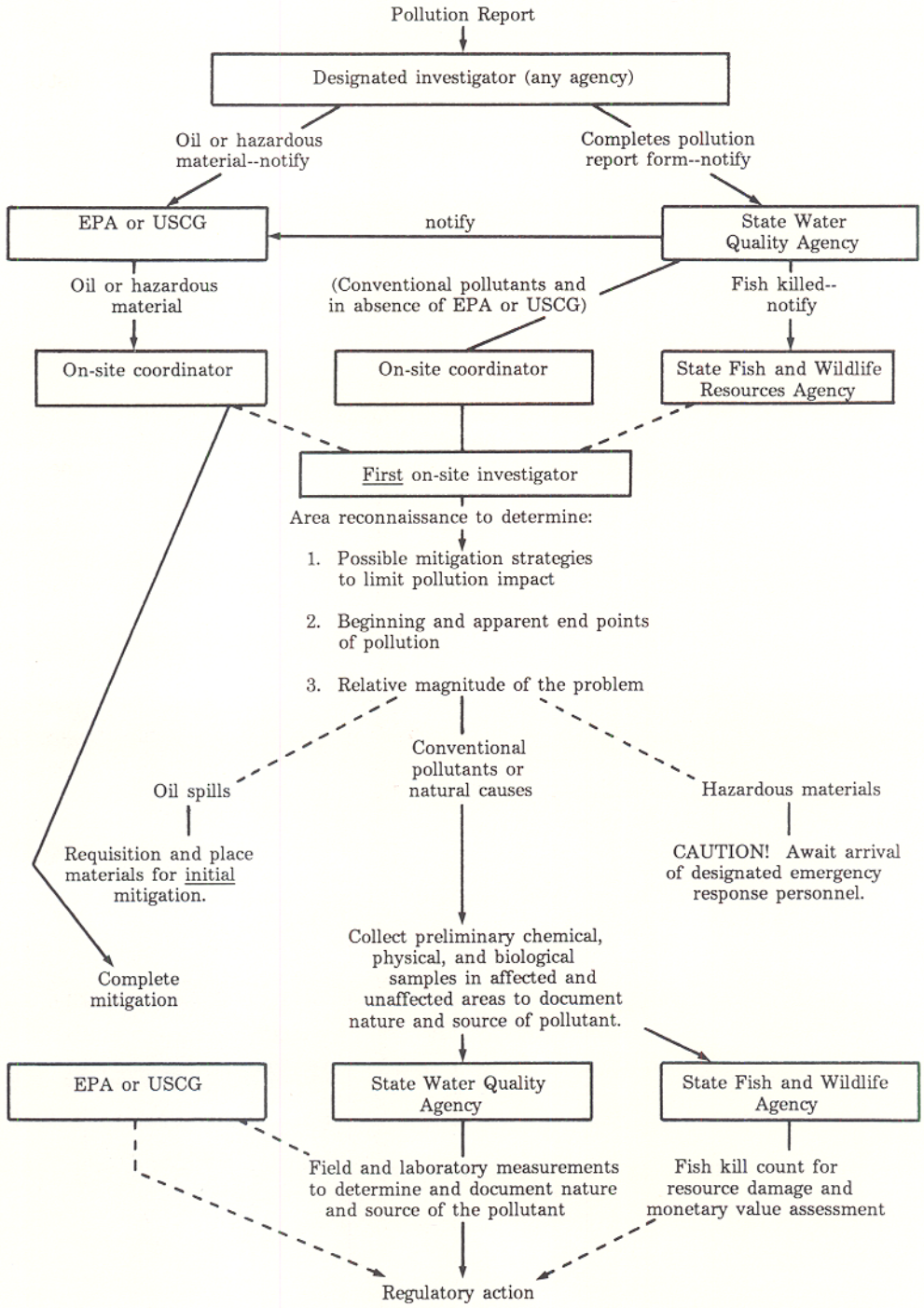


Fig. 2.1. Flow chart for coordination and performance of a fish kill investigation (modified from Hill 1983). (Abbreviations: EPA = U.S. Environmental Protection Agency, USCG = U.S. Coast Guard.)

CHAPTER 3

Interpreting the Scene

Fred P. Meyer and Roger L. Herman

Introduction

In some instances, the cause of a fish kill is readily apparent (e.g., when an obvious toxic discharge is killing fish). The course of action then is to terminate the cause, document the situation, gather evidence, and charge the perpetrator. Because most fish kills are observed after the fact, it is usually necessary to conduct the type of investigation described in this chapter.

What to Look For

The first few hours after an investigator's arrival on the scene of a fish kill may be critical. It is extremely important that as much information as possible be collected as quickly as possible. Since the investigator is often working alone, it is vital that time be used effectively to gather the information and collect the samples that are likely to contribute most toward determination of the cause of the fish kill.

The presence of dead fish is often the first indication of a serious problem in the environment. (*Photo courtesy of the Missouri Department of Conservation.*)

Immediately upon arrival, the investigator should quickly survey the scene and record the following information:

1. Date and time of day.
2. Location: river, miles of river, lake and area affected, county, nearby highways, cities, or other identifying landmarks.
3. Name, address, and telephone number of person who reported or first noted the fish kill.
4. Names of persons who can provide on-scene information.
5. Time when fish kill was first reported.
6. Estimated time when kill began.
7. Water quality characteristics:
   a. Dissolved oxygen concentration
   b. pH
   c. Water temperature
   d. Conductivity
   e. Color of the water
   f. Odor of the water
   g. Salinity (if in an estuary)
8. Condition of each species of fish seen: live, moribund, dead, or decaying.
9. Condition of other organisms in the ecosystem: live, moribund, dead, or decaying.
10. Weather conditions of the day and previous day and night, such as temperature, cloud cover, recent precipitation, wind direction and speed.
11. Physical appearance of dead and moribund fish, such as gills flared, mouths agape, spinal curvature, excessive mucus, lesions, necrotic areas on gills.
12. Any unusual characteristics, behavior, or other observations of fish or other organisms, such as excessively dark color, odd position of fins, swimming at the surface, loss of equilibrium, fish or crustaceans attempting to get out of the water, excessive mucus, snails out of water on vegetation, tadpoles piping at the surface, discolored vegetation.

Fish that are affected by sublethal toxicosis, low dissolved oxygen, a heavy burden of parasites, or a bacterial epizootic may move to shallow water, vegetation, or shaded areas. They usually ignore the approach of humans.

See Chapter 7 for instructions on what additional data are needed and how the information should be documented. An analysis of this information often makes it possible to rule out several potential causes of a fish kill and may make it possible to distinguish between one or two likely or suspected causes. This reduces the number and types of samples that are required and helps reduce the personnel, equipment, and laboratory work needed when time is critical.

In recording fish kills, it is important to establish the magnitude of the mortality. The significance of a fish kill is always directly related to economic, geographical, and political factors associated with the site, as well as to the ecological effects. The losses of 100 fish in a prime trout stream or any losses due to a possible toxic discharge are always important; in other situations, the loss of thousands of gizzard shad may be of little public concern. The American Public Health Association (APHA) et al. (1985) offers the following guide for reporting fish kills:

Minor kill: less than 100 fish
Moderate kill: 100 to 1,000 fish in 16 km of stream or equivalent lentic area
Major kill: more than 1,000 fish in 1.6 km of a stream or equivalent lentic area

The rate or pattern of loss is a helpful indicator (Fig. 3.1). If all fish died abruptly or within a short time (24 hours or less), it is likely that the kill was caused by a sudden, catastrophic event that made the environment fatally toxic to fish. If the mortality began slowly and then rose sharply over the next 5 to 7 days, the most likely causes would be a slowly developing oxygen depletion or a highly virulent infectious agent. Mortality that continues at a low rate over an extended period may be due to a marginal environment, a low-virulence infective agent, or chronic exposure to sublethal concentrations of a toxic substance.

A second important piece of information is that of the sizes and species of fish affected (Table 3.1). In kills caused by toxic substances, small fish usually die before larger ones of the same species; in oxygen depletion, the reverse is true.

Establishing when a kill began and how long it continued is also often important. It is useful to know whether the kill began at night, how long it continued, and whether it was interrupted for a time and then began anew.

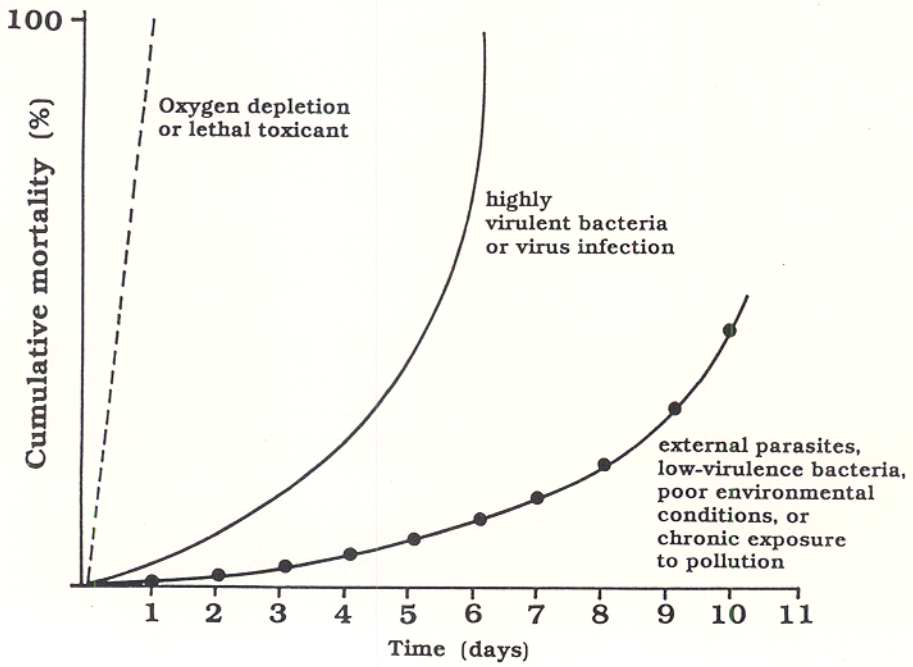


Fig. 3.1. Curves (mortality versus time) associated with three major categories of fish mortality (Wedemeyer et al. 1976).

Table 3.1. *Physical signs associated with fish mortality problems caused by oxygen depletion, toxic algae blooms, and pesticide toxicity (modified from Wedemeyer et al. 1976).*

Physical signs associated with fish mortality	Cause of mortality		
	Oxygen depletion	Toxic algal bloom	Pesticide toxicity
Fish behavior	Gasping and swimming at the surface	Convulsive, erratic swimming, lethargy	Convulsive, erratic swimming, lethargy; if organophosphate pesticide, pectoral fins extended anteriorly
Species selectivity in fish kill	None if depletion is total; common carp and bullheads may survive if depletion is partial	None, all species affected	Usually one species killed before others, depending on fish sensitivity and pesticide level encountered
Size of fish	Large fish killed first, eventually may kill all sizes and species	Small fish killed first, eventually all sizes	Small fish killed first, eventually may kill all sizes
Time of fish kill	Night and early morning hours	Only during hours of bright sunlight, about 9:00 a.m. to 5:00 p.m.	Any hour, day or night
Plankton abundance	Algae dying, little zooplankton present	Abundance of one algal species, little zooplankton present	If insecticide, no zooplankton present, but algae normal. If herbicide, algae may be absent
Dissolved oxygen	Less than 2 ppm, usually less than 1 ppm	Very high, often saturated, or supersaturated near surface	Normal range
Water pH	6.0–7.5	9.5 and above	7.5–9.0
Water color	Brown, gray, or black	Dark green, brown, or golden, sometimes with musty odor	Normal color and little or no unusual odor
Algal bloom	Many dead and dying algal cells	Abundant algae, predominately of one species	Normal bloom of mixed species unless herbicide involved; then algae absent or reduced

Kills caused by toxic substances are usually abrupt. The mortality may begin at any hour and continue until all fish have died or until the substance has been degraded, neutralized, or diluted. Small fish usually die first and affected fish often have convulsions, lose equilibrium, or show other signs of toxicosis (see Chapter 4).

A quick check of limnological or water quality characteristics will yield highly useful information (Table 3.1). If algae are alive and thriving but zooplankton and insects are dead or absent, you should suspect an insecticide as a potential cause. On the other hand, the presence of dead or dying algae, but live zooplankton, would suggest that the substance was herbicidal. If both types of plankton are dead, dying, or absent, an acid, strong alkali, heavy metal, or other highly toxic substance should be suspected.

A review of the previous information should enable the investigator to reach a judgment as to the likely cause of a fish kill and guide decisions about

the appropriate course of action to be pursued and the types of samples to be taken. Specific details regarding procedures to follow are given in later chapters relating to each type of cause.

On-site Investigation

The investigation of a fish kill must be conducted as a forensic investigation. Data collected must be adequate to answer three basic questions: (1) What is the manner of death—natural or otherwise? (2) What is the mechanism of death—toxicosis, asphyxia, or septicemia? and (3) What is the cause of death—what started the lethal sequence of events?

Collections of fish that are affected, but not yet dead, are important to the investigation of any fish kill, but they are not always made or may not always be possible. The types of analyses to be done on the fish depend on the observed and reported circumstances of the kill. Regardless of the suspected cause, fish should be checked for the presence of infectious or parasitic diseases, preferably at the site (Chapter 6). If industrial or agricultural pollution is suspected, chemical analyses are needed, and samples must be collected and preserved accordingly (Chapters 4–6).

When an industrial or municipal discharge is suspected, water samples should be collected above, at, and below the point of discharge, as described in Chapter 4. Then plant managers or other responsible individuals should be contacted immediately to inform them of the problem, to obtain information about the possible contents of the discharge and details of plant operation (particularly just before the

Water chemistry data should be collected as soon as possible after investigators arrive at the site of a fish kill.

Highly toxic substances or high concentrations of less toxic contaminants commonly kill fish of all species and sizes.

kill), and to request permission for access to the property. This action gives plant personnel the opportunity to stop or correct the discharge if there has been an in-plant accident.

Transportation accidents should be handled in the same general way, starting with contacting the hauler, shipper, or consignee to determine what chemicals may be involved and any potential hazards associated with them. The county sheriff or highway department should then also be notified.

Kills due to chemicals used in agriculture or forestry are often difficult to diagnose. Runoff from fields and aerial applications of chemicals may reach bodies of water through ditches or other water conduits. This type of kill is rarely associated with obviously polluted discharges. Checks of information regarding agricultural and forestry practices in the area may suggest toxicants to be included in requested sample analyses. Water samples taken from the area must include both natural and man-made drainage systems that feed water into the area of the kill.

Observations and sampling should not be limited to fish and water. Many fish toxicants also affect other forms of life. Algae, zooplankton, benthic organisms, other aquatic vertebrates, and even rooted vegetation should be examined for signs of toxic or lethal effects.

The mechanism of death in natural kills may be easily determined but the underlying cause may not be immediately obvious. The investigation of non-pollution kills should not stop with the identification of an infectious agent or a determination of oxygen depletion. For example, low flow from a storage dam can be the cause of increased water temperatures in the stream below the dam and thus be the primary cause of a fish kill. Identifying such situations may lead to the modification of water flow management plans to prevent future losses.

Documentation must always be precise and consistent. Sample sites must be clearly identified so they can be revisited to obtain additional samples, verify any physical conditions, or conduct toxicity tests. All samples must be clearly marked so there can be no confusion as to their identity or as to when, where, how, and by whom they were collected. The chain of custody for all data and samples starts with the on-site investigator and must be continuous through any testing or other examinations that may be conducted, until the case is resolved.

Your agency may require an estimate of the number of fish lost, regardless of the cause of death, but if there is reason to believe compensation may be sought or there is a possibility of litigation, a valid estimate of the magnitude of the kill must be made. A guide recommended for this purpose is *Special Publication No.* 13 of the American Fisheries Society (1982).

Some fish kills affect only one or two species of fish; in this incident, only sunfishes were killed.

Dichotomous Key for Fish Kill Investigations

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)
24. Recent temporary major change in water temperature .... Temperature kill (as from shutdown 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 *Ichthyophthirius*), 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*

Chronic exposure to sublethal levels of contaminants may lead to tumors or other adverse effects in surviving fish. Public concern is heightened when melanomas, papillomas, and other anomalies, such as those on this black bullhead, are seen on fish.

CHAPTER 4

Toxic Substances

Joseph B. Hunn and Rosalie A. Schnick

Introduction

Fish kills caused by toxic substances fall into several categories, each with its own set of accompanying environmental evidence. Highly toxic substances act quickly and cause abrupt, extensive mortalities. Some chemicals kill both plants and animals and thus severely and dramatically affect the ecosystem. Other compounds may affect only plants, only animals, or only certain species or sizes of fish. Kills associated with these substances may be abrupt, progressive, or lingering, and may trigger a chain of adverse environmental changes. If toxic substances enter the ecosystem at sublethal levels over an extended time, the environmental effects are more subtle. Fish kills associated with such changes may appear at unexpected times of the year or long after the discharge has ended.

Biological Responses to Toxic Substances

Species of fish vary in their susceptibility to toxic substances. Unless the substance is so highly toxic or the concentration is so high that virtually all fish are killed shortly after contact, a progression of selectivity among fish species is usually evident. Because toxic substances may kill all of the biota, it is important to also check whether other organisms, such as algae, zooplankton, sandworms, snails, insects, crabs, crayfish, frogs, turtles, or snakes, are still alive. Often, some species are less sensitive than others to a toxicant, at least in the early stages of the kill.

Unless the substance is herbicidal or algicidal, the dissolved oxygen, pH, and other water chemistry characteristics may appear normal. If the substance also kills plants, the picture becomes confused by misleading indicators, such as low oxygen, low pH, high CO_2, and dying algae. The observer must be alert and consider all of the evidence to determine the true cause of the fish kill.

A fish kill is sometimes the result of long-term, chronic introduction of toxic material. The rusting 55-gallon drums shown here contained hazardous materials that were released over several years.

An array of information is needed before an investigator can determine whether a toxic substance was responsible for a fish kill. Evidence used to make such a determination must come from on-site investigations and laboratory analyses of samples taken during the investigation. Information developed from preliminary observations may include the following:

Fish
- Rate of mortality was abrupt and most fish died within 24 hours
- Small fish died first
- Some species were affected more quickly than others, although all fish eventually died
- Behavioral changes noted were indicative of toxicant poisoning (Tables 4.1 and 4.2)

Invertebrates
- Zooplankters dead, dying, or absent (suspected insecticide poisoning)
- Benthos numbers greatly reduced with a marked change in species composition
- Crabs, crayfish, and sandworms dead, dying, or absent

Other animals
- Signs of poisoning observed among other vertebrates (e.g., frogs, turtles, snakes)
- Invertebrates (e.g., snails) show signs of poisoning

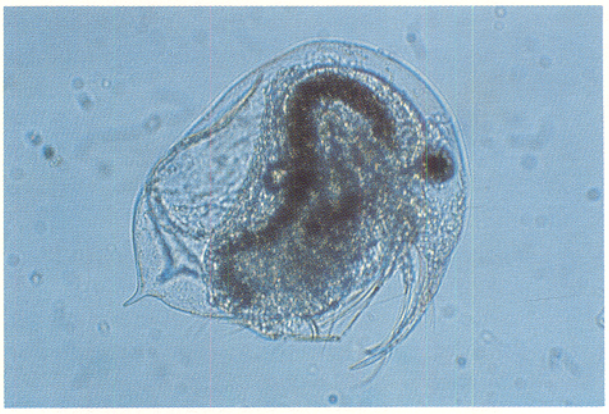
Algae
- Algae alive and normal
- Algae absent or dead (suspected herbicide poisoning)

Table 4.1. *Some observed fish behaviors and water chemistry characteristics associated with fish mortalities (modified from Davis 1986).*

Observations or water chemistry	Possible cause
Large fish coming to surface, gulping air; low dissolved oxygen. Small fish alive and normal	Oxygen depletion caused by excessive organic matter; look for a sewage treatment plant, livestock feedlot, irrigation runoff, decaying plant material, or dying algal bloom after several days of hot, calm, cloudy weather
Large fish coming to surface and gulping air in the presence of adequate dissolved oxygen	May be same as above but enough time has passed to allow for reoxygenation of water. Ammonia kills may also have these characteristics; look for possible drainage from livestock feedlot
Fish swimming erratically and moving up tributary streams to avoid pollution	Usually a heavy metal or chemical wastes discharged from a chemical complex or through a sewage treatment plant
Fish dying after a heavy rain	May be a pesticide or herbicide that has washed off adjacent agricultural fields; a spill dumped from spraying equipment; or chemicals from an aerial spraying operation
Oil sheen on water	Drilling and refinery operations; ruptured pipeline in the area; wash water discharged from oil barges; or a leaking barge
Streambanks and bottom covered with orange-colored substance; high conductivity readings in water samples	Drilling operations; look for discharge of brine water into the stream
Low pH, orange discoloration of water but good water clarity	Acid water discharge from coal mining operation
Fish hyperexcitable, rapid movements followed by death; fish may attempt to swim onto shore	High levels of ammonia or low pH
High levels of chloride, high conductivity, high salinity, and high osmolality in nonmarine waters	Possible return flow of irrigation waters that are hyperosmotic to fish
Low levels of chloride, low salinity, and low conductivity in estuarine or marine waters	Intrusion of fresh water that is hypoosmotic to fish

Table 4.2. *Fish behaviors associated with insecticide poisoning (modified from South Carolina Department of Health and Environmental Control 1979).*

Organochlorine pesticides	Organophosphorus pesticides
Central nervous system disorders	Lethargy
Increased ventilation rate	Loss of equilibrium
Rapid, jerky movements of body and fins	Dark, often reddish, discoloration; hemorrhaging in muscles and beneath dorsal fin
Erratic, uncoordinated swimming movements with spasms, convulsions, and racing	Hypersensitivity—startled fish involuntarily swim rapidly in circles
Increased sensitivity to external stimuli	Tremors, convulsions, and coughing
High excitability	Involuntary extension of pectoral fins and opercula to most forward position possible
Loss of equilibrium with successively longer periods of quiescence until respiratory movement ceases	Spinal abnormalities



Top photo. Cladocerans such as *Bosmina longirostris* are highly sensitive to toxic substances. Their presence outside the affected area but absence in the kill zone is a valuable clue to the possible cause. *Bottom photo.* Plankton nets are used for collecting zooplankton to check for toxic effects.

Chemical Changes Related to Toxic Substances

The toxicity of a substance refers to its potential for having a harmful effect on a living organism. Toxicity is a function of concentration and the duration of exposure. Acute effects occur rapidly as a result of a short-term exposure to a relatively high concentration of a toxicant. Generally, acute effects are severe and usually include mortality (Rand and Petrocelli 1985). However, fish kills may also be induced by the entry of sublethal levels of toxicants through the food chain. Such kills are usually not acute and do not occur at a particular time of year or affect a particular life stage.

Frequently, the introduction of a toxic substance causes no change in the water chemistry, but may leave residues in the water, sediment, or animal tissues. These materials should be checked because the results may yield significant information and may provide the first firm evidence that a toxic substance is involved. Preliminary analyses may provide the following information:

Water

- Water chemistry is normal for the current season and local area
- Some water constituents are abnormal and in a range known to be toxic
- A suspect toxicant has been detected in quantities known to be toxic

- Significant differences exist in the chemical composition of water between the site of the kill and the reference (control) site
- On-site toxicity tests indicate that water from the kill site is toxic, whereas that from the reference site is not

Sediment

- A suspect toxicant is present in sediments from the site of the kill
- The suspect toxicant was not found in sediments from the reference site or is present in equal or lesser quantities at the reference site
- Toxic chemical levels at the site of the kill are higher than those of background samples from the area (Kelly and Hite 1984)

Tissues

- Activity of enzymes (e.g., acetylcholinesterase in brain, ATPase in gills) is reduced in fish from the kill area
- Concentrations of toxic metals (e.g., Cd, Cu, Hg, Zn) in gill tissue are higher in fish from the kill area than in fish from the reference site (suspected metal poisoning)
- Concentrations of the suspect toxicant in tissues are greater in fish from the kill site than in those from the reference site
- Concentrations of the suspect toxicant in fish tissues are known to be toxic

Investigations of kills suspected to have originated from a toxic substance must proceed as though the cause is unknown. All factors must be checked or eliminated unless there is firm evidence that certain causes are not involved. The investigation should proceed through a process of elimination.

The use of autoanalyzers provides rapid and highly sensitive water chemistry determinations.

Diagnosis of Toxic Effects

When the initial field inspection is completed and the probable cause is believed to be a toxic substance or substances, the next step is to establish whether the suspect chemical was present in sufficient quantity to be toxic to fish. A complete water chemistry analysis should help rule out other possible causes and help identify any contributing factors (e.g., dissolved oxygen, pH) that could influence the toxicity of the suspected chemical agents. Analyses that should always be run as soon as possible are listed below (in approximate order of importance) as Priority I. Other desirable, useful analyses that should be run when possible are listed as Priority II.

Routine Water Chemistry Analyses

Priority I	Priority II
Dissolved oxygen	Biological oxygen demand
pH	Calcium
Temperature	Total organic carbon
Ammonia, nitrogen	Chlorine
Alkalinity	Chemical oxygen demand
Color	Hardness
Conductivity	Iron
Nitrite nitrogen	Magnesium
Nitrate nitrogen	Manganese
Total suspended solids	Osmolality
Salinity	Phosphate
Sulfate	
Turbidity	

Changes in pH caused by the discharge of contaminants can drastically alter the availability or activity of toxic substances. Standardized equipment, such as this digital pH meter, promptly provides accurate data.

Results of the analyses of samples taken for Priority I testing can be used to determine whether the water chemistry is within the normal range for substances that are involved in most fish kills—for example, low dissolved oxygen and high ammonia. If all characteristics are within the normal range, it may be necessary to seek further analyses such as those listed as Priority II. If the values resulting from analyses of samples from Priority I and II testing are within the normal range for the area sampled, it is a strong indication that the kill was caused by a toxic substance not usually found in the waters concerned.

Fish kills sometimes occur in situations where all environmental factors seem to be normal. Favorable water chemistry characteristics and high dissolved oxygen concentrations indicate good water conditions; the fish are normal in color and physical condition and have no lesions. The mortality rate may be slow, but continuous. Generally, predatory or omnivorous species older than 2 years are the only fish affected, and small fish and forage species may be alive and well. Such mysterious kills are most commonly seen in late fall or early winter, depending on the latitude.

These seasonal fish kills often occur in waters adjacent to areas where chemicals are used, stored, or applied. Spills, accidental spraying, or runoff can introduce sublethal pesticide levels to the environment that then become involved in the food chain by biomagnification. In kills of this type, the key indicator is that only large predatory fish are affected, whereas young-of-the-year and forage fishes seem to be thriving. Water conditions will appear to be good to excellent.

The most common cause of these unexplained fish kills is chronic exposure to sublethal levels of a pesticide. Although the daily exposure may be low, fish

The loss of large predators may indicate a fish kill caused by biomagnification of contaminants through the food chain. In such kills, young-of-the-year fish of all species may survive. (*Photo courtesy of the Missouri Department of Conservation.*)

bioaccumulate a pesticide in their energy reserve (fat) to levels that are much higher than a single acutely toxic dose. As long as the food intake meets or exceeds their daily energy requirements, the fish will continue to function normally. However, when seasonal water temperatures fall below the feeding range, the fish must rely on stored energy to survive. In fish with a high pesticide residue in their fat, mobilization of the energy reserve may release lethal levels of pesticide into the blood stream. Although signs of toxicosis are sometimes seen, the fish usually seem weak or lethargic, or unconscious. Diagnosis of such a cause requires the analysis of blood samples or brain tissue for pesticide residues. Although analysis of the fat is helpful, the results can be misleading because stored residues may be unrelated to the kill.

Under certain circumstances, selenium, a required element, enters the food chain in excessive amounts. Waterborne concentrations exceeding 3 μg/L have been detected in lentic systems—for example, in power plant cooling reservoirs and certain agricultural drainage waters. Selenium bioaccumulates in the ovaries of sensitive fish species such as centrarchids. Although selenium-laden ova can be fertilized, the young fail to survive, leading to an eventual collapse of the fish population (Lemly 1985; Baumann and Gillespie 1986).

The EPA (1986) published brief summaries of acute and chronic toxicity information for freshwater and marine species for all contaminants for which the agency has developed criteria recommendations. These criteria, which are summarized in Appendix B, are expected to be adequate to protect aquatic life. The summaries are updated to reflect recent changes in EPA's recommendations on acceptable limits for the protection of aquatic life and human health. More detailed information on

Runoff from coal mining areas can cause fish kills. Low pH in the acid mine drainage is usually the causative agent in such kills. Note the reddish iron precipitate and the conspicuous lack of life in the water.

individual water quality criteria established by EPA is provided in documents available from the National Technical Information Service (NTIS). See Appendix C.

The EPA also issued a series of documents relating to water quality criteria based on State regulations. These documents present the criteria for each State in alphabetical order. The documents are available through NTIS (Appendix D). In addition, these water quality standards are available for each State as a separate document or as part of a compilation in one document that can be purchased from NTIS.

Factors that Modify Toxicity

Laboratory and field studies have shown that many factors influence the toxicity of chemicals to fish. The origin of modifying factors may be either biotic or abiotic (Sprague 1985; Mayer and Ellersieck 1986). Biotic factors include species, life stage and size, nutritional state, general health, and parasitism. Abiotic factors include characteristics of the water (e.g., temperature, pH, hardness, alkalinity, osmolality, dissolved oxygen, salinity, dissolved organic carbon), possible binding to suspended or dissolved materials, and formulation of pesticide products.

Water hardness has little effect on the toxicity of organic compounds. However, increased water hardness (as Ca and Mg) can reduce the availability of metals such as Al, Cd, Hg, and Pb (Hunn 1985; Mance 1987). Hardness, alkalinity, and pH all influence the availability of metals, such as Cu (Sprague 1985). Hydrogen ion concentration (measured as pH) influences the toxicity of chemicals that ionize. For example, the toxicity of ammonia, cyanide, and hydrogen sulfide is influenced by the pH of the water. Un-ionized molecules usually are more lipid-soluble than ionized forms and thus penetrate membranes more readily (Hunn and Allen 1974; Spacie and Hamelink 1985). As noted by Mayer and Ellersieck (1986) in a study of 410 chemicals, pH affected the toxicity of only about 20% of the organic chemicals tested, but caused greater changes in 96-hour LC50 values than any of the other water chemistry factors examined.

Results from the analysis of water samples tested for a suspected chemical should yield positive results if that substance is present. Analytical chemistry data generated should include the concentration found, limits of detection, quality assurance, and quality control information that will help determine whether the analysis was accurate and reliable. In comparing the results from the control or reference site with those from the kill site, there should be a definite difference in concentration of the chemical. If there is not, several possibilities exist: (1) the reference site was not a true control; (2) the chemical moved downstream (in running water); (3) the compound was removed by becoming bound to sediment; (4) the substance was biotransformed, degraded, or volatilized; or (5) a combination of these possibilities.

Keup (1974) listed eight factors to consider when an investigator is attempting to interpret on-site evidence at a fish kill: (1) time of water travel (streams); (2) dilution; (3) lateral mixing; (4) season and temperature; (5) habitat characteristics; (6) delayed reactions in fish and invertebrates; (7) synergism and antagonism; and (8) suspended materials. Time of travel and dilution of the chemical can be estimated after the fact by conducting a dye study if the hydrological conditions present during the investigation are similar to those that existed at the time of the kill. For further information on how to conduct dye studies, see Slifer (1970).

Toxicity data from acute tests are usually reported as LC50's in mg/L. An LC50 is the estimated concentration of a substance in water that is lethal to 50% of the test organisms after exposure for a stated period of time (e.g., 24, 48, or 96 hours). Thus, the larger the LC50 value, the less toxic the chemical is to fish; and the smaller the value the more toxic the chemical. The relative acute toxicity of chemicals to fish (96-hour LC50) can be categorized as follows:

Toxicity rating	96-hour LC50
Practically nontoxic	100–1,000 mg/L
Slightly toxic	10–100 mg/L
Moderately toxic	1–10 mg/L
Highly toxic	0.1–1.0 mg/L
Extremely toxic	Less than 0.1 mg/L

It is important to establish some measure of the relative toxicity at the site. A valid pH measurement may be sufficient to establish whether the hydrogen ion concentration was lethal (Table 4.3). In extremely soft water, pH determinations should be made with a special electrode designed for use in waters of low ionic strength. Most substances are toxic to organisms if the concentration is high enough and

Table 4.3. *Influence of the addition of acidic or alkaline materials on the pH of receiving waters of various hardnesses.*

Total hardness (as $CaCO_3$) of receiving water	Resultant pH				
	3.0–5.0	5.0–6.0	6.0–9.0	9.0–11.0	>11.0
Extremely soft 0-9 ppm	A pH of <5.0 may be toxic, depending on species	Aluminum is most toxic to fish; other toxic metals are Cd, Cu, and Zn	At pH 8 and above, suggests algal bloom or alkali input	Indicates strong alkali input	Indicates strong alkali input
Very soft 10–39 ppm	Indicates acid input	Normal or limited acid input	Normal pH	Indicates alkali input	Indicates strong alkali input
Soft 40–159 ppm	Indicates acid input and possibility of CO_2 toxicity	Indicates acid input	Normal pH	Indicates alkali input	Indicates strong alkali input
Hard[a] 160–279 ppm	Indicates acid input and possibility of CO_2 toxicity	Indicates acid input	Normal pH	Indicates alkali input	Indicates strong alkali input
Very hard[a] 280–399 ppm	Indicates strong acid input	Indicates acid input	Normal pH	Indicates alkali input	Indicates strong alkali input
Extremely hard[a] >400 ppm	Indicates strong acid input	Indicates acid input	Normal pH	Normal in alkaline waters	Indicates strong alkali input

[a]As hardness increases, the toxicity of metals decreases.

Fish kills due to insecticides may destroy all fish and invertebrates but have no effect on plants (as shown here by the thriving duckweed among the dead fish).

the length of exposure is long enough. Although data obtained from 24-hour exposures are most appropriate for use in evaluating an acute kill situation, data from 24-, 48-, and 96-hour tests can also be used to estimate the toxicity of a substance suspected of causing the kill. The 95% confidence interval establishes a range for the LC50 and is helpful in determining whether the concentration of chemical found in the field was high enough to cause acute toxicity (Mayer and Ellersieck 1986).

Sources of Toxicity Information

One of the best sources of information on toxicity developed since 1970 is the data base AQUIRE. It includes information on acute and chronic toxicity, bioaccumulation, sublethal effects, chemical substance information, details on test organisms, study protocols, experimental design details, and results. Bibliographic references to the original sources are included. AQUIRE is one of the Chemical Information System components sponsored by the Office of Toxic Substances of EPA. The data base focuses on the toxic effects of chemical substances on freshwater and saltwater organisms, other than aquatic mammals, birds, and bacteria. As of July 1988, about 68,000 records were available on more than 4,000 chemicals.

The following references are sources for toxicity information: McKee and Wolf (1963); EPA (1973, 1977, 1980–1989, 1983–1989, 1986); Thurston et al. (1979); Alabaster and Lloyd (1982); Rand and Petrocelli (1985); U.S. Department of the Interior (1985–1989); Mayer and Ellersiek (1986); Mance (1987); Mayer (1987); and Weed Science Society of America (1989).

Clinical Signs of Toxicosis

Few of the signs related to fish poisoning are unique to a particular compound or group of compounds. For example, if adequate oxygen is available in the water at the time of exposure, cyanide poisoning results in bright red gills and blood because the available oxygen cannot be used at the tissue level. This condition might lead an investigator to assume that water conditions were normal; however, there will be hemorrhages and blood clots in the liver and viscera.

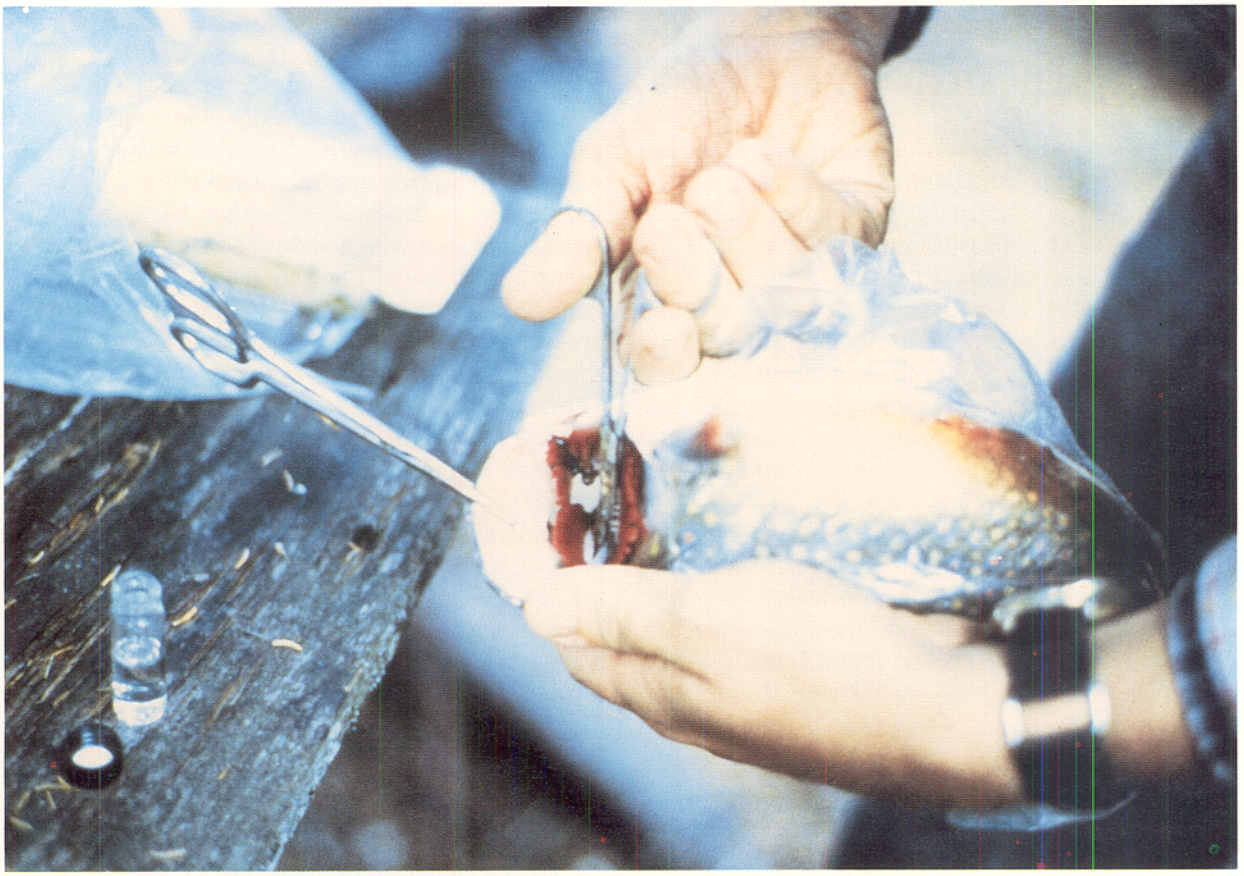
Acetylcholinesterase-inhibiting compounds (e.g., organophosphates or carbamates) reduce brain levels of cholinesterase activity, induce a forward positioning of the pectoral fins in moribund scaled fishes, and may induce spinal abnormalities.

High concentrations of nitrite can induce methemoglobinemia, a condition that is characterized by brown blood. However, hydrogen sulfide can also bind to hemoglobin to produce sulfhemoglobin, which also results in dark, chocolate-colored blood. Exposure to sulfide reduces the level of cytochrome oxidase in fish tissues and increases the levels of thiosulfate in the blood, kidney, and spleen.

The clinical signs listed must be observed in freshly dead or moribund fish because they disappear soon after the fish die. Other signs that have been observed in relation to toxicant-caused fish kills are listed in Table 4.4. It should be noted that the listed signs and behavioral responses (Tables 4.1 and 4.2) are not strictly diagnostic as to the cause of death, but they provide useful information in developing evidence.

Table 4.4. *Clinical signs associated with toxicosis in fish (modified from U.S. Department of the Interior 1970).*

Sign	Possible causative agent
White film on gills, skin, and mouth	Acids, heavy metals, trinitrophenols
Sloughing of gill epithelium	Copper, zinc, lead, ammonia, detergents, quinoline
Clogged gills	Turbidity, ferric hydroxide
Bright red gills	Cyanide
Dark gills	Phenol naphthalene, nitrite, hydrogen sulfide low oxygen
Hemorrhagic gills	Detergents
Distended opercles	Phenol, cresols, ammonia, cyanide
Blue stomach	Molybdenum
Pectoral fins moved to extreme forward position	Organophosphates, carbamates
Gas bubbles (fins, eyes, skin, etc.)	Supersaturation of gases



The gills of fish are delicate, highly sensitive tissues. Injury or other damage caused by corrosive or toxic chemicals is readily evident to a trained observer. Parasites, bacteria, or fungi may also cause gill damage.

Sample Collection for Suspected Toxic Substances

"It is an old axiom that the result of any test can be no better than the sample on which it is performed" (APHA et al. 1985). When a toxic substance is suspected as the possible cause of a fish kill, it is critical that the investigators collect samples properly, use appropriate containers, follow preservation and storage methods that are consistent with accepted methodology, and ship samples properly and promptly. The following sections discuss proper procedures for the collection, handling, storage, and shipment of samples for fish, water, sediments, invertebrates, and plants.

An essential element is a field log in which there is an entry for each sample collected for analysis, its identification number, the site where collected, the date, and the name or initials of the collector. These entries provide backup identification if sample labels are damaged, are lost, or if confusion develops over when and where certain samples were taken.

Fish Samples

A representative size series of moribund or recently dead fish of each species affected should be collected. If possible, healthy fish of the same species and sizes from the unaffected area should also be collected to provide background data. Methods that are used to preserve the various samples should always be noted on the label. For samples to be analyzed for pesticides or other toxic organic substances, the whole fish should be rinsed with clean water, wrapped in aluminum foil (with the dull side toward the specimen), and frozen as quickly as possible. Samples to be analyzed for metals or other elements should be collected separately, placed in polyethylene bags, and frozen. Subsamples of tissues such as brain, gills, or blood that are needed for special analyses should be taken immediately after sampling and frozen in separate clean glass containers. Special analyses may include measurements of enzymatic activity (e.g., acetylcholinesterase in brain or Na,K-ATPase in gill tissue) or metals in gill tissue (e.g., Cd, Hg, Zn, or Cu).

Tissues for histological examination should be taken from moribund fish—never from dead fish (in which postmortem changes are likely to have occurred). Fish that have been dead longer than 10–15 minutes are not suitable specimens. Tissue samples for histological examination should not be frozen. It is imperative that tissue specimens be placed into a suitable fixative as soon as possible, preferably at a ratio of 1 part tissue to 10 parts fixative. A 10% solution of buffered neutral formalin is readily available and is an acceptable fixative. Check with the histopathologist who will do the tissue analyses for his or her choice of fixative and for other instructions on fixation techniques. Fish tissues that were preserved in a fixative for histological examination should be transferred to 70% ethanol for storage. They can then be held for a year or more if the solution is renewed periodically. For further information, see Morrison and Smith (1981) or Yasutake (1987).

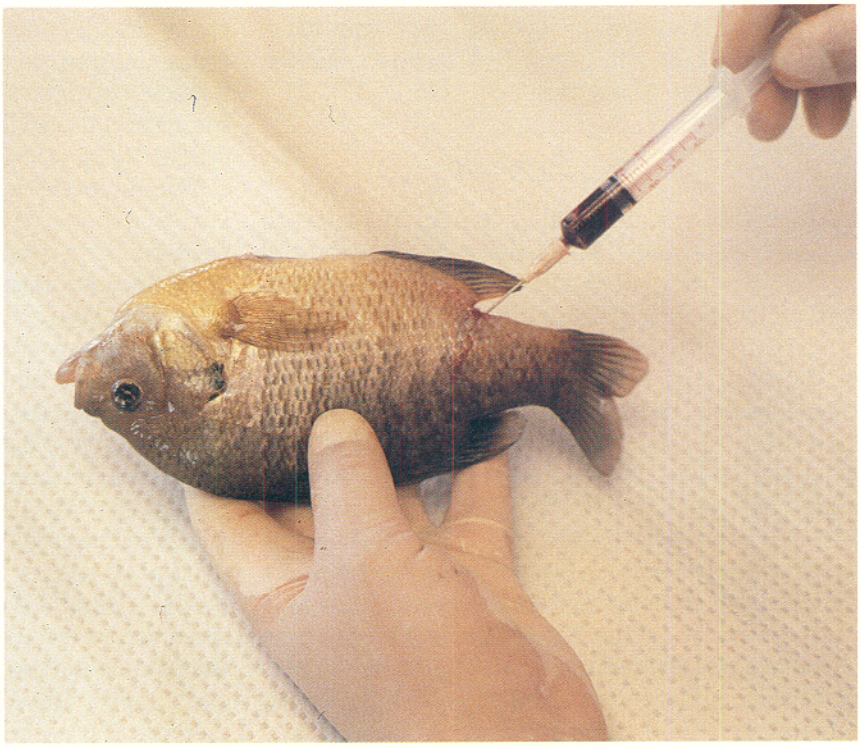
For analytical purposes, it is better to collect several small fish than one large fish from each species that is affected. The numbers collected, amount of tissue needed, and preservation techniques depend on the types of analyses to be performed. The following general guidelines apply:

Inorganic analyses
: Per sample: at least three fish or as many as needed to provide 100 g of whole body tissue as the minimum total sample; collect three samples for each species from each site

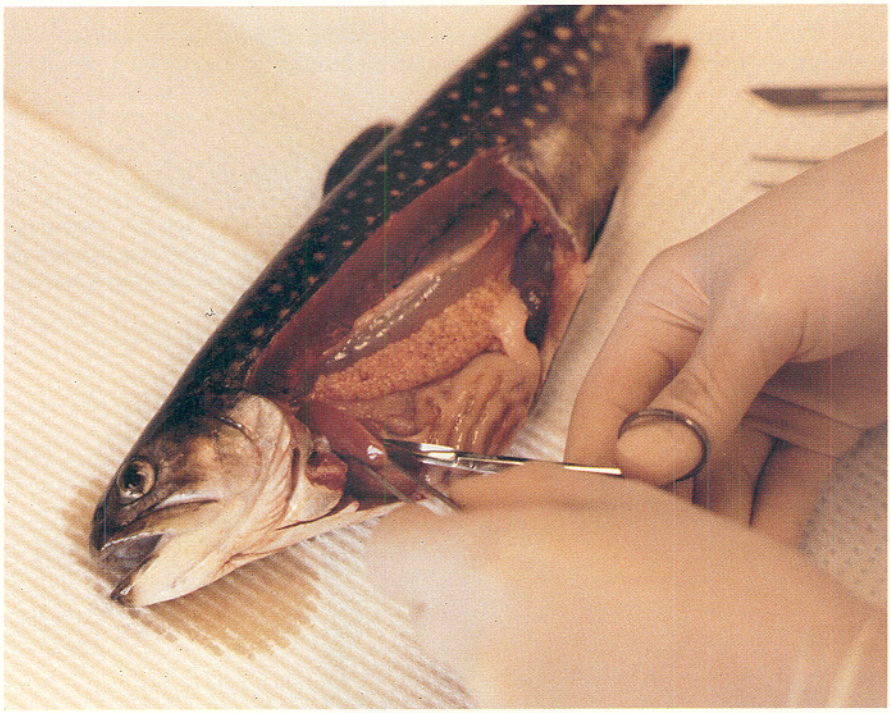
Organic analyses
: Per sample: at least three fish or as many as needed to provide 250 g of whole body tissue as the minimum total sample; collect three samples for each species from each site

If it is suspected that the causative agent is a volatile substance, about 100 g of tissue should be placed in containers that can be sealed airtight and frozen.

The composite samples of three or more fish should be separately wrapped in foil and placed in a single bag, properly labeled, and frozen. Samples of all types should be frozen as quickly as possible and kept frozen at −20° C or lower until analyzed. For a large kill with many species, the investigator must select the species to be collected. Samples should include representatives from all trophic levels that are affected—for example, herbivores, omnivores, forage fish, and predators. It is critical that the same species of fish (and preferably of the same sizes) be sampled from the control or reference area as from the kill area. The numbers and types of samples collected will depend on the extent of the



Blood samples taken from surviving fish often provide insight into the nature and identity of a toxic substance associated with a fish kill.



The liver is a major site for detoxification or biotransformation of toxic substances in fish. Consequently, it is often analyzed for residues of suspected contaminants or their metabolites.

kill, the number of species involved, agency protocol, instructions from the analytical facility, and the estimated costs of analyses.

Water Samples

After tests of water quality characteristics, such as dissolved oxygen, pH, conductivity, salinity, and temperature, have been completed, grab samples of water should be taken. (For information on the type of container needed, sample size, and methods of preservation, see Table 4.5.) At a minimum, samples should be collected above, within, and below the kill area (Figs. 4.1 and 4.2). The specific types of sampling and analyses to be run must be determined on a case-by-case basis by the on-site investigator. Before the sample bottles are filled, each bottle should be rinsed two or three times with the water that is being sampled (unless the bottle contains a preservative or dechlorinating agent). Water samples must be refrigerated at 4° C in amber bottles and stored in darkness (Table 4.5). The number of samples to be taken and sampling methods should be determined by consultation with the agency that is to perform the analyses (Keith et al. 1983). If no guidance is available, as many samples as convenient should be taken over the area. Although it may not be necessary to have all samples analyzed, there may not be another opportunity to collect useful samples. Sampling protocols should be in place, if possible, before investigative sampling is begun. For further information, see Hill (1983), Keith et al. (1983), and APHA et al. (1985).

The minimum volume needed for water samples varies with the type of analysis to be performed on the sample. In general, a 1-liter sample is sufficient. It is important that properly cleaned, prepared containers be used to collect and store the samples. In general, samples to be analyzed for inorganic compounds can be taken with plastic (polyethylene or equivalent) bottles that have been acid washed and rinsed with distilled water. For preservation, samples taken for metals analysis should be acidified to pH 2 with redistilled nitric acid. Samples taken for suspected pesticides or other toxic organics will require glass bottles with Teflon-lined caps. The glass bottles should have been rinsed with hexane and dried before use. If volatile organics are suspected, the sample bottles should be filled to overflowing and capped, leaving no air space. Recommended methods of preservation and storage times are given in Table 4.5. Properly cleaned and stored sample bottles and preservatives should be part of the fish kill investigation kit (see Chapter 12); such containers are commercially available. Ampules that contain premeasured amounts of acid for preserva-

Small, baited traps can be used to collect surviving forage fish from the site of a fish kill or from a control area.

Table 4.5. *Summary of special sampling or handling requirements for water samples*[a] *(modified from and permission to use granted by American Public Health Association et al. 1985).*

Determination	Container	Minimum sample size (mL)	Preservation	Maximum storage (d = days, h = hours, m = months)	
				Recommended	Regulatory[b]
Acidity	P, G(B)	100	Refrigerate	24 h	14 d
Alkalinity	P, G	200	Refrigerate	24 h	14 d
Biological oxygen demand	P, G	1,000	Refrigerate	6 h	48 h
Boron	P	100	None required	28 d	28 d
Bromide	P, G	—	None required	28 d	28 d
Carbon, organic, total	G	100	Analyze immediately; or refrigerate and add $H_2SO_4$to pH <2	7 d	28 d
Carbon dioxide	P, G	100	Analyze immediately	—	—
Chemical oxygen demand	P, G	100	Analyze as soon as possible, or add H_2SO_4 to pH <2	7 d	28 d
Chlorine, residual	P, G	500	Analyze immediately	0.5 h	2 h
Chlorine dioxide	P, G	500	Analyze immediately	0.5 h	2 h
Chlorophyll	P, G	500	30 days in dark; freeze	30 d	—
Color	P, G	500	Refrigerate	48 h	48 h
Conductivity	P, G	500	Refrigerate	28 d	28 d
Cyanide					
Total	P, G	500	Add NaOH to pH>12, refrigerate in dark	24 h	14 d
Amenable to chlorination	P,G	500	Add 100 mg $Na_2S_2O_3$/L	—	—
Fluoride	P	300	None required	28 d	28 d
Grease and oil	G, widemouthed calibrated	1,000	Add H_2SO_4 to pH <2, refrigerate	28 d	28 d
Hardness	P, G	100	Add HNO_3 to pH <2	6 m	6 m
Iodine	P, G	500	Analyze immediately	0.5 h	—
Metals, general	P(A), G(A)	—	For dissolved metals, filter immediately, add HNO_3 to pH <2	6 m	6 m
Chromium VI	P(A), G(A)	300	Refrigerate	24 h	48 h
Copper by colorimetry[b]					
Mercury	P(A), G(A)	500	Add HNO_3 to pH <2, 4° C	28 d	28 d
Nitrogen					
Ammonia	P, G	500	Analyze as soon as possible or add H_2SO_4 to pH <2, refrigerate	7 d	28 d
Nitrate	P, G	100	Add H_2SO_4 to pH <2, refrigerate	48 h	48 h
Nitrate + nitrite	P, G	200	Analyze as soon as possible or refrigerate; or freeze at −20° C	0	28 d

Table 4.5. *Continued.*

Determination	Container	Minimum sample size (mL)	Preservation	Maximum storage (d = days, h = hours, m = months) Recommended	Regulatory[b]
Nitrite	P, G	100	Analyze as soon as possible or refrigerate; or freeze at −20° C	0	48 h
Organic, Kjeldahl	P, G	500	Refrigerate; add H_2SO_4 to pH <2	7 d	28 d
Odor	G	500	Analyze as soon as possible; refrigerate	6 h	—
Organic compounds					
Pesticides	G(S), TFE-lined cap	—	Refrigerate; add 100 mg $Na_2S_2O_3$/L if residual chlorine present	7 d	7 d
Phenols	P, G	500	Refrigerate, add H_2SO_4 to pH <2	[a]	28 d
Purgeables by purge and trap	G, TFE-lined cap	50	Refrigerate; add 100 mg $Na_2S_2O_3$/L if residual chlorine present	7 d	14 d
Oxygen, dissolved	G, BOD bottle	300			
Electrode			Analyze immediately	0.5 h	1 h
Winkler			Titration may be delayed after acidification	8 h	8 h
Ozone	G	1,000	Analyze immediately	0.5 h	—
pH	P, G	—	Analyze immediately	2 h	2 h
Phosphate	G(A)	100	For dissolved phosphate, filter immediately; refrigerate; freeze at −10° C	48 h	48 h
Salinity	G, wax seal	240	Analyze immediately or use wax seal	6 m	—
Silica	P	—	Refrigerate, do not freeze	28 d	28 d
Sludge digester gas	G, gas bottle	—	—	—	
Solids	P, G	—	Refrigerate	7 d	7–14 d
Sulfate	P, G	—	Refrigerate	28 d	28 d
Sulfide	P, G	100	Refrigerate; add 4 drops 2N zinc acetate/100 mL	28 d	28 d
Taste	G	500	Analyze as soon as possible; refrigerate	24 h	—
Temperature	P, G	—	Analyze immediately	—	—
Turbidity	P, G	—	Analyze same day; store in dark up to 24 hours	24 h	48 h

[a] See text for details. For determinations not listed, use glass or plastic containers; preferably refrigerate during storage and analyze as soon as possible. Refrigerate = storage at 4° C, in the dark. P = plastic (polyethylene or equivalent); G = glass; G(A) or P(A) = rinsed with 1 + 1 HNO_3; G(B) = glass, borosilicate; G(S) = glass, rinsed with organic solvents; TFE = Teflon.

[b] U.S. Environmental Protection Agency, Proposed Rules, *Federal Register* 44; No. 244, 18 December 1979.

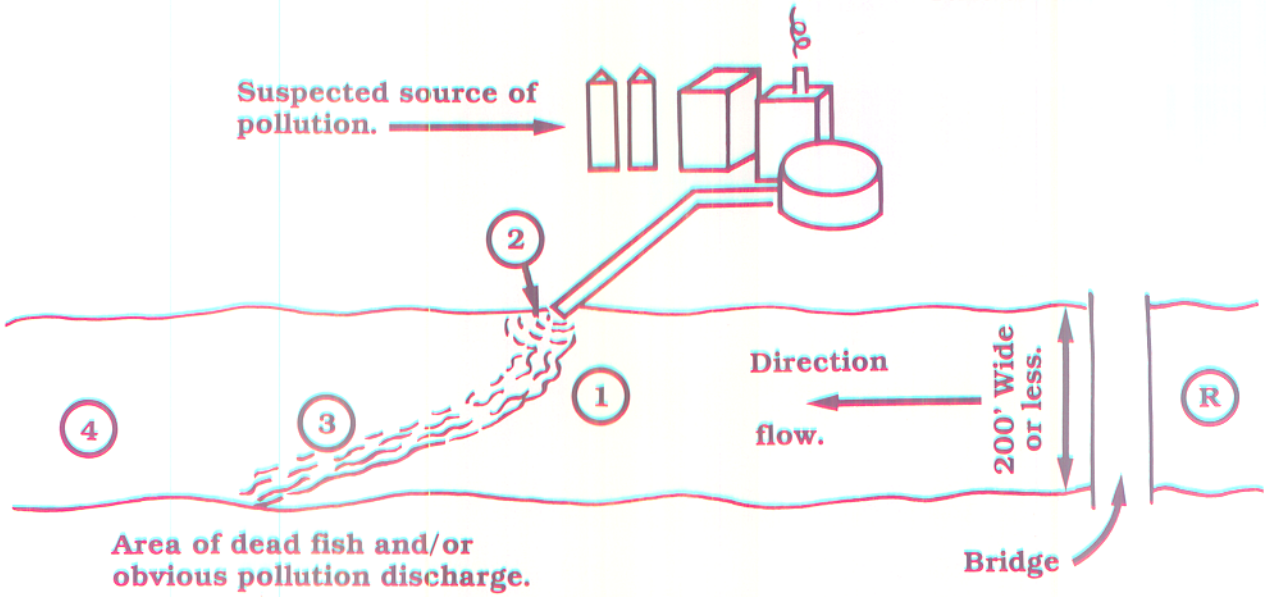


Fig. 4.1. Suggested sites for collecting samples related to a fish kill in which only one source is suspected. The *circled numbers* indicate where samples should be taken to look for the toxic substances. Site *R* is a reference site above the affected area (modified from South Carolina Department of Health and Environmental Control 1979).

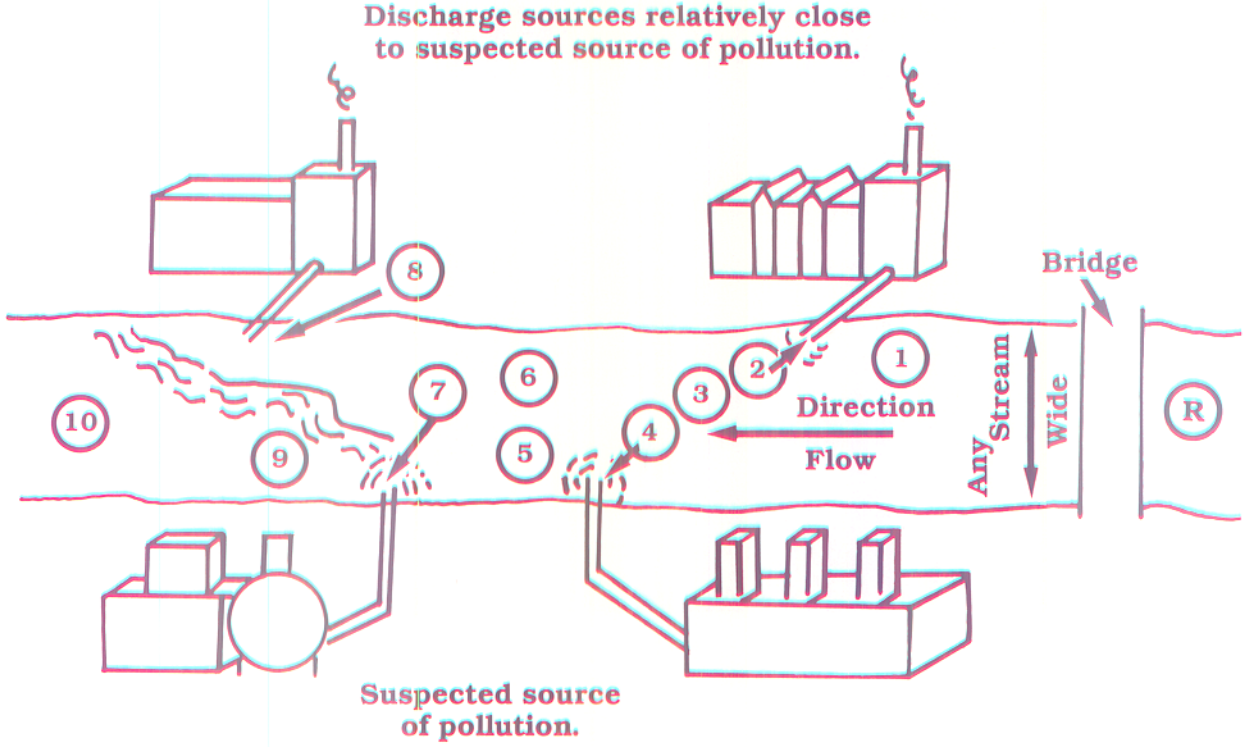
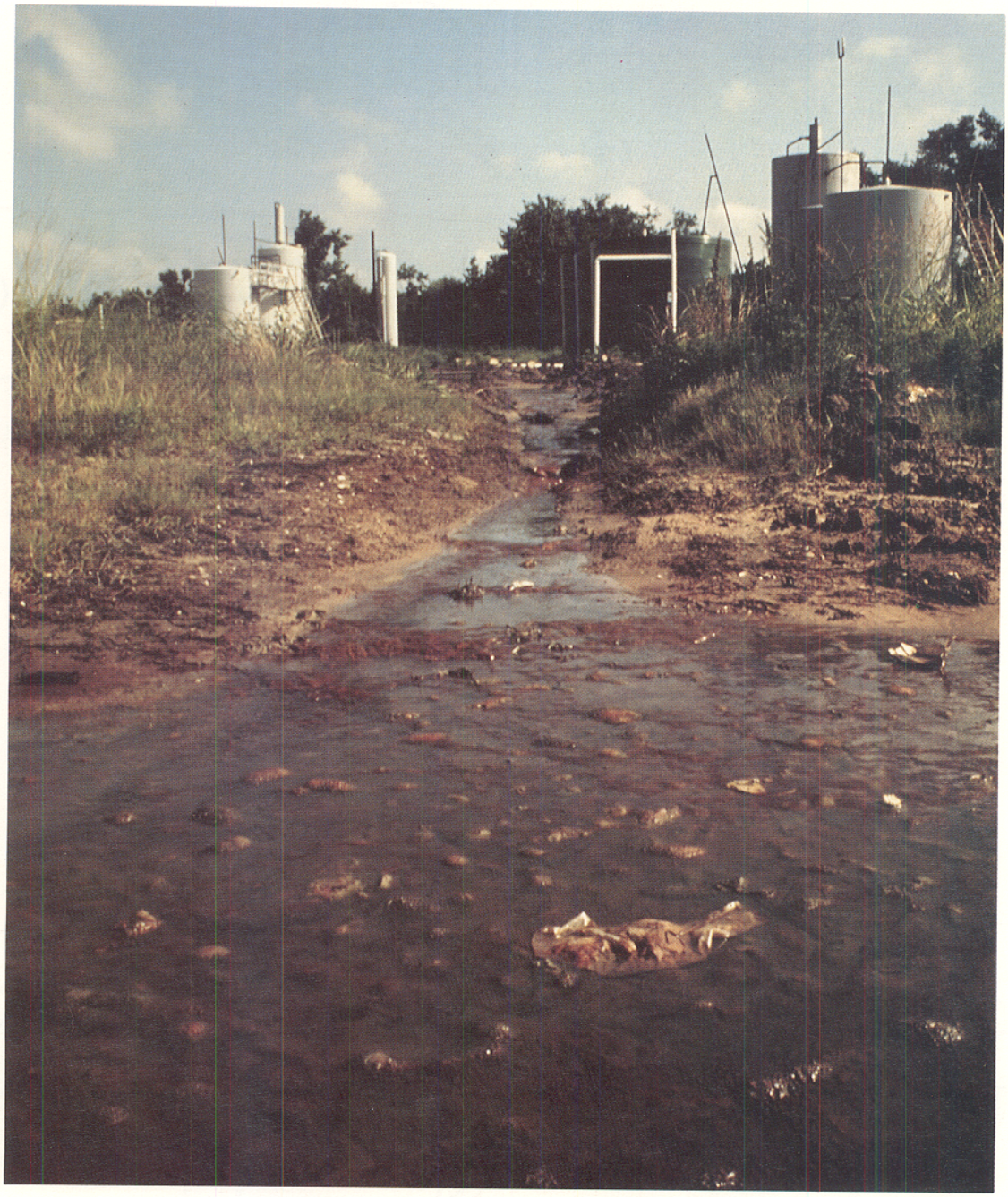


Fig. 4.2. Suggested sites for collecting samples related to a fish kill in which multiple sources might be involved. The *circled numbers* indicate sites where samples should be taken to look for possible toxic substances. Site *R* is a reference site above the affected area (modified from South Carolina Department of Health and Environmental Control 1979).



During an investigation of a fish kill, it is important to check all point-source discharges in the area. Although the flow shown in this photo is relatively low, the contaminants being released are having an obvious adverse effect on the receiving stream.

tion of water samples are also commercially available. Use of these ampules reduces acid leaks in sampling kits.

The on-site circumstances should indicate where and how many samples should be taken. As a minimum, samples should always be taken outside and inside the kill area. The control or reference site (outside the kill area) should always be free from the influence of the suspected toxic water. In a stream, one sample should always be taken above the kill area or above any point source potentially associated with the kill. If involvement of an effluent discharge is suspected, a sample of the effluent should be collected, as well as water samples collected downstream from the outfall (Figs. 4.1 and 4.2), to delineate the contaminated zone. For streams more than about 60 m wide, samples should be taken at two or more points along a transect across the stream. In large streams, it may also be necessary to take samples at various depths. Sampling devices that can be used to take water samples are outlined in Chapter 12; others are given by EPA (1982), Hill (1983), and APHA et al. (1985).

Sediment Samples

It may not be necessary to collect sediment in all fish kill investigations. However, samples should be consistently taken from the same sites where water samples were taken (above, within, and below the kill area). Special sampling sites below point source inputs may be desirable and should always be carefully documented. The method of handling the samples after collection and before analysis is determined by the type of test to be run. Samples should always be kept cool (4° C) or frozen and stored at −20° C or lower (EPA 1982; Palmer 1984; Tetra Tech 1986). If samples are to be used in toxicity tests, they should always be kept cool (4° C), but never frozen (M. K. Nelson, National Fisheries Contaminant Research Center, Columbia, Missouri, personal communication).

Sediments are usually taken with a corer or mechanical grab dredge (EPA 1982; Palmer 1984; Tetra Tech 1986). The needed sample size is usually not less than 50 g (Table 4.6). One-quart wide-mouthed glass jars with screw cap lids are accept-

To determine if benthic organisms have been killed, samples of the bottom materials must be collected with equipment such as this Ponar dredge.

Table 4.6. *Recommended quantities, containers, preservation techniques, and holding times for sediment samples to be analyzed for selected variables (modified from Tetra Tech 1986).*

Variable	Minimum sample size (g)[a]	Container[b]	Preservation	Maximum holding time (d = day; m = month)
Particle size	100–150[c]	P,G	Cool, 4° C	6 m[d]
Total solids	50	P,G	Freeze	6 m[d]
Total volatile solids	50	P,G	Freeze	6 m[d]
Total organic carbon	25	P,G	Freeze	6 m[d]
Oil and grease	100	G only	Cool, 4° C, HCl; Freeze	28 d[d] 6 m[d]
Total sulfides	50	P,G	Cool, 4° C, 1N zinc acetate	7 d[d]
Total nitrogen	25	P,G	Freeze	6 m[d]
Biochemical oxygen demand	50	P,G	Cool, 4° C	7 d
Chemical oxygen demand	50	P,G	Cool, 4° C	7 d

[a] Recommended field sample sizes for one laboratory analysis. If additional laboratory analyses are required (e.g., replicates), the field sample size should be adjusted accordingly.
[b] P = polyethylene, G = glass.
[c] Larger samples are required for sandy sediments than for muddy ones.
[d] This is a suggested holding time. No U.S. Environmental Protection Agency criteria exist for the preservation of samples or quantities needed for determination of this variable.

able containers. The caps should be lined with Teflon sheeting (metal analysis) or aluminum foil (organic analysis). All jars, lids, sheeting, or foil should first be washed with a nonphosphate, laboratory-grade detergent, and triple rinsed with tap water. They should then be rinsed with reagent grade nitric acid (1:1) and tap water, followed by a rinse with 1:1 hydrochloric acid (reagent grade), and a triple rinse with distilled water. The containers and materials should then be rinsed with acetone, followed by pesticide grade hexane, and dried in a contaminant-free area. Commercially prepared containers are available. Clean jars should be stored in the sample kit with lined caps screwed on the jars.

When widemouthed glass jars are used, the jars should be filled almost to the top with sediment, topped off with water from the site, and sealed with a Teflon-lined cap or aluminum foil beneath the lid. After appropriate labeling, the samples should be stored at 4° C. If samples are to be held for long-term storage, jars should be only two-thirds full, including the cover water. Samples should then be immediately frozen and stored on dry ice for transport. For short-term storage (less than 7 days), they should be refrigerated at 4° C; for long-term storage, they should be frozen and kept frozen until analyzed.

Invertebrate Samples

Samples of benthic invertebrates can be used to determine the extent of the kill and to document recovery after the kill. Samples should be taken in the same areas in which water and sediment samples were taken. If sufficient invertebrates, especially unionid mussels, are available, tissue can be used for residue analyses. Tissue samples should be frozen in a suitable clean container and properly tagged and labeled.

In most investigations, benthic invertebrate samples are not needed for toxicant residue analyses. If information on residues in the benthos is desired, a sample of at least 100 g is required for analyses. Generally, large invertebrates such as crayfish or unionid mussels suffice as samples for analytical purposes. Samples should be frozen in the same type of prepared containers as those used for sediments, and stored at −20° C until they are analyzed.

It is usually difficult to collect enough zooplankton for residue analysis. Generally, a record of its

The survival or death of invertebrates, such as crayfish, is a valuable clue to the cause of a fish kill.

Fish kills sometimes affect large areas. Many millions of fish were killed along several hundred miles of the Mississippi River and over large areas in its delta at the Gulf of Mexico by the dumping of a large quantity of a pesticide.

presence or absence and whether living or dead is sufficient.

Zooplankton samples can also be used to document the nature of the cause and the extent of the kill. The presence or absence of live animals can be useful information in determining the cause of the kill (see Chapter 3). The choice of sampling gear used to collect the zooplankton depends on the types of organisms present and the body of water to be investigated. To collect zooplankton, 30 liters of water are filtered through an 80-micron mesh plankton net. For a discussion of sampling techniques, see APHA et al. (1985) or Weber (1973). To preserve zooplankton, use 70% isopropyl alcohol or 5% buffered formalin. Do not store the sample in formalin longer than 48 hours before transferring it to 70% isopropyl alcohol.

Plant Samples

Phytoplankton and macrophyte samples are not normally used for residue analyses. However, in certain situations, for example, petroleum hydrocarbon contamination, residues can be rinsed from the plant surfaces and used to document the presence of particular hydrocarbons.

Phytoplankton

Samples of phytoplankton should be examined for the presence and abundance of live algae. Closing samplers, pumps and filters, or fine-mesh plankton nets can be used to collect samples. For quantitative determinations, the volume of water filtered must be recorded. If live samples are wanted for analysis, the samples should be refrigerated after collection or kept chilled at 4° C. For fixing and preserving samples, Lugol's solution is recommended (Weber 1973; Vollenweider 1974; APHA et al. 1985). (See Appendix E for the formula for Lugol's solution.)

Macrophytes

The distribution, abundance, and general physical condition of macrophytes should be noted if it is suspected that the plants are causing a decrease in the dissolved oxygen concentration, especially in early morning hours.

CHAPTER 5

Fish Kills Due to Natural Causes

Roger L. Herman and Fred P. Meyer

Introduction

Mortality from natural causes is the largest single cause of death of individual fish in a population. Unless fish are killed by some disturbance of the environment, by angling, or by other human intervention, they are most likely to die as a result of predation or old age.

Although natural phenomena can lead to fish kills, the most common effect of environmental changes in natural waters is the stress imposed on the fish. If the stress level is high enough, a weakening of the immune response may predispose affected fish to infectious diseases. If fish are carrying a significant burden of parasites, harboring a subclinical bacterial infection, or are already weakened by malnutrition, the resultant effect of an environmental stressor is sometimes a fish kill. The magnitude of the kill may far exceed the losses that might be expected from the pathogen observed; the primary cause is then the environmental stressor—not the apparent pathogen or parasite.

Fish kills do, however, occur as a direct result of natural causes. Causative agents that have been identified are oxygen depletion, gas supersaturation, toxic algal blooms, turnovers, toxic gases, natural toxic substances, sudden or excessive temperature changes, lightning, bacterial infections, fungi, viruses, parasites, and others. Usually there is sufficient evidence at the site to help the investigator accurately determine if the kill was due to a natural cause. Some of the common natural causes are discussed here.

Oxygen Depletion

Perhaps the most common natural cause of fish kills is oxygen depletion. It occurs when the total demand for oxygen by biological and chemical processes exceeds the oxygen input from aeration and photosynthesis or when the water is unable to hold sufficient dissolved oxygen to maintain aquatic life through the night. Oxygen depletion is usually associated with abundant growth of rooted vegetation, heavy algal blooms, or high concentrations of organic matter. The oxygen required during the decay of plants and breakdown of organic matter by the bacterial flora, coupled with consumption by fish and other biota, may exceed the oxygen available in the water. Circumstances that foster development of natural oxygen depletion include calm, cloudy, hot weather or low water levels, as may occur during a drought or an extended period without rainfall. Oxygen depletion is highly seasonal in occurrence unless there is extreme eutrophication (a high release of organic nutrients) such as that resulting from untreated or partly treated sewage. Oxygen depletion in natural waters is most common during June, July, and August, but may also occur in December, January, or February.

The environmental evidence associated with summer oxygen depletion may include the following:

1. Kill occurred abruptly in early morning, usually between 2:00 a.m. and sunrise. If the kill is incomplete, it usually subsides soon after sunrise, but then may resume the following night.
2. Large fish of a species died first; small fish may still be alive, attempting to gulp air in shallow water.
3. Species selectivity is evident; species with the highest oxygen requirements die first.
4. Dissolved oxygen concentration is low—usually between 0 and 1 ppm.
5. The pH is between 6.0 and 7.0.
6. Concentration of free carbon dioxide is high.
7. Color of the water changes from light green to pea-soup green, brown, gray, or black.
8. The site and water have a sour-cabbage odor.
9. Decaying vegetation (black and odorous) may be abundant, or many dead and dying algae can be detected under a microscope.
10. Zooplankters are dead or dying.

Winter oxygen depletion occurs when ice and snow cover prevent photosynthesis or other aeration. Mortalities may occur at any time of the day. Other environmental indicators are the same as those listed above.

Care must be exercised to avoid confusing an oxygen depletion due to a natural cause with a depletion caused by an herbicide, which can result in a kill that begins at any time and continues unabated throughout the day and night.

A fish kill that results from natural causes, such as oxygen depletion, is usually preceded by indicators that should alert an investigator. Before lethal oxygen depletion occurs, heavy growths of aquatic vegetation or thick blooms of blue-green algae may be present for several days or weeks. Dissolved oxygen may exceed saturation between noon and 2:00 p.m. and approach the critical lower limit for fish survival just before daybreak. Accompanying this phenomenon is a wide shift in pH with readings of 10 or above at midday and 6.9 or below at daybreak. These signs are readily apparent to a trained observer and provide advance warning. In contrast, fish kills due to toxic substances are abrupt, large-scale, catastrophic events that occur without warning.

Toxic Algal Blooms

In certain unique situations, a single species of toxic alga may become dominant in the flora. Some blue-green algae and certain dinoflagellates release toxins that kill or inhibit other algae. When competition for nutrients becomes intense, the level of toxin released climbs. Susceptible species of algae gradually disappear until only the single dominant species remains, usually in high abundance. As the alga uses up the available nutrients, the species competes with itself and the level of toxin released continues to rise. Eventually, the water may become toxic to zooplankton, insects, fish, and sometimes even to animals that drink the water. Red tides, which occur in marine waters because of blooms of the dinoflagellate *Gymnodinium brevis*, are a common example.

Mortalities due to toxic algal blooms are unique in that production of the toxin is strongly related to photosynthetic activity. Kills begin at about 9:00 a.m., continue through the day until 4:00 p.m., and then subside, only to be repeated the following day. Unless some factor intervenes, the phenomenon continues until the algal bloom ends or an oxygen depletion occurs. Often there is a large-scale die-off of the problem alga, sometimes followed by signs of a classical oxygen depletion (e.g., low O_2, low pH, high CO_2, dark water color, sour-cabbage odor). Unless the observer has information about the early phases of the fish kill, the role of the toxic alga may be overlooked.

In toxic algal blooms, pH is very high (9.5 to 11.0) at midday, dissolved oxygen is near saturation or above, and water temperatures are above 27° C. A single species of alga is present in large numbers. Species of *Anabaena*, *Aphanizomenon*, *Dinobryon*, *Glenodinium*, *Gleotrichia*, *Gymnodinium*, and *Microcystis* are some that have been reported to cause toxic blooms.

Turnovers

Occasionally, weather-related disturbances trigger fish kills. In shallow lakes, high-velocity winds can break the thermal stratification and cause a turnover. Cold, heavy rainfall following prolonged hot weather or a severe hailstorm can also cause a summer turnover that brings anoxic water and decaying organic materials into the total water column and greatly increases the total oxygen demand. Oxygen depletion can result, in spite of the aeration by wave action. Typical signs are low dissolved oxygen, decaying organic matter, foul odor, color change, and others, as normally seen during an oxygen depletion.

Hydrogen Sulfide Poisoning

Severe weather can also cause different kinds of fish kills. Disturbance of thermal stratification often releases large quantities of hydrogen sulfide (H_2S). High dissolved H_2S, even in the presence of adequate dissolved oxygen, can cause a "brown blood" condition and mortalities in fish. The brown color of the blood is caused by the formation of sulfhemoglobin, which drastically reduces the ability of the blood to carry oxygen. Some fish usually survive and ultimately recover. The largest fish are most severely affected. Environmental signs include (1) an odor of H_2S in the water—especially downwind from the site, (2) black, decaying organic matter on the windward shore, (3) disoriented, dying fish, and (4) fish

with dark, chocolate-colored gill filaments. Acidifying a sample of the brown blood with acetic or hydrochloric acids will release a distinctive H_2S or rotten-egg odor. Signs of an oxygen depletion may be observed, but are not always present. The dissolved oxygen is sometimes above 3 ppm in cases of H_2S poisoning.

Toxic Natural Substances

Problems occasionally develop because of thermal stratification. In areas where manganese is abundant in soils of the watershed, dissolved manganous oxide may accumulate in the anoxic, acid hypolimnion to levels that are toxic to fish. Generally, because no fish are in the anoxic zone, the potential hazard usually goes unrecognized. However, if the stratification is disturbed (e.g., by a cold rain, a turnover, or an internal seiche), a fish kill may occur. If a turnover or internal seiche brings the toxic water to the surface or above the intake of penstocks in a dam, a fish kill may result in the river or at a hatchery below the dam. Such fish kills are particularly difficult to diagnose because the mortality is sporadic, environmental characteristics appear normal, and there are no lesions on affected fish. Diagnosis is based on the detection of toxic levels of manganese in the water.

Gas Supersaturation

The solubility of gases in water is inversely related to temperature and directly related to atmospheric and hydrostatic pressures. As illustrated by the bubbles that form in a glass of cold water set in the sun, warm water holds less gas than cold water. When a container of carbonated beverage is opened, pressure is released and gas bubbles form (the beverage fizzes). If a diver surfaces too rapidly from a deep dive, bubbles of nitrogen form in the blood vessels because the solubility of nitrogen in the blood decreases as the hydrostatic pressure is reduced. This results in a condition known as "the bends" that can be lethal. Fish can suffer from the same condi-

Supersaturation of water with dissolved gases may be lethal to fish. This yellow perch shows typical lesions associated with gas bubble disease. Note the presence of large gas bubbles around and behind the eye.

tion, but it is called gas bubble disease or gas bubble trauma. In fish dying from this disorder, obvious gas bubbles develop in the fins, under the skin, or around the eyes. With magnification, bubbles can be seen in the capillaries of the gills. Exophthalmia or popeye can occur without visible bubbles. An excellent discussion of problems caused by gas supersaturation in water was published by Marking (1987).

Fish kills attributable to gas bubble disease can be caused in several ways. If a thermocline forms during warm weather, fish that remain in the cold water below the thermocline sometimes develop gas bubble disease if they move to the warmer surface waters. Water drawn from deep-water intakes of high dams has been subjected to the pressure of the water column and is usually cooler than surface water. When such water is discharged into a surface stream, the hydrostatic pressure is reduced and the rising temperature reduces the solubility of the dissolved gases. Fish subjected to these conditions develop gas bubble disease. If the stream has riffles immediately below the dam, only a short length of stream is affected because turbulence through the riffles releases the excess dissolved gases. Heated discharges from power plants attract fish during cold weather. Movements of fish from cold water into the warm discharge plume sometimes also induce gas bubble disease.

Nitrogen supersaturation is usually involved in gas bubble disease, but oxygen supersaturation can also cause problems. If aquatic plants (such as the stonewort, *Chara* sp.) are abundant and weather conditions are ideal for photosynthesis, the plants may supersaturate the water with oxygen. If the water temperature rises or if the pressure changes, fish in the area may develop oxygen-related gas bubble disease just as they do with supersaturation of nitrogen.

Other Environmental Stressors

Sometimes, an environmental stress may go unrecognized because no direct mortality occurred. Oxygen concentrations below 4 ppm, spawning, migrations, or elevated or depressed water temperatures may be significant stressors that reduce the resistance of fish to pathogens. For example, threadfin shad require warm water. If the temperature falls to 10° C or lower, the fish become severely stressed and may die; the weakened survivors then frequently develop bacterial or fungal infections that result in a fish kill. Postspawning fish also have reduced resistance to pathogens; it is not uncommon to observe significant numbers of dead fish in spring. Kills of fall-spawning species may also occur. Such kills are usually restricted to adults of a single species, but multiple species may be affected, depending on the chronology of their spawning.

Fish kills can also be related to abnormal or unusual characteristics of population structure or density. Occasionally, a single year class of a species may be so successful that it dominates ensuing year classes. Such dominant year classes may be so abundant that their numbers exceed the carrying capacity of the habitat. When this occurs, individual fish become stunted, are in poor condition, and are highly susceptible to stresses and secondary infections. The collapse of the dominant year class may occur as a large-scale, catastrophic die-off, seemingly associated with a particular pathogen. Although the cause of the fish kill may seem to be disease-related, the primary factor is merely a natural adjustment in the population dynamics of a single species.

CHAPTER 6

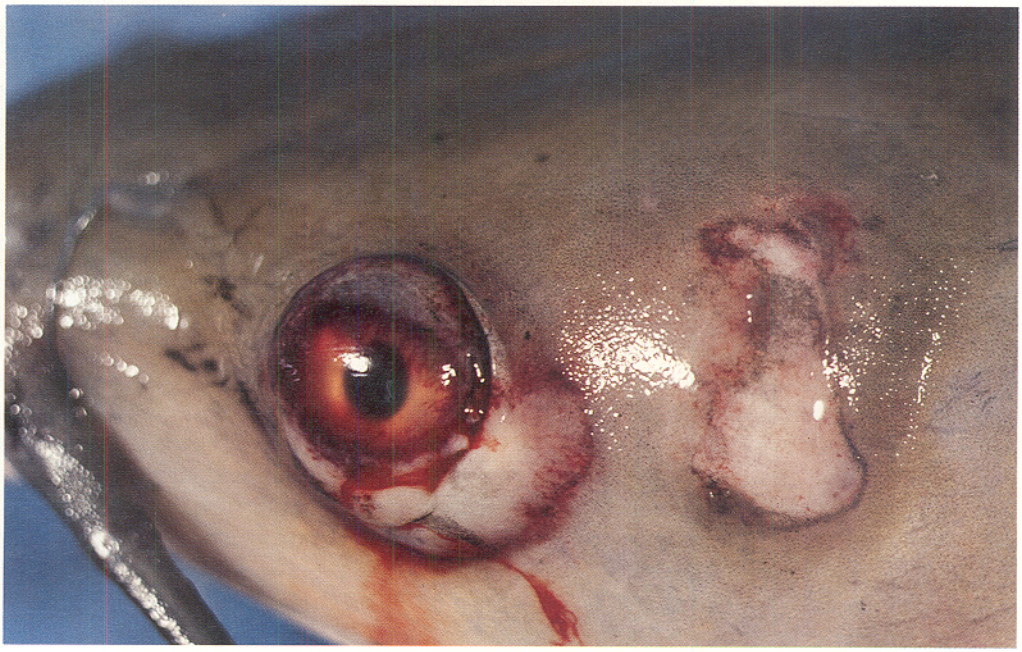
The Role of Infectious Agents in Fish Kills

Roger L. Herman

Introduction

Outbreaks of bacterial disease are seldom the result of a single factor. Three factors are involved in every potential disease situation: susceptible hosts, pathogenic organisms, and predisposing environmental conditions. All must be present when an epizootic occurs. Snieszko (1964) listed decreased immunological response, poor genetic resistance, temperature stresses, pollution, unfavorable water chemistry, and other adverse conditions as some of the possible predisposing factors. Adverse conditions may include factors such as crowding, inadequate food supply, spawning activity, storms, and seasonal changes. Although bacteria may be the ultimate cause of death in a particular situation, some other factor is often more important. Consider, for example, the massive losses of tilapia that occur when the water temperature falls below the optimum for these species. A sudden cold wave may result in massive losses. Survivors or moribund individuals often yield heavy cultures of bacterial pathogens and, unless the observer is alert to the circumstances involved, a diagnosis of a bacterial epidemic might be given. Temporary intrusions of salt water into freshwater environments (or vice versa) can cause similar situations.

In the examples given above, outbreaks of disease that result in mass mortalities in natural waters are associated with stressful environmental changes, high population densities, or shortages of food. Whenever any of these factors compromises the immunological capability of the fish, disease often occurs. Pathogens rarely overwhelm a healthy population of fish. Therefore, it is important to look for underlying factors that may have contributed to the occurrence of a pathogen-caused fish kill in natural waters.



Bacterial infections are usually characterized by lesions on or in the body of fish. This channel catfish shows the type of lesion associated with bacterial hemorrhagic septicemia.

In fish kills caused by parasitic or infectious agents, losses are seldom abrupt. Rather, there is a gradual buildup in the rate of loss as the weakest, most severely affected animals die first. Often only a single species is affected. Occasionally, an affected population is subjected to a second stressor and a seemingly abrupt fish kill may develop after a lingering, chronic loss has persisted for some time. In all such disease cases, moribund fish are heavily infected with the pathogen or parasite. Lesions may be present, but microscopic examinations and bacterial or cell cultures are usually required to identify the causative pathogen.

Occasionally, when the pathogen is a highly virulent bacterium or virus, the mortality rate may begin slowly, but increase logarithmically and reach catastrophic levels in a relatively short time. Even so, the course of the fish kill is not as abrupt as in an oxygen depletion or situations related to toxic substances.

A variety of infectious agents have been identified as the cause of fish kills in natural waters, among which viruses, bacteria, fungi, and parasitic organisms are prominent. The likelihood that they may be involved in a fish kill is discussed here.

Viral Agents

Viruses have seldom been documented as the causes of major fish kills in nature. However, they most often infect very early life stages of fish, and major losses of fry and fingerlings could occur without visible evidence. Examples of instances in which viral agents have been involved follow.

Infectious pancreatic necrosis virus has often been isolated from captive and wild freshwater and marine fishes and from some invertebrates. This virus is best known as the cause of a disease of young cultured salmonids that destroys the pancreas. It is also the cause of spinning disease in wild Atlantic menhaden, so-named because of the erratic swimming of infected fish. Outbreaks of infectious pancreatic necrosis in menhaden are usually associated with low dissolved oxygen and changes in water temperature.

Infectious hematopoietic necrosis virus has killed 2-year-old, wild kokanees and an unidentified virus was implicated in a large mortality of wild rainbow smelt in late summer in Canada.

The isolation of a virus requires the inoculation of infectious material onto living cell cultures. Not all cell lines will support each virus. When dealing with a suspected, but unknown, viral disease, several different types of cell cultures must be innoculated, just as different types of bacteriological media must be inoculated when an unknown bacterial disease is suspected.

Unlike bacteriological media that can be prepared and stored for extended periods in anticipation of need, cell cultures must be maintained in an active, fresh condition. Therefore, a fish kill investigator who is not associated with a laboratory routinely working with fish cell cultures will be unable to process samples for virological assay at the kill site. Instead, the investigator should select and properly package fish for shipment to a laboratory equipped to isolate and identify fish viruses. Moribund animals showing lesions and aberrant behavior should be selected for analysis. Fish dying from viral infections may have hemorrhagic lesions, but ulcerated, necrotic lesions are rare. The fish should be bagged or wrapped in plastic and packed with wet ice. They must not be frozen. The samples should be transported to the laboratory as soon as possible.

Bacterial Agents

Most bacterial diseases of fish are stress-related. This means that fish kills related to bacterial pathogens are associated with a significant environmental situation or change. Usually the stressful, but sublethal, situation occurred 10 to 14 days before the start of the epizootic. An investigator should be alert for seasonal stresses related to climate or weather or to normal physiological changes in fish, such as those related to migration or spawning.

Massive winter and spring kills of gizzard shad are classic examples of fish kills associated with the bacterium *Aeromonas hydrophila*. This organism is a ubiquitous facultative pathogen that frequently causes disease when the defense systems of fish are compromised by stressful environmental conditions, nutritional deficiencies, low temperatures, or reduced winter feeding. When spring water temperatures increase rapidly, the pathogen responds more rapidly than the immune system of the fish. As a result of this difference in physiological responses, outbreaks of hemorrhagic septicemia due to *A. hydrophila* may occur. The disease name is descriptive of the gross appearance of infected fish—hemorrhages and hyperemic (red) areas on the

Lymphocystis is a disfiguring viral disease of walleyes. However, this disease is not the cause of fish kills, though it causes significant public concern about the edibility of infected fish.

Edwardsiella tarda, a bacterial pathogen, causes gross hemorrhagic lesions on the body of fish. As the infection progresses, the lesions often become necrotic.

body, fins, and internal organs. The bacterium is easily isolated from the kidney and other organs by culture on artificial media.

Flexibacter columnaris also causes disease in cultured and wild fishes. It is a serious problem in migrating salmon of the Pacific Northwest, particularly where dams have transformed the rivers into a series of lakes, warmed the water, and otherwise modified the environment to favor this bacterium. It may also cause mortality among other species during the spring spawning season. Fish infected by this pathogen have grayish lesions on the fins or body that progressively destroy the skin and gills. Scrapings from the lesions show characteristic gram-negative, filamentous rods that have a flexing movement and aggregate in "haystacks" or columns when observed in wet mounts (hence the species name).

Other bacterial fish pathogens that may be involved in fish kills in natural waters are *Pasteurella piscicida* and *Aeromonas salmonicida*.

In taking samples for bacteriological study, two or three moribund fish should be selected from several different areas where the fish are showing lesions or aberrant behavior, as well as three or four seemingly normal fish for use as controls.

Fish should be checked visually for external lesions or other evidence of disease. If lesions are present, scrapings of material should be taken from the edge of the lesions and gram-stained or examined as wet mounts to check for bacteria.

The presence of large numbers of gram-negative rods or gram-positive rods or cocci suggests that these bacteria are responsible for the lesions. Material from near the edge of the lesion should be streaked onto brain heart infusion agar or blood agar. If long, thin, gram-negative rods are present in the lesions, further isolation should be made by streaking isolates on tryptone yeast extract agar. Closed lesions are preferable to open ulcers as sampling sites. The lesion (boil, pustule, etc.) should be swabbed with isopropyl alcohol or another disinfectant, then lanced with a sterile scalpel. A sterile loop inserted through the incision should be used to collect an inoculum for streaking on appropriate media.

All petri plates should be identified on the outside bottom of the plate. A permanent marker should be used to include the date, site location, fish species, organ sampled, and any other identification deemed necessary.

Inoculated plates must be stored bottom-up and protected from temperature extremes. They should be transported to laboratories as soon as possible for appropriate incubation and for identification of isolated bacteria.

Gill lamellae should be examined in wet mounts under ×100 magnification and compared with the appearance of gill lamellae from healthy fish; abnormalities, such as hyperplasia, hypertrophy, hemorrhages, or necrotic areas, should be looked for. If bacteria are present, gill material should be streaked on appropriate media, depending on the morphological type of the bacteria seen.

After the collector has checked for external lesions and taken isolates from any that are present, the entire external surface of the fish should be disinfected with Roccal or chlorine. The abdominal cavity should be opened by using aseptic techniques, and internal organs should be visually examined for gross lesions.

Stained smears should be made from any observed internal lesions and from the kidneys, liver, and spleen, and examined for bacteria. If bacteria are present, an inoculum should be streaked on brain heart infusion agar or blood agar, unless the bacteria are long, thin, gram-negative rods. If these are present, tryptone yeast extract agar should be used. The surface of the lesion or organ to be sampled is cut aseptically, a sterile loop is inserted, and the inoculum is streaked on appropriate agar plates.

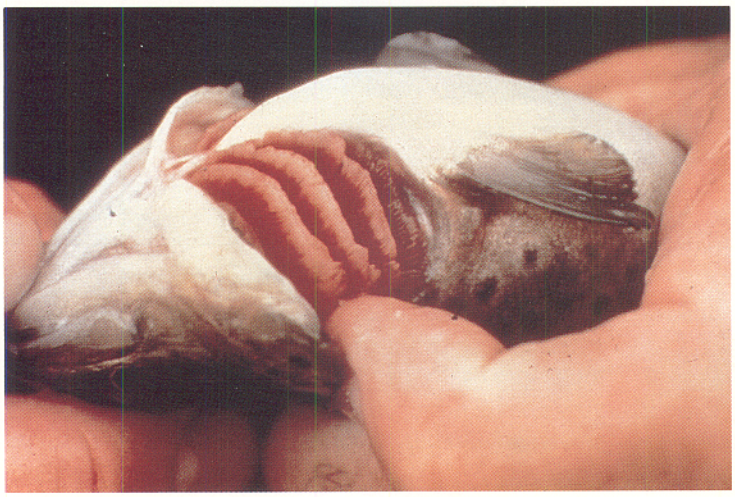
If the fish cannot be examined on site, bag or wrap them in plastic and pack them in wet ice. Do not freeze the specimens because many bacteria do not survive freezing. The use of dry ice for preservation may freeze the specimens and render them useless. On-site examination is best conducted in a trailer, van, or building where airborne contamination of the bacteriological media is minimized.

Fungal Agents

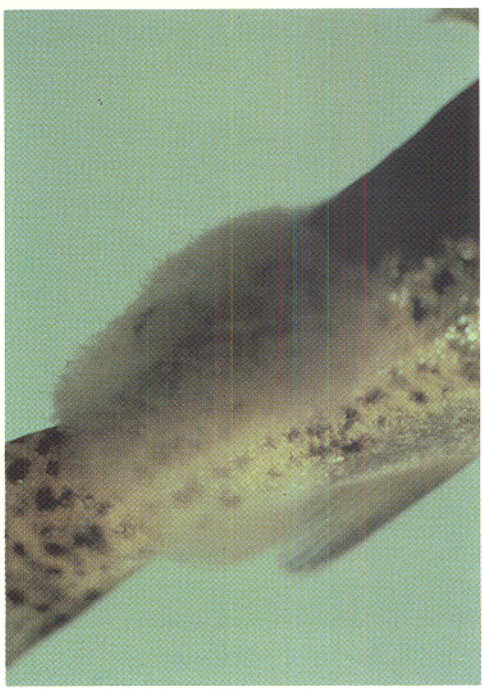
Fungal agents rarely cause major fish kills in nature. If fish are injured, diseased, or die of any cause, fungi rapidly invade the lesions or carcass and may lead an investigator to ascribe greater significance to the fungal growths than they warrant. Fungi are also opportunistic, secondary invaders around lesions caused by injuries, bacteria, or parasites. Again, in such situations, the fungi are of little significance. Occasionally, however, fungi may be the primary cause of a fish kill.

Branchiomycosis, a gill disease caused by fungi of the genus *Branchiomyces*, sometimes kills large

Checking for bacterial pathogens involves the use of specialized media and requires expertise and laboratory facilities for doing such work.



Highly caustic substances or extremely irritating compounds may cause severe damage to the gills of fish. Note the necrotic areas on the outer ends of the gill fragments.



Injured, moribund, or dead fish often develop gross secondary fungal infections. However, fungi alone seldom cause extensive fish kills.

numbers of fish, usually of a single species. Kills of northern pike have been observed in Wisconsin (F. P. Meyer, National Fisheries Research Center, La Crosse, Wisconsin, personal communication) and in striped bass in Arkansas (Meyer and Robinson 1973). In this disease, fungal filaments are readily visible in microscopic investigations of wet mounts of gill tissue. Although stained histological preparations are needed to identify the species involved, they are not required for making a diagnosis.

Ichthyophonus hoferi is a fungus responsible for sporadic mass mortalities in Atlantic herring, in the North Atlantic Ocean. Aquatic fungal infections caused by *Saprolegnia* are common secondary infections associated with external injuries. *Saprolegnia* may invade adjacent tissues and eventually kill infected animals, but it is not considered a primary cause of fish kills.

Generally, fungal infections can be recognized as such in wet mounts under low magnification with a compound microscope. However, species identifications of fungi are difficult and often require that the organism be grown on artificial media. As a result, fungal pathogens are rarely identified beyond the genus level.

Also, because fungi are opportunistic secondary invaders in wounds, abscesses, ulcers, and parasite-induced lesions, an investigator should always check beyond the obvious fungal growths to determine if some other factor may be the primary cause.

Parasitic Agents

Parasites are generally not the cause of major fish kills in natural waters. Their primary effect is to act as stressors, but parasites may render fish vulnerable to secondary infections or weaken their tolerance of environmental changes.

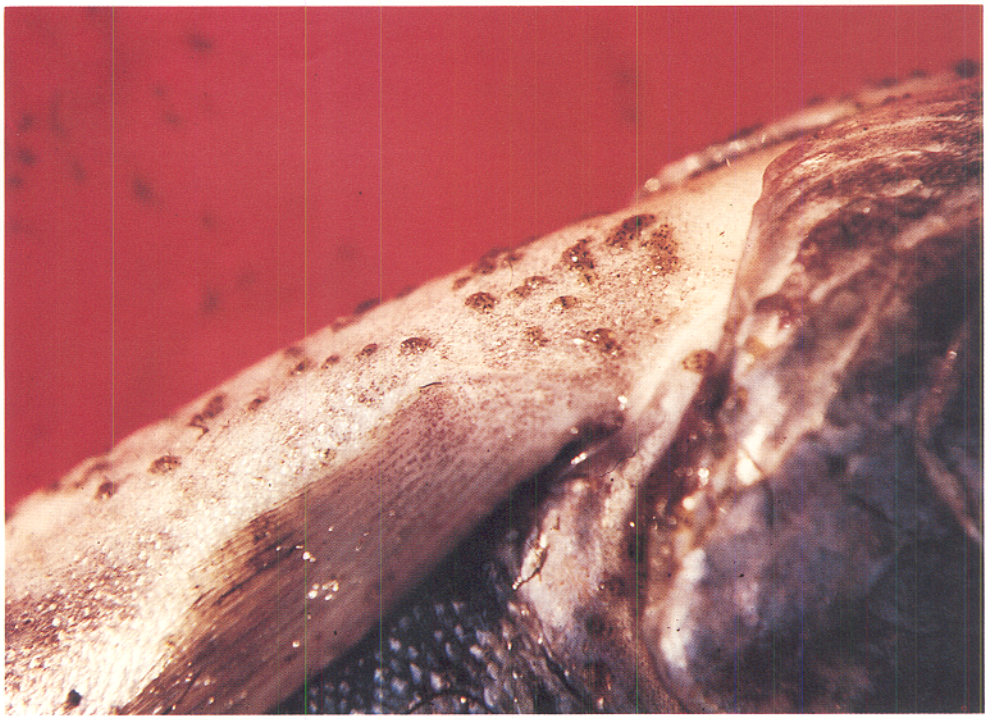
Ichthyophthirius multifiliis (Ich) is a ubiquitous, freshwater parasite that shows no host specificity and is difficult to treat. The parasites are seen as white spots under the epithelium of the fins, body, and gills. In the wild, kills of fish caused by *Ichthyophthirius* usually occur in ponds or lakes, but epizootics have been reported in rivers. As in *Aeromonas hydrophila* infections, *Ichthyophthirius* infestations are most common in late winter and early spring when fish are still in relatively poor condition due to the stresses of overwintering. Kills due to *Ichthyophthirius* infestations are less species specific than those caused by bacterial infections.

The fish louse, *Argulus* sp., has caused numerous fish kills in lakes and ponds (Hugghins 1959). This parasite attacks many species of fish and is often overlooked because it closely resembles a fish scale. Fish infested with fish lice have red, inflamed areas over their body caused by feeding of the parasites. Otherwise, the dead fish may appear normal. Diagnosis requires detection of the parasites on moribund fish because fish lice, like other external parasites, leave the host fish soon after it dies.

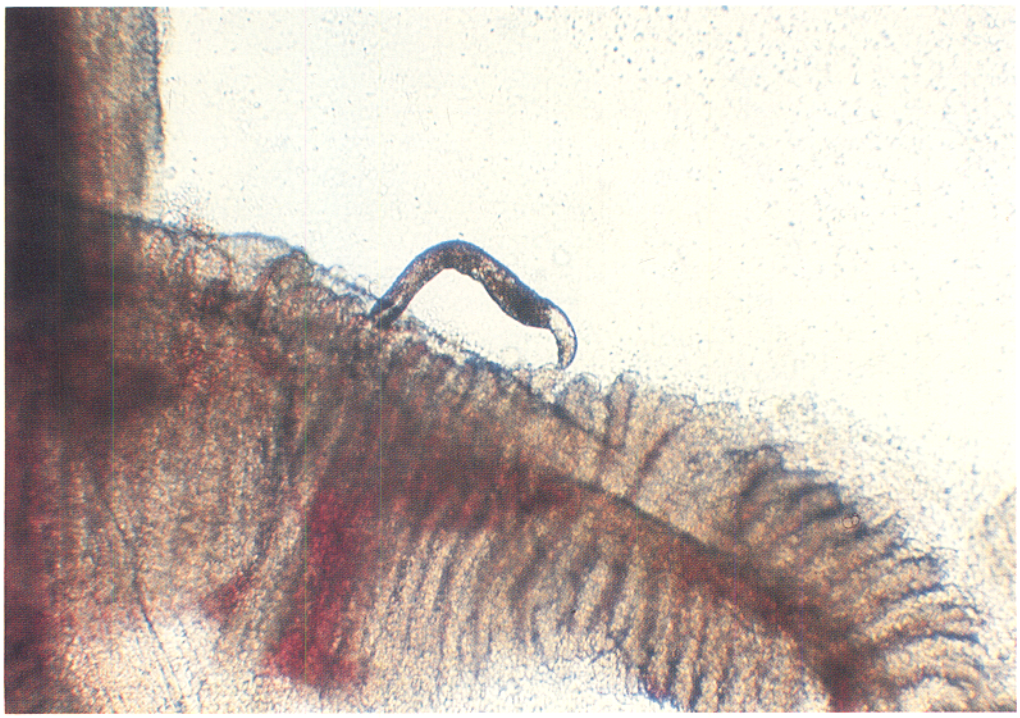
Any checks for parasite-related causes of fish kills should be done on moribund but living specimens from which the parasites have not yet detached. Small parasites, such as *Ichthyobodo* (formerly *Costia*), may become obscured by the release of mucus as tissues begin to die. Other postmortem changes may render the tissue unfit for study as either wet mounts or histological sections.

A visual examination of the body, fins, and gills of the fish can be used to check for the presence of leeches and parasitic copepods. The detection of protozoans and monogenetic trematodes requires a microscope. Magnifications of $\times 25$ and $\times 100$ should be used to search gill tissue, fins, and scrapings from external lesions for organisms. Monogenetic trematodes can easily be seen, although their movement may not be noticeable. Ciliates and flagellates may move rapidly, but sessile forms, such as *Ambiphrya* (formerly *Scyphidia*), move little (except for their cilia). When attached to the epithelium, the flagellate *Ichthyobodo* may not move. This lack of movement, in combination with its small size, makes it difficult to see in unstained material. Phase contrast optics are useful for examination of wet mounts for small organisms.

Cysts (externally or internally) found during an examination can be opened and checked for the presence of larval worms or sporozoans by using wet mounts. The brain should not be overlooked as a potential site for parasites. In checking the gastrointestinal tract for worms, the entire tract should be excised, placed in a shallow container with clean water, and opened along its full length. Many acanthocephalans, cestodes, nematodes, and trematodes are large enough to be immediately noticeable. Some trematodes can be found only by examining scrapings of the gut lining. Such scrapings may also reveal sporozoans such as *Eimeria*.



The fish louse, *Argulus*, is a highly destructive parasite. When numbers are large, this parasite sometimes causes extensive kills involving many fish species.



Although external fish parasites, such as this *Cleidodiscus* on the gills of a channel catfish, may be obvious and fairly numerous, they seldom cause epizootics of kill fish.

Identification of parasites to class or order is often sufficient, but identification to genus and species requires special techniques and expert knowledge. A good reference for use in making tentative identifications was published by Hoffman (1967).

Fish that were dead at collection or that have been dead for more than 1 hour (even if refrigerated) are not suitable for examination because of the loss of parasites when the fish dies.

If a laboratory is nearby, moribund fish should be put in individual plastic bags, placed on wet ice, transported to the laboratory, and examined soon after arrival. The quality of samples deteriorates rapidly with time: specimens 1 hour old may be adequate, whereas those 4 hours old are virtually useless.

If it is not possible to make on-site checks for parasites or to expedite the transport of affected fish to a laboratory, the investigator is forced to preserve the specimens for later study. The samples should not be frozen as the freezing and thawing process destroys tissues, usually kills the parasites, and contributes to major postmortem changes.

It is best to place whole small fish directly into a preserving solution such as 10% buffered formalin. If the fish are longer than 8 cm, a 3-cm incision should be made through the abdominal wall to allow the preservative to enter the body cavity. If the fish are longer than 15 cm, the outside gill arches on both sides, one pectoral and one pelvic fin, and a piece of the caudal fin should be removed and placed into the preservative. If attached parasites are observed, they should be excised along with about 1 cm^3 of the tissue around the site of attachment. Loose parasites should be added to the container along with the other materials. A ratio of 10:1 (preservative to tissue) or more should be maintained to ensure that there is adequate solution to preserve the tissues and specimens. Before the container is closed, a label should be inserted with complete information on the fish species, date collected, site collected, preservative used, and the name and initials of the collector. The jar should then be tightly capped and tagged with an outside label with the same information as that on the inside label.

The study of preserved specimens or tissue is difficult at best. Wet mounts should be made of the loose material in the bottom of the jar to look for external parasites that released from the fish upon contact with the preservative. Wet tissue should then be examined under a dissecting microscope to search for parasitic copepods, worm parasites, leeches, isopods, or other large organisms. It may be necessary to clear some materials by passing them through an alcohol–xylol dehydration series to identify the organisms. In other instances, histological preparations may be required.

Histological Study

Moribund animals showing aberrant behavior or lesions are best for histological examination. If no animals are found alive, select the freshest specimens. The sample should reflect the size range and species composition of the affected population. If fish cannot be preserved in the field, they should be bagged or wrapped in plastic and placed in wet ice. Do not freeze tissue samples. Freezing disrupts cells and makes the specimens worthless for histological examination.

Fish less than 30 mm long can be adequately preserved by placing the animal directly in the fixative. Larger fish should have the abdominal wall slit from the anus to the gills. The visceral mass should be pulled from the cavity after the esophagus has been severed (the intestine should be left attached), and the gas bladder should be punctured. This procedure exposes all organs to the fixative and keeps the internal organs associated with the specimen. Fish 40 mm long or longer may require incisions through the dorsal muscles from head to tail. Because muscle tissue is dense, the fixative penetrates too slowly if the incisions are not made. Placing large fish in preservative without opening the abdominal cavity results in autolysis of the internal organs, thus reducing the diagnostic value of a specimen.

Fish longer than 100 mm should be carefully dissected and the organs fixed separately in portions no more than 5 mm thick to ensure rapid penetration of the fixative. The ratio of volume of fixative to volume of fish or tissue should be 10:1 or more.

Each sample container must be labeled inside and outside. The label should not be affixed to the lid. The outside label should be glued or tied to the jar. Tape is convenient, but often it can be removed too easily. The information should be written on the label with a soft lead pencil or with permanent ink and should include the date of collection, contents, location, the identification code being used for the case, and the identity of the person who collected

Necropsies performed on moribund or freshly dead fish often provide useful clues to the cause of death.

the sample. An inside label of good quality paper stock (file card stock works well) should contain the same information.

Additional information on the preparation of tissues for histological study was given by Morrison and Smith (1981) and Yasutake (1987).

Making a Diagnosis

Information detailing sampling conditions and observations of gross condition should accompany each sample of fish submitted for necropsy. A data sheet that details the results of pathological examination, identification of bacterial or viral cultures, and lists any observed parasites should be provided from each laboratory where the fish are necropsied. An example of a pathology report is shown in Fig. 6.1. The results of these examinations will be helpful when a determination is made of the mechanism of death.

Death due to bacterial or viral infection is usually fairly easy to diagnose if the causative agent is a known fish pathogen. The agent can be easily isolated from most fish examined and the pathology will be typical of infection by the isolated agent. Death due to parasite infestation can be more difficult to diagnose as fish in good condition can often carry a large number of parasites without apparent adverse effects.

If the causative agent is an unknown new pathogen, the diagnosis may be difficult, particularly if special media or new cell lines are required for its culture. In such cases, a presumptive diagnosis may be all that is possible. Although diagnosis of death due to exposure to a toxicant can sometimes be easily made, identification of the toxicant on the basis of the observed pathology is difficult and often impossible. Many of the pathological changes seen in toxicosis of fish are nonspecific. The results of pathological examinations and chemical analyses of the fish, water, and sediments,

in combination, may lead to a presumptive diagnosis if the pathology is compatible with a diagnosis of death due to exposure to the suspect chemical found in analyses.

When the mechanism of death is an infectious process, the final report should include an explanation of the circumstances involved in causing the deaths. This would include a statement about how the fish were sufficiently stressed to allow the infection to progress to an acute disease state. If the situation was one in which human activity created the circumstances, recommendations should be included for changes in management practices to prevent recurrences.

In fish kills caused by an infectious agent, moribund fish often show distinctive lesions such as petechial hemorrhages or ulcers. This gizzard shad has bacterial hemorrhagic septicemia.

Accession no. 89-103

Submitted by L. Barclay Date 5 May 1989

Submitter's code VFO-001-031 Chain of custody: yes X no ___

Species Gizzard shad Length 18-25 cm Weight _____

GROSS EXTERNAL EXAMINATION

Skin: ( ) Normal ( ) Excessive mucus ( ) Abnormal color _____
(X) Lesions: ( ) Single ( ) Multiple ( ) Closed ( ) Open
(X) Hemorrhagic ( ) Necrotic ( ) Ulcer ( ) Blister ( ) Tumor
( ) Lost scales ( ) Abrasions
Location: Vent
Wet mount/smear: _____

Eyes: ( ) Normal (X) Exophthalmia ( ) Cataract ( ) Hemorrhagic
( ) Opaque cornea ( ) Lens lost ( ) Parasites (X) Bilateral

Fins: (X) Normal ( ) Frayed _____ ( ) Hemorrhagic _____
( ) Eroded _____ ( ) Deformed _____
Wet mount/smear: _____

Gills: ( ) Normal (X) Pale ( ) Mottled ( ) Hemorrhagic ( ) Necrotic
( ) Excessive mucus ( ) Hyperplasia ( ) Telangiectasia ( ) Gas emboli
( ) Cysts ( ) Large parasites _____ ( ) Fungus visible
Wet mount/smear: few trichodinids

GROSS INTERNAL EXAMINATION

Adipose tissue: ( ) Normal ( ) Excessive (X) Reduced ( ) Petechial hemorrhage
Color _____ ( ) Cysts

Liver: ( ) Normal ( ) Enlarged ( ) Reduced Color: (X) Pale ( ) Mottled
( ) Other _____ Texture: _____
( ) Lesions: ( ) Single ( ) Multiple ( ) Tumor ( ) Necrotic
( ) Hemorrhagic ( ) Cyst (parasite) ( ) Cyst (fluid)

Spleen: (X) Normal ( ) Enlarged ( ) Reduced ( ) Raspberry surface
( ) Cyst (parasite) ( ) Cyst (fluid) Color: _____
Stained smear _____

Intestine: ( ) Normal ( ) Distended (fluid) (X) Distended (mucoid)
(X) Flaccid (X) Hemorrhagic ( ) Cysts (parasite) ( ) Tumor

Kidney, posterior: ( ) Normal (X) Enlarged ( ) Lesions: ( ) Single
( ) Multiple ( ) Gritty, white ( ) Cyst (parasite) ( ) Cyst (fluid)
( ) Tumor
Stained smear Gram-negative rods numerous

OTHER: Gall bladder distended, bile green

Fig. 6.1. Example of a fish kill necropsy report.

Sampled for BACTERIOLOGY XX VIROLOGY ____ HISTOLOGY XX

Results Bacteriology Gram-negative rod, oxidase positive, glucose fermentation without gas, growth in presence of vibriostat 0/129 *Aeromonas hydrophila*

Results Virology ____

Histological evaluation:

Gills--hypertrophy of the respiratory epithelium, edema

Liver--diffuse necrosis with Gram-negative bacteria, no vacuolization of hepatocytes.

Posterior kidneys--massive necrosis of hematopoietic tissue and tubular elements with Gram-negative bacteria.

Intestine--mucosa sloughing, inflammation of lamina propria, vascular dilation and, hemorrhages.

Diagnosis: septicemia, *Aeromonas hydrophila*

Pathologist ____ Ph.D. Date 9 May 1989

Title Certified Fish Pathologist

CHAPTER 7

Quality Assurance and Rules of Evidence

Susan D. Haseltine

Introduction

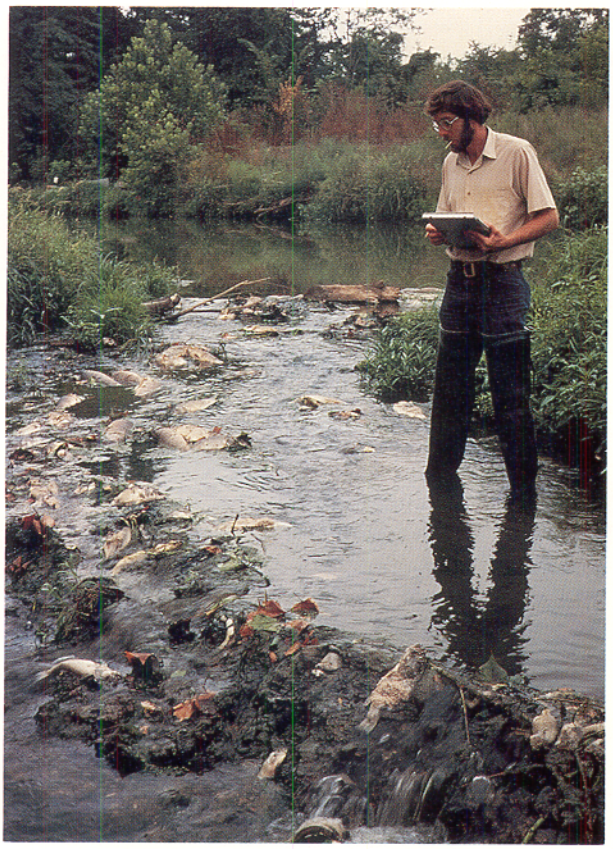
The primary purpose of any fish kill investigation is to gather information on which to base a determination of the cause. If the information is developed properly, it not only can be used as evidence in hearings and litigation, but also becomes part of the scientific literature. Evidence consists of all photographs, field records and observations, sampling and testing procedures, test results, and any other relevant information related to the investigation of a fish kill. The investigator must be able to demonstrate conclusively that the data are valid and applicable to the samples collected. If full documentation cannot be shown, valuable evidence may be declared inadmissible in a court of law.

Documentation Guidelines

All preliminary assessments of reported fish kills should be regarded as exploratory. Even if the cause of mortality seems straightforward, the full implications of the investigation and the final diagnosis of cause of mortality cannot be determined until all tests are completed. It is important that full documentation of all aspects of the fish kill, the environmental conditions, the sequence of events, and associated phenomena be carefully executed. This information is impossible to establish after the fact. The information may also serve as the basis for recommended corrective actions. Many fish kills lead to legal actions, and the extensive background and technical information developed by the investigator may be used as evidence in the courts. Proper documentation of field information, samples, test results, and chain-of-custody procedures for all samples are critical to successful litigation. Overall, the most valuable data gathered during a fish kill investigation are those recorded at the time of the event by alert observers in the field. The more comprehensive, rigorous, and well-documented this information is, the more reliable the final determination of the cause will be.

Site History and Information

Two essential ingredients in the documentation of on-site investigations are thoroughness and timeliness. Any information, no matter how seemingly irrelevant, may prove useful as the evidence accumulates. All types of information should be recorded as accurately and quantitatively as possible. Because many types of evidence are transitory or perishable, rapid preliminary decisions must be made to key in on critical measurements. A judgment concerning the priority and chronology of needed work should be based on the initial evaluation of knowledgeable observers. Accuracy and precision of all measurements should be ensured by following the manufacturer's or agency's calibration, standardization, and maintenance procedures for the instruments (e.g., scales, pH meters) used to collect quantitative data. These procedures should be thoroughly documented in the field and laboratory notes of the investigators. Quantification may include numeric, colorimetric, taxonomic, and range measurements. Saving time by omitting calibration or standardization steps reduces the accuracy of the final determination of the cause of fish mortality. Photographs or videotapes can be used to document graphic evidence and give investigators a useful record of the event. Photographs should be taken as close up as possible with the specimen in a sharp frame of reference (e.g., habitat, orientation on scene). Specimens should fill the frame or be clearly identifiable within the picture. Photographs should clearly delineate any features of special concern to on-site investigators. If videotapes are used, a running commentary with close-up shots can be invaluable in later phases of the investigation. Care should be taken to identify each tape orally by date, time, and exact location (county, township, range, or other local



The site of a fish kill should be inspected for clues other than the presence of dead fish. The investigator must be alert to detect changes in the environment, to look for other organisms that might be affected, or to search for organisms that have survived the incident.

geographical references; latitude and longitude for large-scale events). Recorders should state their name and affiliation, at both the beginning and the end of the footage.

Although several approaches can be used in recording data from an investigation of a fish kill, the most reliable form is a bound notebook into which standardized data forms that address all potential aspects of the investigation are permanently affixed. (An example of a habitat assessment form is given in Appendix F.) All data should be recorded in indelible ink; persons responsible for each measurement and sample collected should be identified. A complete record of the investigation should include the following information:

Case number
: A numeric or alphanumeric identifier unique to the organization of the principal investigator should be assigned; this number should be written on or affixed to every piece of paper, specimen, and report generated during the investigation. All specimen numbers should include the case number and other unique identifiers needed to distinguish specimens from one another.

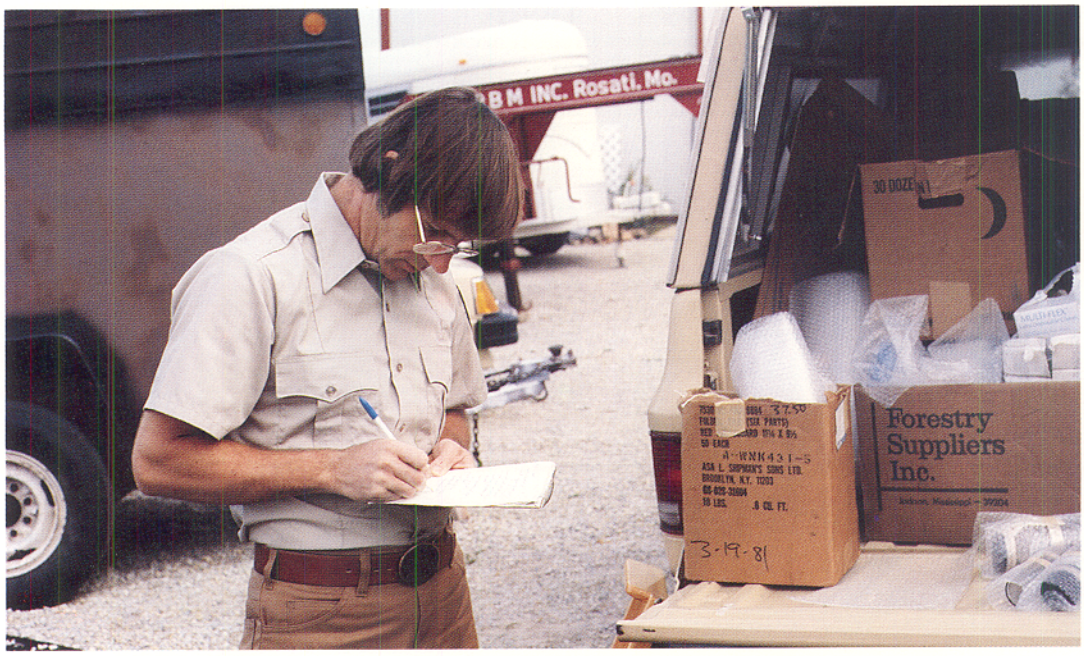
Names of reporting individuals
: The name, address, telephone number, and agency affiliation of each person contributing information about the kill should be recorded. This is particularly important if legal implications are associated with the kill; witnesses may later be asked for affidavits or testimony.

Chronology, species, size, sequence, and location of fish kill
: Estimates of the date and chronology of the kill over time and during a diurnal period are critical to diagnosis. It is essential to record any unusual event (climatic, industrial, agricultural, municipal) or any abrupt change in water conditions that occurred about the time the kill began. The sequence in which fish species died, size and species differences, and changes in the location of the kill over time should be noted. The location should be as specific as possible as to county, township, range, and section, and should be identifiable on a local road map. A map should be drawn or marked by the investigator to establish the extent of area affected. A map or sketch should also be marked to indicate sampling sites as specimens are collected.

Extent of the fish kill
: The magnitude and characteristics of the fish kill should be estimated by using the guidelines provided by the American Fisheries Society (1982). It is particularly important that, in addition to the numerical assessment, photographs or videotapes be taken and fully identified to demonstrate the extent of the fish kill and the effect on species of specific concern. If threatened or endangered species occur in the area, a special notation should be made. Miles of waterway or surface acres affected by the kill should be estimated; the location of any point sources of discharge in or near the affected area should be noted. If possible, a record of the times, nature, and magnitude of discharge from each source should be obtained.

Water quality; limnology
: Limnological characteristics of the water should

When investigating a fish kill, it is important to document the extent of the kill and the numbers, species, and sizes of fish affected. Sometimes so many fish are killed that it is impossible to collect all of them or to even count the numbers. Investigators should then follow estimation procedures recommended by the American Fisheries Society (1982).



Supplies and materials are required for the investigation of any fish kill. It is important to accurately record what containers were used to preserve and store samples, the sample numbers, the case number assigned to the kill, and the name of the person who made the collections.

be assessed immediately (e.g., dissolved oxygen; pH; conductivity; color; temperature; presence of live or dead algae, zooplankton, or insects). If mortality is still occurring during an investigation, additional checks of the water should be made in the early morning, at midday, and in the evening. Any observed changes may be significant in determining the cause of the fish kill. In addition to making these measurements, the investigator should note any anomalies in the system, such as unusual odors, discoloration of plants, dead animals, and the presence of unusual organic layers in sediments.

Vegetation and other organisms

The condition and quantity of all types of aquatic vegetation should be noted. Also, information about the presence and condition (live, dead, decaying) of other vertebrates may be useful in eliminating a number of potential causes of the fish kill.

Condition of fish

A general estimate of the condition of most fish of various sizes and species can be used in interpreting other environmental results: Are there live fish of any species? Are there decaying or freshly dead fish? Do living, but impaired, specimens remain in the system? Moribund specimens make excellent samples for pathology and toxicology. They should be collected, if available. The investigator should note any lesions or other clinical signs that are apparent; the posture of fish at death; and the behavior of affected fish that are not yet moribund or dead. Inspections of other affected animals (vertebrate and invertebrate) should be made in the same way. Photographs are useful in helping to fully describe clinical or behavioral signs.

Background interviews

After the initial site assessment has been made, background interviews should be conducted with any persons who may have specific information about environmental changes associated with the fish kill. People who might add specifics to the timing, extent, or characteristics of the kill should also be interviewed. Care should be exercised to inform all persons or organizations that might be affected by the investigation. Investigators should obtain all necessary authorizations before entering property to collect samples and to do other investigative work. Followup interviews should not delay the submission of initial samples to appropriate diagnostic or support laboratories for analysis.

Investigative summary reports

All data collected in an investigation are potentially useful in narrowing the scope of the investigation. Some of the initial data may not be relevant to the final diagnosis if a complex environmental situation is involved. Therefore, the production of a summary report of the initial investigation, with presentation of the data pertinent to suspected causes, is advisable. The following sections should be included: (1) date and time of preliminary investigation; (2) members of the investigative team; (3) location, sequence, extent, and magnitude of the kill; (4) environmental changes associated with the kill; (5) methods used to investigate and sample the site; (6) observed water quality or other limnological characteristics; (7) condition and characteristics of affected fish; (8) location and time of collection of all samples taken; (9) potential causes of the kill; and (10) suspected cause of the kill.

Followup investigations

For some fish kills, extra site visits are required for two reasons: (1) to follow up on initial sample analysis for confirmatory evidence, and (2) to discover new information that might lead investigators to suspect other possible causes of the kill. In such instances, data should be recorded in the same way they were recorded during the initial survey and the same documentation procedures should be followed. A supplemental report with the same case number and other identifiers as those used in the initial report should be issued to describe the dates, conditions, methods used, and results developed in the followup investigation. This document should be filed as an addendum to the initial report and sent to all offices and laboratories that received the initial report.

Collection and Identification of Samples

Information regarding samples collected at a field site must clearly satisfy four requirements: (1) each collection of samples must be accompanied by a concise background of the events surrounding the kill and a description of the chronology, location, and

characteristics of mortality; (2) each sample must be uniquely identified and related in time and space to the kill; (3) all samples, subsamples, or replicates must be clearly identified and associated by number to the primary sample; and (4) any samples that are likely to become part of a criminal or civil investigation must be accompanied by chain-of-custody forms that have been properly executed (see the next section on legal requirements and Appendix I).

To meet these requirements, the investigator should be able to provide the analytical laboratory with a synopsis of the events related to the fish kill and a list of the samples it will receive. The following information must be available:

1. A unique case number assigned to the fish kill.
2. A unique catalog or lot number for each sample.
3. Submitter's name, address, affiliation, and telephone number.
4. Location of die-off: State, county, nearest road, waterway, or body of water.
5. Environmental information associated with kill: weather, water-flow changes, any pesticide application or discharge, point sources of pollutants, limnology, or water quality characteristics.
6. Extent, timing, and species affected in the kill.
7. Other significant findings.
8. Habitat description: Any features not listed above; primary land use in the area should be mentioned.
9. A complete list and description of the samples sent with unique numbers, weights (if applicable), collector, collection date, collection location, and preservation technique. Work requested should also be included with the listing of the samples.
10. The specific analysis needed for each sample (e.g., chemical, histological, microbial, viral).
11. Any unique quality assurance requirements that are not common practice for the laboratory that will be processing the samples.
12. The time within which results are needed.
13. The names and addresses of the persons to whom the results should be sent.

An example of a synopsis or catalog of samples is given in Appendix G. In addition to information provided in the synopsis sent to each laboratory along with the samples, each sample should be labeled both inside and outside its container. For samples for which it would be inappropriate to place a label inside the container (e.g., in a water sample), a double-packaging system should be used and a second label placed between the two containers. Lids should not be labeled because these can easily be interchanged in the laboratory or field. Labels should include the following information:

1. A unique identifier that includes the case number; subsamples and replicates should have the same identifier and be clearly marked (e.g., A, B, C).
2. Time, date, and location of sample collection.
3. Name of the collector.
4. Description of the sample (as specific as possible).
5. How the sample was collected and preserved (if appropriate to analysis).
6. Any unusual or distinguishing characteristics of the sample that may not remain evident after transport.
7. Weight of the sample (if appropriate to analysis).

The samples and an enclosed synopsis and catalog should be shipped to the analytical-support laboratories. A duplicate copy should be sent by airmail to the designated laboratory official who will receive the samples, along with copies of the complete shipping information for the package. It is recommended that shippers phone the receiving laboratory to confirm that the package was shipped and to provide the name of the carrier to the laboratory.

Packing and shipping should follow procedures prescribed by the receiving laboratory to preserve specimen integrity. Shipments should never originate on Friday, Saturday, Sunday, holidays, or on days preceding holidays. See Chapter 9 for general guidance in the packaging and shipment of samples.

Legal Requirements

If, during the initial investigation, evidence exists that a fish kill occurred as a result of criminal or negligent behavior, the on-site team should immediately contact the appropriate State enforcement agency in the area. If the kill is on Federal lands or waters or involves endangered species or anadromous fishes, the Federal Special Agent for Law Enforcement at the nearest Regional Office of the U.S. Fish and Wildlife Service should be notified (see Appendix H). It is the responsibility of law enforcement officials to determine if prosecution might be

warranted or whether another agency should be contacted. The documentation of potential witnesses, background information, and samples collected is invaluable in producing a strong case. If there are legal questions, the use of chain-of-custody forms (Appendix I) that ensure the integrity of all samples from collection site to final analysis will be required. Such forms from the appropriate enforcement office must be completely filled out with the following information:

1. Legal case number and title.
2. Law enforcement district and official.
3. Source of specimen (person, site location, matrix description, or other information as appropriate).
4. Time and date of collection or seizure.
5. Description and unique identifier for samples to be used as evidence.
6. A complete list of the persons who have had responsibility for guaranteeing the security and integrity of that sample through time (with dated release and receipt signatures for each party) is required. When not being processed, the samples should be secured from unauthorized personnel. When shipped or delivered between responsible parties, the method of transport should be indicated. Receiving parties must guarantee the continuing integrity of each sample. All results from analysis of the samples become part of the case file and should be promptly reported to appropriate law enforcement officials. Persons providing evidence must be able to describe their methodology and defend the validity and significance of their findings in court.

CHAPTER 8

Where to Send Samples for Analysis

Rosalie A. Schnick

Destination of Samples

It is essential that laboratories that can provide various types of analyses under contract be identified in a readily accessible list. Agency policies on submissions of samples should be in place before it is necessary to send samples for analysis. If a fish kill occurs in the public domain and is being investigated by a State or Federal employee, the agency may have its own analytical service laboratory, may use the services of another agency's laboratory, or may have a standing contract for such services with a commercial laboratory. Government employees should check with their own agency for guidance before they ship samples for analysis. A laboratory may require that it be notified as soon as possible after a fish kill of the numbers and kinds of samples being collected, the types of analyses that will be required, the method of delivery, when the samples should arrive, and when the results are needed.

The selection of a contract laboratory to analyze environmental samples is critical to the validity of results developed in relation to a fish kill. The ability of the laboratory to analyze for the types of substances likely to be the cause of a fish kill and to perform analyses in the sample matrices involved is especially important. Because regulatory activities and legal constraints have recently increased, quality assurance requirements must be met (see Chapter 7 for details).

A number of analytical laboratories have developed expertise in performing analyses under contract. Availability is thus not a problem, but the selection of a laboratory that will adequately meet specific needs may require careful thought. The following criteria should be considered when a laboratory is selected (Keith 1988):

A. Criteria for Selecting Analytical and Diagnostic Services
   1. Credibility.
   2. Record of experience in processing the types of matrices involved in aquatic samples (sediment, fish tissue, water, nonfish tissue, and plant tissue).
   3. Quality assurance documentation.
   4. Conformity to needs.
   5. Reliable, timely service.
   6. Knowledgeable contact person at the laboratory.
   7. Price quotations.

B. Procedures to Follow When Evaluating Contractors
   1. Analytical Services Contractors
      Keith (1988) listed the following procedures to follow when evaluating a laboratory to perform analytical services on environmental samples:
      a. Obtain a copy of the laboratory's Quality Assurance Plan.
      b. Ask for information concerning State and National certification.
      c. Obtain a list of current client references and phone some of them about the firm's timeliness of service and acceptability of results.
      d. Visit the laboratory if possible.
      e. Ask for sample reports so you can check to see if quality control data are provided.
      f. Check to see whether confidence intervals, sensitivity of method, detection levels, or limits of quantification are provided.
      g. Verify the experience and capability of the laboratory's staff by checking with past clients.
      h. Verify that the laboratory has done or can do the kind of analysis desired.
      i. Provide audit samples for performance evaluation.
      j. Define your needs and then evaluate price quotations from several contract laboratories. Make certain that the following are included in the price quotation: sample col-

Plasma emission spectrophotometry is often used when a toxic substance is suspected, but the nature of the agent is unknown.

Before samples can be analyzed, the use of extensive sample separation techniques, such as preparative liquid chromatography, may be required.

lection kits with precleaned containers and preservatives; analysis of various blanks; use of standards traceable to the National Bureau of Standards; multipoint calibration curves; disposal of excess samples; customized reports; and computerized reports.

Einerson and Pei (1988) said, "There is not a definite correlation between the quality of the work and the price of the work." They recommended that quality be emphasized in a selection, ahead of price considerations. A good contact at the laboratory is essential and a continuing monitoring program should be established.

2. Diagnostic Services Contractors
Many of the same procedures used to select analytical services contractors can be used when you are selecting diagnostic services contractors. These procedures include the following:

a. Determine if the laboratory will accept your samples on short notice. Does it have adequate facilities, equipment, and staff to do the desired work promptly?
b. Obtain a copy of the laboratory's Quality Assurance Plan and make certain the laboratory can meet quality assurance requirements.
c. Ask for information about State or Federal certification.
d. Obtain a list of current client references and phone some of them.
e. Visit the laboratory if possible.
f. Check sample reports to determine if quality control data are provided.
g. Verify the capability and experience of the laboratory's staff by checking with past clients.
h. Verify that the laboratory can do the kind of cultures and diagnosis you require.
i. Define your needs and then determine whether price quotations meet your needs.

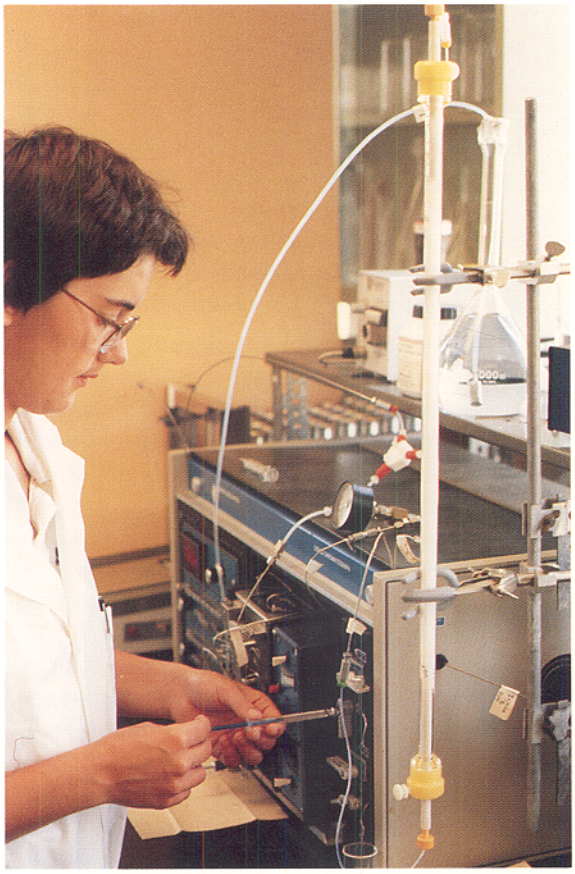
Certain procedures may be required under State or Federal regulations. You may need to contact a State-certified diagnostic laboratory before you are allowed to use a private contractor.

C. Potential Sources of Contractors

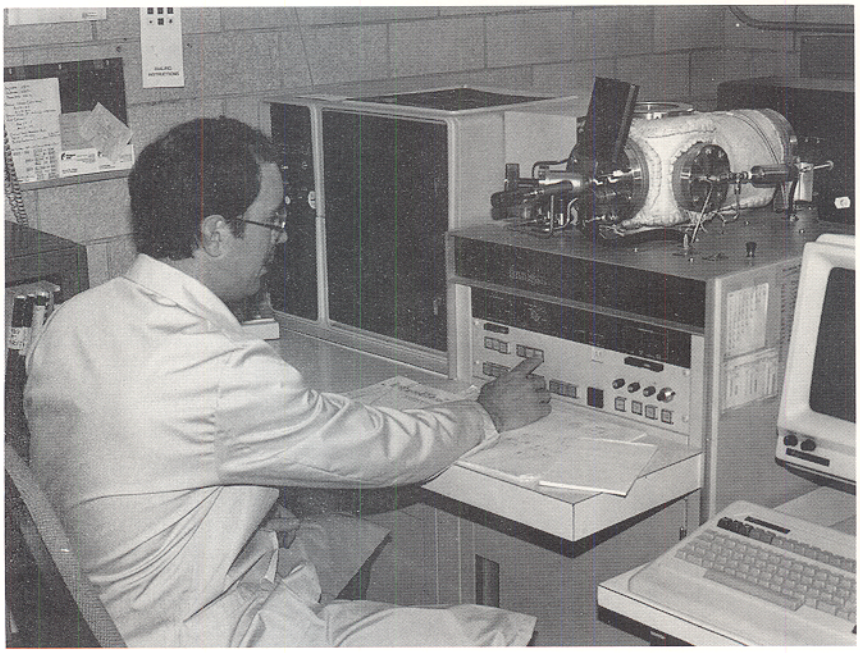
1. Analytical Services
Each State has its own laws and regulations governing the analysis of samples, especially if the samples are taken from State waters. Investigators should check with the State pollution agency for recommendations about where to send the samples. Fish kills on private lands may require that the owner or investigator pay for analytical services.

Some States have their own analytical laboratory; local universities may also offer such services. Listings for private firms are also available. The American Society for Testing and Materials (1988) publishes an annual directory of laboratories that test for various substances. Several categories included in the directory will help identify laboratories that can analyze environmental samples. These categories include animal and fishery products, animal tissues, human tissues, chemicals and chemical products, chromatography, and biological materials. The directory lists the laboratories alphabetically by State and provides helpful alphabetical and subject indexes. It is the responsibility of the contracting agency to determine whether the laboratory can meet quality assurance requirements.

2. Diagnostic Services
Most States offer some form of diagnostic services for aquatic animals. Laboratories that provide disease diagnostic services are listed in two directories that are published annually. *Aquaculture* magazine issues an annual Buyer's Guide and Industry Directory that lists available diagnostic service centers (Honer 1988). The list is arranged alphabetically by State and includes the name, address, and telephone number of each laboratory (Appendix J). However, no details are given about the services provided by each. The National Veterinary Services Laboratories (1989) distributes an annual Directory of Animal Disease Diagnostic Laboratories. Laboratories are listed by State and city. Information provided includes the name of the laboratory, name of director, address, telephone number, affiliation, who may submit specimens, the major species accepted for examination (domestic, wildlife, or zoo animals),



High-performance liquid chromatographs can be used for direct analysis, molecular separations, or the cleanup of extracts from samples collected in the field. There may be an additional charge for preparing sample extracts for analysis.



Sophisticated analytical equipment may be required to identify unknown toxic substances in samples collected at a fish kill.

and the services offered. Services may include bacteriology, clinical pathology, gross pathology, histopathology, mycology, parasitology, serology, or virology. Interested persons should contact the National Veterinary Services Laboratories directly to obtain a copy of the annual directory.

D. Quality Assurance and Quality Control Considerations

Proper procedures must be followed to ensure that the data collected are credible (see Chapter 7 for details on how to proceed).

Cost and Time Required

Although it is not possible to do a complete survey of public and private contractors for costs to analyze contaminants, representative costs charged are available. The following items help determine the estimated cost of having analyses made:

1. Analyses for organic contaminants in various matrices

   Costs for analyzing samples for organic contaminants range from $11 to $450 per procedure for less than 15 samples and $10 to $350 for more than 15 samples (see Table 8.1 for details). If a rapid return of results is needed, the investigator can expect to pay double the prices shown in Table 8.1. Additional charges are assessed if extensive sample preparation or cleanup is involved or if specialized procedures or equipment must be used. If a specific identification or delineation is requested, an added fee may be assessed.

2. Analyses for inorganic contaminants in various matrices

   Cost estimates range from $5 to $48 per procedure for less than 15 samples and from $4 to $38 for more than 15 samples (see Table 8.2 for details). Additional charges will be made if extensive sample preparation or cleanup is required or if specialized procedures or equipment must be used. If a specific identification or delineation is requested, an added fee may also be assessed.

3. Costs for expedited or "RUSH" service

   Charges vary according to the laboratory. A laboratory normally charges twice the normal price or adds a significant surcharge for fast service.

4. Estimated time required for analyzing contaminants

   Laboratories normally perform the analyses within 90 days for the first 300 to 500 samples.

If the level of a contaminant is high, serial dilutions may have to be made before the samples can be analyzed.

Table 8.1. *Approximate costs for organic analyses per procedure in 1989 (modified from Patuxent Wildlife Research Center 1989).*

Procedure[a]	Number of samples: 15 or fewer	Number of samples: 16 or more
Dissection (optional)	$13	$13
A. Homogenization		
Animal	18	16
Plant	16	14
Soil or sediment	8	7
B. Percent moisture	8	8
C. Organochlorine analyses		
Tissue, nonfish	284	272
Tissue, fish	302	288
Soil or sediment	225	217
Water	200	192
D. Aromatic hydrocarbons		
Tissue	249	235
Soil or sediment	204	193
Water	184	174
E. Aliphatic hydrocarbon		
Tissue	149	138
Soil or sediment	116	106
Water	109	102
F. Dicofol		
Tissue	193	171
Soil or sediment	128	113
Water	139	123
G. Endosulfan I and II		
Tissue	193	171
Soil or sediment	128	113
Water	139	123
H. Photomirex and degradates		
Tissue	193	171
Soil or sediment	128	113
Water	139	123
I. Octachlorostyrene		
Tissue	193	171
Soil or sediment	128	113
Water	139	123
J. Chlorophenoxy acid herbicide		
Plant tissue	192	171
Soil or sediment	192	171
Water	134	123
K. Kepone		
Biological tissue (nonfish)	192	171
Fish tissue	234	214
Soil or sediment	138	123
Water	134	123
L. Oil and grease	27	24
M. Quantification of individual arochlors	30	28
N. Mass spectrometry confirmation	255	255
O. Dicofol with OC scan	11	10
P. Endosulfan I and II with OC scan	16	14
Q. Endosulfan sulfate with OC scan	32	28
R. Photomirex with OC scan	27	26
S. Octachlorostyrene with OC scan	19	16
T. Organophosphate pesticide scan, including mass spectrometry	300	250
U. Carbamate pesticide scan, including mass spectrometry	300	250
V. Combined organophosphate and carbamate scans, including mass spectrometry	450	350

[a] OC scan = organochlorine scan.

The delivery time usually is less than 90 days; for expedited service, it is normally 15 working days.

5. Forms required for use in reporting data and conclusions

Each laboratory has its own forms that need to be filled out to ensure proper identification of samples, analysts, and data.

Diagnostic services are available from a number of sources—Federal, State, and private. Federal and State laboratories generally provide assistance without charge, but they may have restrictions on the types of samples they will accept or on the agencies to whom they provide services. The laboratory should always be contacted before the samples are sent. There are few practicing veterinarians in

Table 8.2. *Approximate costs for inorganic analyses per procedure in 1989 (modified from Patuxent Wildlife Research Center 1989).*

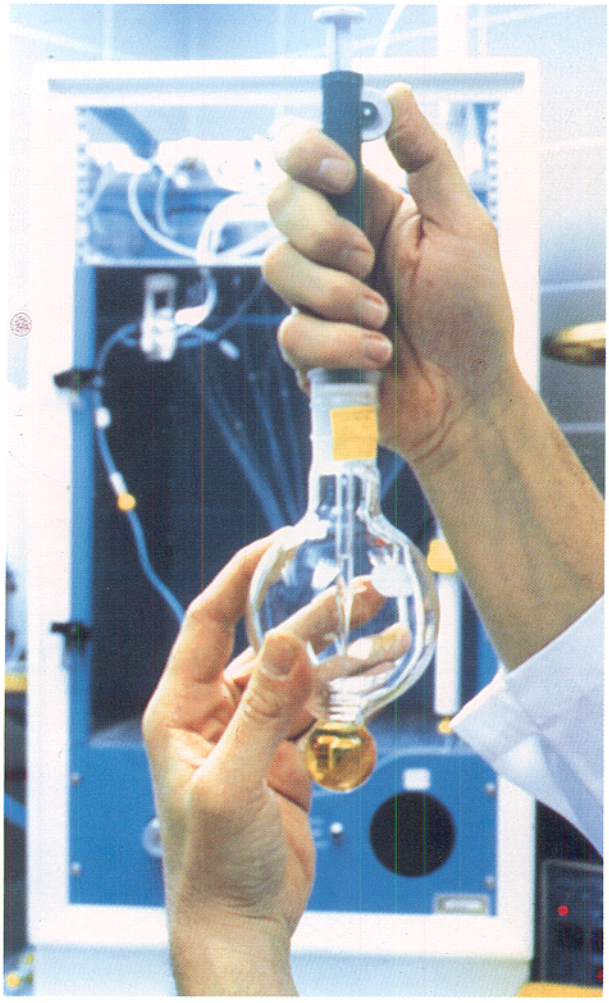
Procedure	Number of samples	
	15 or fewer	16 or more
Dissection (optional)	$34	$26
A. Homogenization		
Animal	12	9
Plant	9	7
Soil or sediment	11	8
B. Lyophilization	7	6
C. Percent moisture	7	5
D. ICP analysis[a]		
Animal tissue	44	33
Plant	44	33
Soil or sediment	44	33
Water	43	32
E. Precon for ICP[a]		
pH (3 or 6)	12	9
F. Arsenic analysis		
Animal tissue	29	23
Plant	29	23
Soil or sediment	30	24
Water	29	23
G. Selenium analysis		
Animal tissue	29	23
Plant	29	23
Soil or sediment	30	24
Water	29	23
H. Mercury analysis		
Animal tissue	27	22
Plant	27	22
Soil or sediment	28	23
Water	27	22
I. Other metals by HGA[b]		
Animal tissue	28[c]	22
Plant	28	22
Soil or sediment	28	22
Water	28	22
J. Total volatile solids		
Soil or sediment	9	9

[a] ICP = Inductively coupled plasma emission spectroscopy. ICP yields a scan of many elements (16–23) at various detection limits that are usually higher than those determined by atomic absorption methods.
[b] HGA = Graphite furnace analyses for separate elements. Prices are on a per element basis. This analysis provides a lower limit of detection than the ICP for most elements.
[c] Prices per element; if three elements (e.g., cadmium, lead, copper) are required, multiply these price ranges by three.

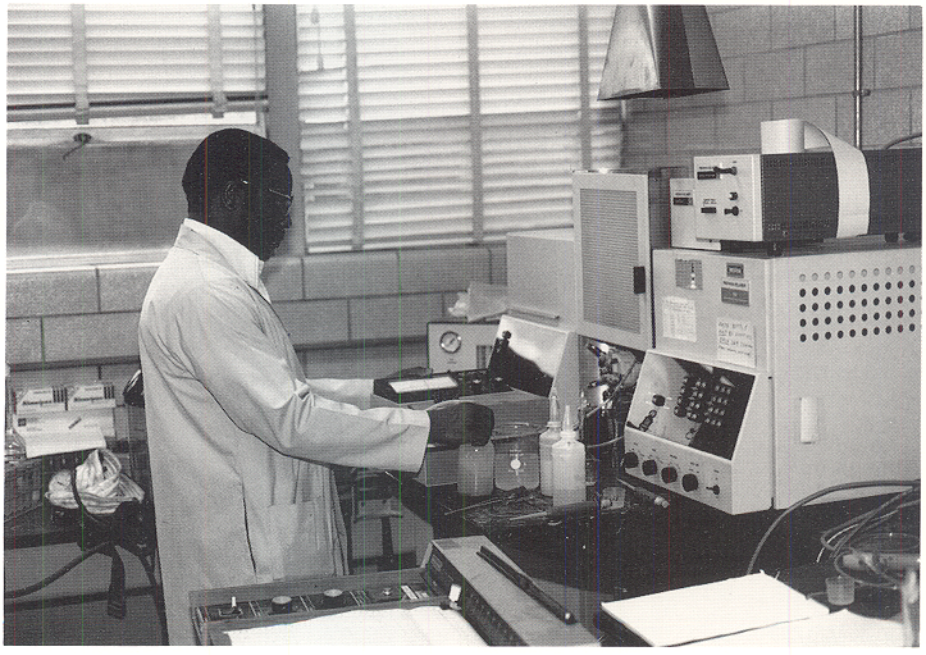
fisheries, but a number of universities provide diagnostic assistance for a fee. If a fee is charged, the rate depends on the type of pathogen involved, the level of identification requested, and the number of samples to be processed. Checks for viruses will be expensive because laboratory work involves cell cultures, serological tests, and possibly electron microscopy.

Costs to the investigating agency for histological examinations of tissues vary, depending on the policy of the agency furnishing the service. State agriculture departments or universities may have veterinary or human health services that can make the evaluations. They may absorb the cost or they may have a policy of charging the investigating agency for partial or full reimbursement. The assessment of costs should be determined before samples are submitted.

Costs also vary if commercial laboratories are used. Slide preparations are generally $3 to $5 each. The pathologist's report is an added cost that may be calculated by the slide, fish, or hour. For a large number of samples, the total cost per slide could be less than $10, but might be $25 or more.



Before a sample can be analyzed, extraction and cleanup procedures are usually performed.



Atomic absorption spectrophotometry is a useful procedure when environmental samples are checked for metals and minerals.

CHAPTER 9

How to Ship Samples

Lee A. Barclay

Introduction

Shipping samples from one point to another is fraught with potential pitfalls. Careful planning and attention to details reduce lost or damaged shipments and preserve valuable evidence that may be needed in future legal proceedings. The shipper should have complete information on hand about the carrier, flights, schedules, forwarding companies, and the telephone numbers of accountable personnel for each involved party before a shipment is made. Notify the receiving laboratory of the scheduled arrival time and ask that you be phoned immediately after receipt of the shipment. If the samples do not arrive when expected, both you and the laboratory should contact the carrier to begin an immediate search for the lost samples. Shipments should never be made on Fridays, weekends, or during holiday periods.

Chain of Custody and Other Legal Considerations

A chain of custody must be maintained on all potential evidence to ensure that it will be admissible in court. It may become necessary to prove that the evidence collected at a particular site is the same as that on which the testimony is based. When potential evidence is passed from one person to another for storage or testing, a chain-of-custody form (Appendix I) must be signed and dated by each person involved. Each transfer must be clearly documented on the form. Every person who handled the evidence may be required to testify in court, before data from a particular sample can be admitted as evidence. Information on chain-of-custody forms, evidence tags, and other similar material can probably be obtained from an agency's law enforcement division.

Handling of Samples

Sample Selection

The samples required will depend on the suspected cause of the fish kill. If a toxic chemical is suspected, specific types of samples must be collected and handled according to precise guidelines. Information on collecting and packaging samples for chemical analyses is provided in Chapter 4 in the section entitled "Sample Collection for Suspected Toxic Substances." For kills in which infectious or parasitic organisms are believed to be involved, Chapter 6 provides information on how the types of organisms affect the kind of samples needed and how the samples are to be handled. General information on how to select and ship fish samples was published by Wellborn (1985). (See Appendix K.)

Preservation

Several points must be remembered in preserving samples:

1. Plan sample preservation procedures before making any collections
   - Consult the laboratory's analytical staff. Sample preservation requirements may vary with the kinds of analyses to be performed.
   - Have all necessary equipment and supplies when you go to the collection sites.
   - Prepare a checklist of needed supplies and containers and consult it when preserving samples for shipment. Do not rely on your memory; memories can be faulty.
2. Do it now and do it fast
   - Some contaminants are highly ephemeral, others are less so. Nevertheless, the sooner steps are taken to prevent chemical deterioration or to keep degradation to the feasible

minimum, the better are the chances of obtaining valid analytical data.

3. Maintain active preservation
   - Often samples must be stored for considerable periods before analyses can be arranged. Check samples frequently to make sure they do not thaw, dry out, or otherwise deteriorate.

4. Have needed equipment and supplies available
   - Freezer capable of maintaining a temperature of −20° C or lower and that can be locked to meet chain-of-custody requirements.
   - Buffered formalin (10%).
   - Wet ice—universally available.
   - Dry ice—not available at all places or during all seasons. You should develop a list of sources in your area, including dates and times when dry ice is available.
   - Durable ice chests are preferred containers. Styrofoam ice chests are adequate only if boxed. Heavy-gauge cardboard boxes are suitable for short periods if lined with Styrofoam (generally available in 4-foot × 8-foot sheets, 1 to 2 inches thick, from building supply stores). Reusable Styrofoam-lined cardboard shipping containers are available from commercial vendors.

Packaging

Proper packaging is a key element in shipping. Samples intended for chemical analyses must be wrapped, packaged, and stored in a way that will prevent deterioration and cross-contamination. Samples that are handled improperly often are not worth collecting or keeping. Minimizing the risk of cross-contamination demands that any wrapping or containers in direct physical contact with sample materials be chemically clean and chemically inert.

Scrupulous adherence to chemical sanitation is mandatory if analyses are to be performed for suspected organic contaminants. Planning and preparation are necessary to ensure that appropriate containers and packaging supplies are on hand and in field-ready condition.

Glass containers or other fragile materials must be kept separated and immobilized in the shipping containers. Foam rubber sheets, "bubble wrap," or crumpled paper will serve. The shipping container should be strong enough to sustain rough handling. If sample materials must be kept chilled or frozen, the jars or plastic bags can be packed in wet or dry ice as described below.

Chilled samples

Some materials must be kept chilled, but should not be frozen (e.g., fish or wildlife samples for necropsy). Durable ice chests are preferred. Thick Styrofoam ice chests may be acceptable, but they must be packed in strong cardboard boxes. Ice should be sealed in plastic bags to prevent leakage as the ice melts. Add packing material (such as plastic "peanuts") to reduce shifting when shipping containers are handled.

Frozen contents

In most situations, frozen materials must be packaged in dry ice. Although dry ice is expensive, the cost is small when measured against the value of lost evidence or destroyed samples. There is no rule of thumb governing the quantity of dry ice to use, but do not skimp. Allow for shrinkage and wrap the dry ice in heavy paper to reduce evaporation. Pack enough to keep the samples frozen for 24 hours beyond the scheduled time of arrival; 4.5 kg in a Styrofoam box (38 × 38 × 38 cm) provides about 48 hours of freezing potential. Do not pack dry ice in airtight containers; doing so might cause the containers to burst. Always use gloves when handling dry ice.

Transportation of Samples

Transporting samples from one point to another (as from collection site or field office to laboratory) can be frustrating and wasteful. Investments in sample collection and preservation come to naught if the materials are lost en route. Shipments sometimes go astray, usually with disastrous results when the samples are perishable. Planning and careful attention to details reduce the likelihood of lost or damaged shipments.

The cost of shipping samples is important, but maintaining the integrity of the samples must be the paramount consideration. Low up-front costs can be very high if the samples are lost or decompose en route.

Hand Delivery

When feasible, personally delivering the samples to the analytical laboratory is the best alternative. Little packing is required, the chain of custody is easily maintained, receipt of the samples can be acknowledged at once, and the shipping containers used are immediately available for further use.

Air Shipment

A. Carriers
   1. Air express (e.g., Emery, Federal Express, DHL)

      Air express is preferred over air freight. This service should be used whenever possible because air express agencies are dependable and have an excellent system for tracking lost shipments. They also provide door-to-door pickup and delivery service.

   2. Air freight (use only scheduled airline service)

      Air freight is satisfactory for direct city-to-city shipments. However, air freight shipments are sometimes delayed en route when higher priority shipments preempt all available space on the aircraft. If possible, the shipper should avoid shipments that require a change of planes en route, and—at all costs—shipments that require a change in airlines en route.

B. Preparation for Air Shipments
   1. Be sure that the recipient's name, street address, and telephone number are marked plainly on containers.
   2. If applicable, mark PERISHABLE on the outside container in bold letters.
   3. If the contents are packed in dry ice, note this on the containers as follows:

      DRY ICE
      _____ POUNDS

   4. If shipping on a Government Bill of Lading, have this form filled out before you turn the shipment over to the carrier.
   5. Complete the airbill. Identify the contents as BIOLOGICAL SAMPLES and indicate that they are perishable. Put a dry ice notation on the airbill. If the shipment is to be picked up on arrival, note this on the airbill and include the name and telephone number of the recipient. Special "Hold and Notify" labels may be required.
   6. Get a copy of the airbill, flight numbers, and time schedules before you release the shipment.

C. Consignment to Air Carrier
   1. Phone the receiving laboratory (or other recipient) to ensure that someone there will take and accept the shipment. Mondays through Thursdays are preferred days for shipping unless special arrangements have been made.
   2. Phone the carrier to determine three items of information: (1) departure time, routing (e.g., flight numbers if known), arrival time, and waybill number; (2) how and where the shipment will be delivered; and (3) method of payment required or permitted.
   3. Before shipment, obtain information on the airline identity, flight numbers, expected arrival time, and the number of the airline bill of lading. If a forwarding or air express company is involved, be sure to get its name and after-hours and daytime telephone numbers.

Bus Shipment

In local areas, many towns and cities have daily bus service that accepts parcels and can give 24-hour delivery. This method of shipment is usually reliable.

U.S. Postal Service

Avoid the U.S. Postal Service if samples are perishable. If the contents are fragile, pack them with special care. Check for size limitations on packages.

Followup

As soon as practicable after you consign a shipment to a carrier, you should advise the receiving laboratory by phone that the shipment is en route. It is important to give the laboratory the airbill or waybill number and the names and telephone numbers of the carriers. You should also describe the shipment (number, sizes and types of containers, and relevant labels). If something goes awry, the carrier will use this information to trace the shipment. Also

advise the recipient whether the shipment was sent collect, prepaid, or on a Government Bill of Lading. If you are making a collect shipment, mail the original airbill to the recipient, but be sure to keep a copy. Have the recipient phone back to advise you of receipt or nonreceipt of the shipment.

Safety Considerations

Dry ice can be dangerous. Always use gloves when handling it. Do not seal shipping containers; be sure the expanding gas can escape so that the containers do not burst during shipment.

CHAPTER 10

Writing the Report

Fred P. Meyer and Bernard L. Berger

Introduction

Preparation of a written report after the completion of field and laboratory studies is sometimes regarded as a necessary evil. As a result, this portion of a fish kill investigation is often given low priority because of other demands. Investigators must realize that a written record is the only way information developed in a fish kill investigation can be made available to other interested parties or provide clear documentation for future workers. The written record also provides documentation if litigation results from the fish kill.

The completion report should be sufficiently detailed and well written to survive peer review and withstand challenges from opposing parties. If possible, sections describing the work done by specialists should be written by the persons who did the work. Interpretation of the data should be done in consultation with those who performed the analyses.

Content

A well-written completion report is excellent preparation for testimony in court. It should be written in straightforward, semitechnical language so that attorneys, professional scientists, and informed lay persons can clearly understand what was observed, what was done, why it was done, what conclusions were reached, and the basis for making those conclusions. Writers should report only factual information and any inferences that can be drawn from and supported by the data. Accusatory or inflammatory language, editorializing, emotional statements, and personal opinions should be avoided.

The report should be professional in appearance. The material should be securely bound to ensure that no pages are lost during handling or filing. A cover or cover sheet should be added to clearly identify the case involved.

The completion report of a fish kill investigation must clearly, objectively, and explicitly present all pertinent information. It should include the location and area affected; the species, sizes, numbers, and value of the fish that were killed; and a detailed description of the on-site scene. The report should list the following: what samples were collected, where the samples were processed and by whom, what analyses were done and by what methods, and what data resulted from the analyses. The completion report must discuss the relevance of all observations, evidence, and laboratory data; summarize the findings; and present conclusions. Photographs and tables of data should be included to document the nature and extent of the kill, as well as any laboratory results that were used in reaching the final conclusion. Citations of pertinent references should be included if decisions made in the case were based on such documents.

Any recommended followup actions should also be listed, along with an explanation of the reasons for taking the actions. The following types of questions should be addressed: (1) Should the fishery be closed and, if so, for how long? (2) Are surviving fish safe to eat? Will a health advisory need to be issued (see Fig. 10.1)? (3) What damage has been done to the ecosystem? (4) Will the killed fish be replaced by stocking? (5) What action will be taken to prevent future recurrences? (6) Should a claim for damage be filed against the responsible party?

Spills or blatant discharges that cause acute kills usually generate mandated corrective measures. However, chronic toxicity or inadvertent kills may not trigger remedial initiatives. An example of such a situation might be low stream flows during dry weather that result in toxic concentrations of chemicals that would be diluted to nontoxic levels under normal flow conditions. If the stream flow is controlled by reservoir releases, the rate of release

Pollution often destroys the aesthetics of a stream and sometimes renders the fish unfit for human consumption. It may be necessary to prohibit fishing if an investigator indicates a public health hazard.

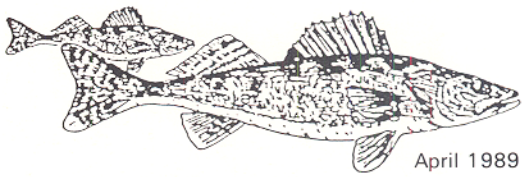
might be increased during low flows to maintain the required dilution.

Although not all fish kills are due to pollution, chemical spills, or toxic contaminants, the public usually is concerned about potential health and human safety issues that should be addressed. There also may be important social or political ramifications. A general news release summarizing the findings and the final recommendations should be drafted and submitted to the appropriate agency official for review. A decision about whether the draft is to be released to the news media should be made by upper-level management unless the investigator has been authorized to speak for the agency.

Disposition of the Report

Although completion reports of fish kill investigations usually do not merit publication in scientific journals, copies of the final reports should be sent to the State pollution control agency and Form 7500-8 (Fig. 10.2) should be filed with the EPA. Copies should also be placed in a permanent file of the investigating agency so that the report can be promptly retrieved upon demand. For retrieval or reference, information on the cover of the report should include the body of water or stream affected, the date of the fish kill, the case number, the agency involved in the investigation, and the name of the author.

Healthy advisory for people who eat sport fish from Wisconsin waters



April 1989

This publication explains which sport fish species in Wisconsin lakes and rivers do not meet health standards for a number of toxic pollutants. It describes health precautions you should consider before you decide to eat fish you've caught from waters where contaminants pose a problem.

It's important to note that this guide features two different sets of health advice: one for fish contaminated with PCBs and pesticides (pages 1, 2 and 3), and another for fish contaminated with mercury (pages 3 through 7). Generally, people who should take the most precautions are children aged 18 or less, women who intend to have children, and women who are pregnant or breastfeeding.

PCB and pesticide contamination in fish

	Group 1 These fish pose the lowest health risk.	**Group 2** Women and children should not eat these fish.	**Group 3** No one should eat these fish.
	See page 3 for specific health advice on each group of fish.		
LAKE MICHIGAN	Lake trout up to 20" Coho salmon up to 26" Chinook salmon up to 21" Brook trout Rainbow trout Pink salmon Smelt Perch	Lake trout 20 to 23" Coho salmon over 26" Chinook salmon 21 to 32" Brown trout up to 23"	Lake trout over 23"* Chinook salmon 32 to 35" Chinook salmon over 35"* Brown trout over 23" Carp Catfish
GREEN BAY south of Marinette and its tributaries (except for Lower Fox River), including the **Menominee, Oconto, and Peshtigo Rivers**, from their mouths up to the first dam	Rainbow trout up to 22" Chinook salmon up to 25" Brook trout up to 15" Smallmouth bass Northern pike up to 28" Perch Walleye up to 20" Brown trout up to 12" Bullhead *White sucker*	Splake up to 16"	Rainbow trout over 22" Chinook salmon over 25" Brown trout over 12" Brook trout over 15" Carp* Splake over 16" Northern pike over 28" Walleye over 20"* White bass
LOWER FOX RIVER from its mouth at Green Bay up to the DePere Dam		Northern pike *White sucker*	White bass* Walleye Carp* Drum* Channel catfish*
LOWER FOX RIVER from the DePere Dam up to the Neenah-Menasha Dam	White bass Walleye up to 15" Northern pike Perch White sucker	Walleyes over 15" Bullheads	Carp over 17"
EAST AND WEST TWIN RIVERS from their mouths up to the first dam	Perch Northern pike Crappie Smallmouth bass		Carp Catfish*
	NOTE: Follow Lake Michigan advisory above for trout and salmon.		
MANITOWOC RIVER from its mouth up to the first dam	NOTE: Follow Lake Michigan advisory above for trout and salmon.		Catfish*
SHEBOYGAN RIVER in Sheboygan County from the dam at Sheboygan Falls to the Coast Guard station in the City of Sheboygan, including **Greendale and Weedens Creeks**	Coho salmon up to 26" Chinook salmon up to 21"	Rainbow trout Brook trout Coho salmon over 26" Chinook salmon 21 to 32"	*Bluegill* *Crappie* Rock bass* Carp* Smallmouth bass* Walleye* Northern pike* Brown trout Catfish* Chinook salmon 32 to 35" Chinook salmon over 35"*
MILWAUKEE RIVER in Milwaukee County (includes **Milwaukee Harbor**) from its mouth up to the North Avenue dam, including the Kinnickinnic **and Menomonee Rivers**	Perch NOTE: Follow Lake Michigan advisory above for trout and salmon.		Crappie Northern pike Carp* Redhorse Smallmouth bass

Wisconsin Department of Natural Resources • Wisconsin Division of Health PUBL-IE-019 89REV

Fig. 10.1. Example of a health advisory (modified from Wisconsin Department of Natural Resources 1989).

PCB and pesticide contamination in fish

	Group 1 These fish pose the lowest health risk.	Group 2 Women and children should not eat these fish.	Group 3 No one should eat these fish.
	See page 3 for specific health advice on each group of fish.		
MILWAUKEE RIVER from the North Avenue dam in Milwaukee County upstream to the Lime Kiln Dam at Grafton (Ozaukee County)	Rock bass up to 8.5"	Redhorse	Northern pike Carp
CEDAR CREEK from the Milwaukee River up to Bridge Road in the Village of Cedarburg			All species*
ROOT RIVER in Racine County from its mouth upstream to the Horlick Dam in the City of Racine	Carp up to 21"		Carp over 21"*
	NOTE: Follow Lake Michigan advisory on previous page for trout and salmon.		
PIKE RIVER in Kenosha County from its mouth up to Carthage College in the City of Kenosha			Carp
	NOTE: Follow Lake Michigan advisory on previous page for trout and salmon.		
LAKE SUPERIOR	Lake trout up to 30"		Lake trout over 30"
	NOTE: Also see advice for mercury-contaminated walleye in the St. Louis River, Douglas County, page 4.		
UPPER FOX RIVER above Swan Lake in Columbia County downstream to Portage			Carp
UPPER FOX RIVER from Portage in Columbia County north to but not including Buffalo Lake	Northern pike	Crappies Bullhead	Largemouth bass Carp
BIG GREEN LAKE in Green Lake County	Lake trout under 32" Carp		Lake trout over 32"
WISCONSIN RIVER from the Nekoosa Dam to the Petenwell Dam (Petenwell Flowage)	See advice on mercury-contaminated fish in the Wisconsin River on pages 3 through 7 under Adams, Juneau, Lincoln, and Wood Counties.		Carp
WISCONSIN RIVER at Wisconsin Dells to the Prairie du Sac Dam (includes Lake Wisconsin)		Lake sturgeon	
ST. CROIX RIVER from Stillwater, Minnesota, to the Mississippi River at Prescott, Wisconsin	Drum *White bass* *Carp up to 26"* *Walleye* *Flathead catfish up to 26"* Sauger *Buffalo up to 23"*		*Channel catfish* *Buffalo over 23"* *Carp over 26"* *Flathead catfish over 26"*
	NOTE: Also see additional advice for mercury-contaminated fish in the St. Croix River under Douglas, Pierce, Polk, and St. Croix Counties, pages 4 through 6.		
MISSISSIPPI RIVER off Pierce and Pepin Counties **from Prescott down to and including Lake Pepin** (Pools 3 and 4)	Drum Walleye *Sauger* White bass up to 13" Flathead catfish up to 30" *Buffalo up to 18" (Pool 3)* *Buffalo up to 20" (Pool 4)* *Channel catfish up to 16" (Pool 3)* Channel catfish up to 21" (Pool 4) *Carp up to 21"*	*Channel catfish 16" to 23" (Pool 3)* *Channel catfish 21" to 23" (Pool 4)*	White bass over 13" *Buffalo over 18" (Pool 3)* *Buffalo over 20" (Pool 4)* Flathead catfish over 30" *Channel catfish over 23"* *Carp over 21"*
MISSISSIPPI RIVER from below the dam at Alma to the dam at Trempealeau (Pools 5, 5A, and 6)	Flathead catfish Carp up to 24" Channel catfish up to 21" *Buffalo* *Drum* *Walleye* *Sauger* White bass	*Carp over 24"* *Channel catfish 21 to 25"*	*Channel catfish over 25"*
MISSISSIPPI RIVER from below the dam at Trempealeau to the dam at Lynxville (Pools 7, 8, and 9)	*Buffalo* Walleye Crappie Flathead catfish *Channel catfish up to 24"* *Drum* White bass Carp	*Channel catfish over 24"*	

HEALTH ADVICE for the charts on pages 1 & 2	
GROUP 1:	Contaminant levels in 10 percent or less of tested Group 1 fish are higher than one or more health standards. EATING GROUP 1 FISH POSES THE LOWEST HEALTH RISK. Trim fat and skin from Group 1 fish before cooking and eating them.
GROUP 2:	Contaminant levels in more than 10 percent but less than 50 percent of tested Group 2 fish are higher than one or more health standards. CHILDREN UNDER 15, NURSING MOTHERS, PREGNANT WOMEN, AND WOMEN WHO INTEND TO HAVE CHILDREN SHOULD NOT EAT GROUP 2 FISH. You should also limit your overall consumption of other Group 2 fish, and trim skin and fat from these fish before cooking and eating them. (NOTE: See specific health advice for mercury-contaminated fish in the Petenwell Flowage and Lake Superior elsewhere in this publication.
GROUP 3:	Contaminant levels in 50 percent or more of tested Group 3 fish are higher than one or more health standards. NO ONE SHOULD EAT GROUP 3 FISH. *Ninety percent or more of Group 3 fish marked with an asterik (*) contain contaminant levels higher than one or more health standards.

U.S. Food & Drug Administration and Wisconsin Division of Health Standards for Contaminants Commonly Found in Sport Fish

PCBs	2 parts per million (ppm)
DDT	5 ppm
Toxaphene	5 ppm
Chlordane	0.3 ppm
Dieldrin	0.3 ppm
Mercury	0.5 ppm
Dioxin	50 parts per trillion

SOURCE: Wisconsin Division of Health and Wisconsin Department of Natural Resources April 1989

MERCURY CONTAMINATION IN FISH

HOW TO USE THE MERCURY ADVISORY

1. Measure each fish you catch from the tip of its nose to the end of its tail.
2. Look at the list of lakes that begins below, which names all Wisconsin waters that are subject to a health advisory for mercury in fish. See if the lake you caught your fish from is on the list. If it isn't, then DNR hasn't tested fish in your lake, or tested fish meet health standards. (Data on tested lakes is available from water resources staff specialists in DNR district offices.)
3. If your lake *is* on the list, check to see what health advice corresponds to the mercury content of the fish you caught. Do this by finding the number — either 1, 2, 3, or 4 or the symbol "★" — in the list that corresponds to the size and species of your fish and the lake and county in which you caught it. Match that number to the group number below (GROUP 1, GROUP 2, etc.) to find out whether you should eat the fish you caught and how often.

HEALTH ADVICE FOR MERCURY-CONTAMINATED FISH

GROUP 1: **Pregnant women should eat no more than one meal a month of Group 1 fish.** *Everyone else may eat unlimited amounts of Group 1 fish.* Skin-on fillet samples average 0.5 ppm mercury or less.

GROUP 2: **Pregnant or breastfeeding women, women who plan to have children, and children under 18 should not eat Group 2 fish.** *Everyone else should eat no more than 26 meals of Group 2 fish a year. Eat no more than 13 of these 26 meals in any one month. Space the remaining 13 meals over the rest of the year at the rate of one or two meals a month.* Skin-on fillet samples average 0.5 to 0.75 ppm mercury.

GROUP 3: **Pregnant or breastfeeding women, women who plan to have children, and children under 18 should not eat Group 3 fish.** *Everyone else should eat no more than 13 meals of Group 3 fish a year. Eat no more than 7 of these 13 meals in any one month, and space the remaining 6 meals over the rest of the year at a rate of one meal a month.* Skin-on fillet samples contain an average of 0.75 to 1.0 ppm mercury.

GROUP 4: **NO ONE SHOULD EAT GROUP 4 FISH.** Skin-on fillet samples contain an average mercury level above 1.0 ppm.

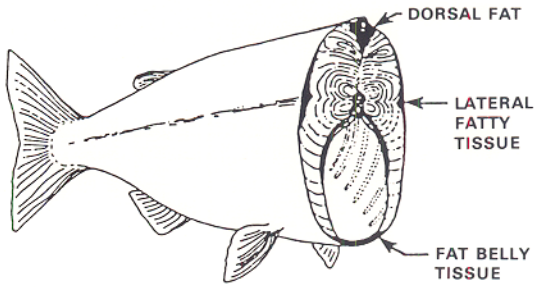
* : This symbol means not enough information on a particular size and species of fish was available to issue health advice.

COOKING, CLEANING, AND EATING PCB-CONTAMINATED FISH

PCBs and many pesticides usually build up in a fish's fat deposits and just underneath the skin rather than in muscle tissue. By removing the fat and skin before you cook and eat these fish (see directions below), you can reduce PCB and pesticide levels, though not always enough to meet health standards.

To reduce PCBs in fish you catch:

- Remove all skin.
- Cut away the dark fat on top of the fish along its backbone.
- Slice off fat belly meat along the bottom of the fish.
- Cut away the dark, V-shaped wedge of fat located along the lateral line on each side of the fish.
- Bake or broil skinned, trimmed fish on a rack or grill so more fat drips off. Discard any drippings. Fish may also be cooked in liquids, but discard the resulting broth.



COOKING, CLEANING, AND EATING MERCURY-CONTAMINATED FISH

Mercury is distributed throughout a fish's muscle tissue (the part you eat) and organs, rather than in fat and skin. **You cannot reduce mercury levels by removing fat or skin or by cooking a fish a certain way.**

NOTE: If you catch fish from **both** Groups 2 and 3 in the mercury advisory, use the conversion chart below to figure out how many combined meals of fish from these groups you may eat in one month or one year.

Monthly Consumption

If you eat this many **Group 2** fish meal in one month...	...eat no more than this many **Group 3** fish meals that same month:
0	7
2	6
4	5
6	4
8	3
10	2
12	1
13	0

Annual Consumption

If you eat this many **Group 2** fish meals in one year...	...eat no more than this many **Group 3** fish meals that same year:
0	13
2	12
4	11
6	10
8	9
10	8
12	7
14	6
16	5
18	4
20	3
22	2
24	1
26	0

FISH ADVISORY
Wisconsin Dept. of Natural Resources
P.O. Box 7921
Madison, WI 53707

Form Approved OMB No. 2040-0087 Approval expires 5-31-89

United States Environmental Protection Agency
Washington, DC 20460

(For OWRS Use Only)

EPA

Report of Pollution-Caused Fish Kill or Abnormality

1. Location *(Name of body of water; latitude-longitude)*

2. Date of Kill or Abnormality

3. Nearest Town/Range/Section/County

4. State/ZIP Code

5. Public Drinking Water Supply Affected? ☐ Yes ☐ No

6. Waterbody Type: ☐ Impoundment ☐ Estuary/bay ☐ Stream/canal ☐ Wetland ☐ Ocean/gulf ☐ Lake ☐ Other *(specify)*

7. EPA River Reach Number *(or, if unknown, USGS Hydrologic No.)*

8. Has a kill or abnormality been observed at this site before? ☐ Yes ☐ No ☐ Don't know

9. Primary Land Use(s) at Site of Kill or Abnormality:
- ☐ Agricultural
- ☐ Industrial
- ☐ Mining
- ☐ Office/shopping
- ☐ Residential
- ☐ Silvicultural
- ☐ Urban
- ☐ Wildland
- ☐ Other *(specify)*

10. Cause(s) of Kill or Abnormality:
- ☐ Bacteria/viruses
- ☐ Cyanides and Phenols
- ☐ Inorganic Chemicals (Metals)
- ☐ Mixed Chemicals
- ☐ Nutrients
- ☐ Organic Chemicals
- ☐ Oxygen Deficiency
- ☐ Pesticides, Herbicides, Etc.
- ☐ Petroleum (Oil and Grease)
- ☐ pH
- ☐ Radionucleides
- ☐ Sedimentation/Silting
- ☐ Temperature
- ☐ Turbidity
- ☐ Unknown
- ☐ Other *(specify)*

11. Specific Pollutant(s)

12. Source(s) of Pollution:
- ☐ Agricultural Applications
- ☐ Animal Feedlot/Waste Operations
- ☐ Aquatic Weed Treatment
- ☐ Construction (Road, Bridge, Other)
- ☐ Dredging
- ☐ Erosion
- ☐ Eutrophication
- ☐ Industrial *(Check Category in item 21 on reverse side)*
- ☐ Irrigation
- ☐ Land Disposal-Industrial
- ☐ Land Disposal-Municipal
- ☐ Land Form Alteration
- ☐ Land Treatment (Effluent Disposal)
- ☐ Mine Drainage
- ☐ Pipeline Rupture
- ☐ Power/Energy Discharge
- ☐ Power/Energy Intake
- ☐ Sewage Treatment, Advanced
- ☐ Sewage Treatment, Primary
- ☐ Sewage Treatment, Secondary
- ☐ Silvicultural Operations
- ☐ Storm or Combined Sewer
- ☐ Transportation ☐ Rail ☐ Air ☐ Truck ☐ Barge or boat
- ☐ Urban Runoff
- ☐ Unknown
- ☐ Other *(list)*

Fish Kill

13. Type of Fish Killed

Game	%
Commercial	%
Other	%
Total	100%

14. Estimated Total Number Killed:

15. Severity ☐ Total ☐ Heavy ☐ Moderate ☐ Light

17. Extent of Area Affected: A. Miles of stream; B. Acres of lake

18. Duration of Critical Effect: A. Days; B. Hours

16. Species Affected/Est. No. Each *(indicate if rare/endangered)*

Abnormality

19. Abnormality(ies) Observed: ☐ Tumor ☐ Disease ☐ Lesions ☐ Deformities ☐ Eye disorder ☐ Other *(specify)*

20. Additional Remarks *(Include observed effects on other biota)*

Reporting Official	Agency Mailing Address	Date of Report

EPA Form 7500-8 (Rev. 4-86) Previous editions are obsolete

Fig. 10.2. U.S. Environmental Protection Agency form for reporting a fish kill caused by pollution.

21. Industrial Categories *(Refer to Question 12; mark (x))*

- ☐ 01 Aluminum forming
- ☐ 02 Asbestos manufacturing
- ☐ 03 Battery manufacturing
- ☐ 04 Builders' paper
- ☐ 05 Carbon black manufacturing
- ☐ 06 Cement manufacturing
- ☐ 07 Clay, gypsum, refractory, & ceramic products
- ☐ 08 Coal mining
- ☐ 09 Coil coating (and canmaking)
- ☐ 10 Concrete products
- ☐ 11 Copper forming
- ☐ 12 Dairy products processing
- ☐ 13 Electrical & electronic components
- ☐ 14 Electroplating (job shop & printed board circuit)
- ☐ 15 Explosives manufacturing
- ☐ 16 Feedlots
- ☐ 17 Ferroalloy manufacturing
- ☐ 18 Fertilizer manufacturing
- ☐ 19 Fish hatcheries
- ☐ 20 Fruit & vegetable processing
- ☐ 21 Glass manufacturing
- ☐ 22 Grain mills
- ☐ 23 Gum and wood products
- ☐ 24 Hospital
- ☐ 25 Ink formulating
- ☐ 26 Inorganic chemicals
- ☐ 27 Iron and steel
- ☐ 28 Leather tanning and finishing
- ☐ 29 Meat processing
- ☐ 30 Metal finishing
- ☐ 31 Metal molding & casting (foundries)
- ☐ 32 Mineral mining & processing
- ☐ 33 Nonferrous metals forming
- ☐ 34 Nonferrous metals manufacturing
- ☐ 35 Oil and gas extraction
- ☐ 36 Ore mining and dressing
- ☐ 37 Organic chemicals
- ☐ 38 Other mining
- ☐ 39 Paint formulating
- ☐ 40 Paving & roofing (tars & asphalt)
- ☐ 41 Pesticide chemicals
- ☐ 42 Petroleum refining
- ☐ 43 Pharmaceuticals manufacturing
- ☐ 44 Phosphate manufacturing
- ☐ 45 Photographic
- ☐ 46 Plastics and synthetic materials
- ☐ 47 Plastics molding and forming
- ☐ 48 Porcelain enameling
- ☐ 49 Pulp and paper
- ☐ 50 Rubber processing
- ☐ 51 Seafood processing
- ☐ 52 Soaps and detergents
- ☐ 53 Steam supply & noncontact cooling water
- ☐ 54 Sugar processing
- ☐ 55 Textiles
- ☐ 56 Timber products processing
- ☐ 57 Transportation
- ☐ 58 Water supply
- ☐ 59 Other *(specify)*

U.S. Environmental Protection Agency
401 M Street S.W.
Washington, D.C. 20460

BUSINESS REPLY MAIL

FIRST CLASS PERMIT NO. 11663 WASHINGTON, D.C.

POSTAGE WILL BE PAID BY ADDRESSEE

U.S. ENVIRONMENTAL PROTECTION AGENCY
ATTN: MONITORING BRANCH (WH-553)
401 M STREET S.W.
WASHINGTON, D.C. 20460

CHAPTER 11

Preparing for Testimony

Lee A. Barclay

Introduction

Involvement with fish kill investigations often means that investigators will be required to participate in legal hearings. Participation in judicial hearings and in quasi-judicial administrative hearings and proceedings of Federal and State regulatory agencies is often required to establish the facts surrounding a fish kill, to determine responsibility, and to fix assessments for damages.

This chapter is not intended as a legal reference. Its purpose is to give practical guidance to field biologists and other professionals regarding what to expect when they become directly involved in some form of litigation and are asked to present the results of their research or investigations. The discussion is directed primarily toward participation in administrative hearings and courtroom proceedings. The intent is to provide guidance on (1) how to prepare for legal testimony, (2) how to conduct yourself during the proceedings, and (3) what will be asked during cross-examination. Prior knowledge of these subjects helps potential investigators develop sound field and laboratory investigation procedures and avoid having valuable scientific work rendered less useful because of failure to follow accepted protocols. The specific preparation by a witness for a particular hearing must, of necessity, involve the trial counsel before you are to testify, and is influenced by the substance of the testimony. Investigators should always remember that, as witnesses, they are servants of the court.

The traditional way in which environmental issues are litigated is in a Federal or State court. In recent years, however, there has been a strong trend toward having contested facts in environmental cases resolved before an agency instead of in a courtroom trial—for example, in administrative trial-type hearings of State and Federal agencies. The rules for presenting expert testimony in trials or in adjudicatory-type administrative proceedings are similar. In each situation, the expert witness is asked to testify about his or her knowledge about technical questions relevant to the issues being tried. It may be helpful to remember that conclusions and opinions generally are not permissible forms of testimony. An exception to this rule may be made for expert testimony under the theory that laymen would be unable to draw conclusions in certain technical areas without the assistance of experts. It is only when the person testifying is truly an expert in the field that opinion testimony is permitted. In such cases, the witness is drawing upon personal expertise in making a conclusion when the laymen (judge or jury), given the same facts, could not reach a conclusion. The proper role of an expert witness is to contribute to the technical base on which decisions will be made.

Before the trial or hearing, you and your attorney must decide whether you will be presented as an expert witness or as a lay witness in possession of technical evidence. Usually your agency must specifically approve your testifying as an expert witness.

Gathering Information in Support of Testimony

On-site, first-hand observations and data usually provide far more persuasive evidence in judicial hearings than evidence derived from the literature. However, familiarity with the literature and other sources of information is essential to well-rounded testimony. In preparation for cases that are likely to end up in court, the investigator must not only search out all available information from cooperators and other sources, but must also conduct the most detailed and comprehensive field investigations that time, manpower, equipment, and resources permit (see Chapter 3 for guidance in conducting on-site procedures).

Preparing Material for Legal Briefs or Submission for the Record

The witness must prepare testimony and records in close harmony with an attorney. Since hearing officers and judges have wide latitude in what they will allow in format, time of submittal, number of copies, and other matters related to presentation for the record, only a few general guiding principles can be set forth here.

1. The points of fact or opinion to be developed and emphasized must be jointly selected by the attorney and witness to identify the major facts and supporting evidence, decide how the information can be presented most persuasively, develop responses to weak points in the presentation, and avoid subjects with which the attorney and witness are not fully conversant.
2. The points of evidence selected for use must be thoroughly researched by the witness and fully discussed with the attorney to reach a common understanding of the significance of the evidence and develop the most appropriate presentation.
3. The points selected must also be critically reviewed with counsel to identify potential weaknesses and develop rebuttal answers to questions that may be asked by opposing attorneys.
4. With guidance from counsel, the witness must prepare testimony and recorded material strictly in accordance with the standards and requirements of the hearing officer or court.

Oral Testimony

Advice concerning oral testimony has been excerpted and modified from a brochure entitled *Preparing to Testify*, by the U.S. Attorney's Office (U.S. Department of Justice 1984). This document offers the following advice:

1. Before you testify, refresh your memory of the fish kill, the situation observed, the distances, and exactly what happened, so that you can recall the facts clearly and accurately when you are asked. If a question is about distance or time, and your answer is only an estimate, be sure you say it is only an estimate. Beware of suggestions by attorneys about distances or times when you do not recall the actual distance or time. Do not agree with their estimates unless you have independently arrived at the same estimates.
2. Speak in your own words. Do not try to memorize what you are going to say. Doing so will make your testimony sound rehearsed and unconvincing. Be yourself. Before the trial, review the matters about which you expect to be questioned.
3. Most important of all, remember that you are sworn to tell the truth, so tell it. Every fact should be promptly and clearly stated. Do not try to figure out whether your answer will help or hurt either side. Answer all questions to the best of your ability.
4. Do not exaggerate. Avoid making statements that you may later have to correct. Be particularly careful in responding to questions that begin, "Wouldn't you agree that. . .?" Give your answers in your own words and do not allow an attorney to put words in your mouth.
5. When giving testimony, a witness is usually first asked a series of questions by the lawyer who called him or her to serve as a witness. This is called direct examination. After this dialogue, the witness is questioned by the opposing lawyer (the defense counsel) in cross-examination. The process may be repeated two or three times to help clear up any confusion. The basic purpose of direct examination is for you to tell the judge and jury what you know about the case. The basic purpose of cross-examination is to attempt to raise doubts about the accuracy of your testimony. Do not become angry if you feel your word is being doubted in cross-examination—it is the goal of defense counsel to raise questions about the validity of your testimony and your credibility. Maintain your composure and do not lose your temper.
6. A witness who becomes angry is likely to exaggerate or will seem to be less than objective or to be emotionally unstable. Keep your temper at all times. Always be courteous, even if the attorney questioning you is discourteous or insulting. Do not appear to be a "wise guy" or you will lose the respect of the judge and jury.
7. Although you are responding to the questions of an attorney, remember that the questions and answers are really for the judge's or jury's

benefit. Always speak clearly and loudly so that every juror can easily hear you.

8. Listen carefully to the questions you are asked. Be sure you understand the question, have it repeated if necessary, and then give a thoughtful, considered answer. Do not answer without thinking. Although answers should not be rushed, there should not be an unnaturally long delay to a simple question if you know the answer.
9. Explain your answer if necessary. Give the answer in your own words. If a question cannot be truthfully answered with a yes or no, say so, explain why not, and then give your answer.
10. Answer only the question asked you. Do not volunteer information that was not actually requested unless you and your attorney wish to have it entered into the record.
11. If your answer was not correctly stated, correct it immediately. If your answer was unclear, clarify it immediately. It is better to correct a mistake yourself than to have the opposing attorney discover an error in your testimony. If you realize you have answered incorrectly, say, "May I correct something I said earlier?"
12. The judge and the jury are interested only in what facts you observed or personally know about. Do not give your opinions or conclusions or repeat what someone else told you, unless you are specifically asked.
13. Witnesses sometimes give inconsistent testimony—something they said before does not agree with something they said later. If this happens, do not get flustered. Explain honestly why you misspoke or were mistaken. The jury normally accepts that people make honest mistakes.
14. Stop instantly if the judge interrupts you or when an attorney objects to a question. Wait for the judge to tell you to continue.
15. Give positive, definite answers when possible. Avoid saying "I think," 'I believe," or "In my opinion," if you can make a direct factual statement. If you know, say so. If you do not know, also say so—do not make up an answer. Be positive about the important things you naturally would remember. If you are asked about minor details that a person would not be expected to remember, say so if you do not remember.
16. When being questioned by defense counsel, do not look at your attorney or at the judge for help in answering a question. You are on your own. If the question is improper, your attorney will object. If a question is asked and there is no objection, answer it. Never substitute your own ideas about what you believe the rules of evidence are.
17. Sometimes an attorney asks, "Have you discussed this case with anyone?" If you say no, the judge or jury will know that this is unlikely. A prosecutor usually talks to witnesses before they take the stand and many witnesses have previously talked to one or more police officers or law enforcement agents. It is perfectly proper for you to have talked with the prosecutor, police, or family members before you testify. You should, of course, always respond truthfully to this question. State frankly that you have talked with whomever you have talked with—your attorney, the defendant, other witnesses, relatives, or anyone else. All you are expected to do is to tell the truth, as clearly as possible.
18. After testifying in court as a witness, do not discuss what was said during testimony with other witnesses until after the case has been decided. Do not ask other witnesses about their testimony and do not volunteer information about your own.

Good fishing is the end result of good water quality.

CHAPTER 12

Equipment Needed for Field Assessments

Georginia R. Ardinger

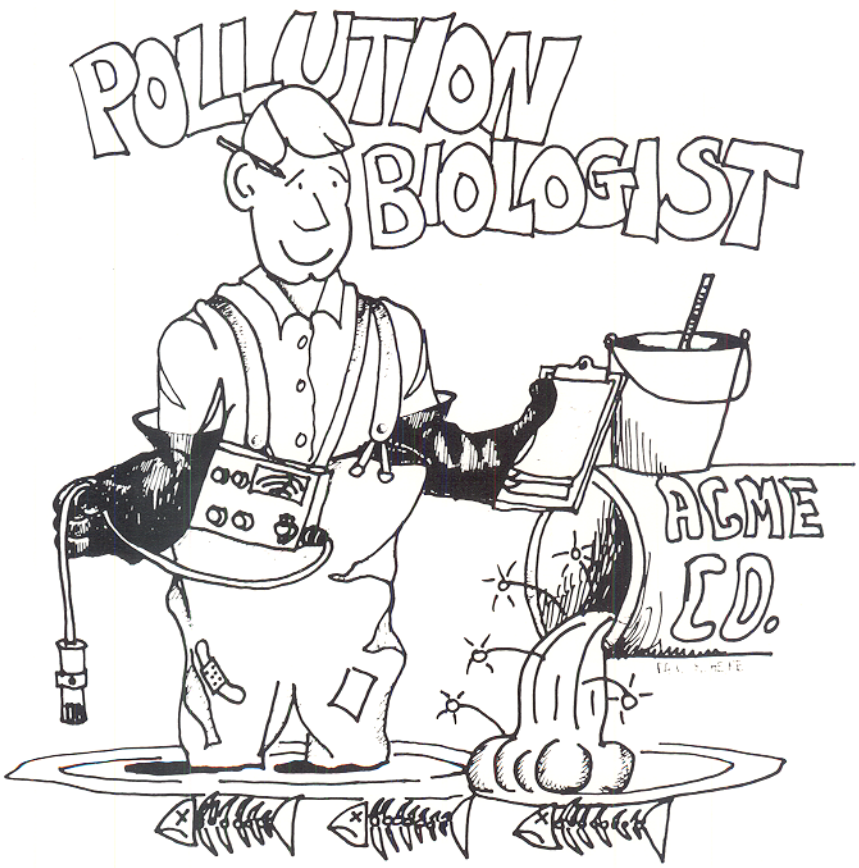
Before going to the site of a fish kill, make sure that you are prepared and have the equipment needed. Remember that your investigation could result in litigation and that your records, methods, and analyses may be used and challenged in court.

The lists that follow should assist investigators in making a determination of the needed supplies. Not all of the equipment and supplies will be needed for every fish kill investigation. Make a checklist of the items you will need. It is better to have too many supplies than not enough; a shortage will delay the investigation and could result in the loss of critical evidence.

It is vital that all supplies and materials be checked or replaced regularly to ensure that they are fresh and maintained in a state of readiness. Bacteriological media should be stored as slants in 20-mL screw-cap tubes and tightly capped to prevent drying. The tubes should be replaced monthly with fresh media. If wrappings or seals are broken on any package of sterile items, the items should be immediately replaced.

None of the supplies should be allowed to freeze. Formalin solution should not be subjected to temperatures lower than 4.4° C. At that temperature or below, a paraformaldehyde precipitate forms, rendering the solution useless.

Equipment should be operated and serviced monthly to ensure that it is working. Calibration should be checked at each servicing. Batteries should be tested monthly and replaced every 6 months. Acids, preservatives, and disinfectants will keep indefinitely if stored in tightly capped containers.



General List of Supplies and Equipment Needed for Investigating Fish Kills

Field diary (bound)
Forms (waterproof) for (1) notification, (2) custody record, (3) fish kill investigations, and (4) counting
American Fisheries Society, Special Publication 13
Collecting permit
Fish identification key
Insect identification key
Names and telephone numbers of persons to be contacted in the field
List of available sources for analytical or diagnostic services
Waterproof marking pen
Pencils
Penknife
35-mm camera and film
Tape recorder
Video camera (optional)
Maps of the area
Clipboards and paper
Wristwatch
Stopwatch
Compass
100-foot* measuring tape
Hand calculator
Tally counter
Flagging (blaze orange)
Two-way radio
Boat and motor
Life jackets
First aid kit
Toilet paper
Paper towels
Rubber hip boots or waders
Rubber gloves
Respirator with appropriate cartridges
Rain gear
Strong flashlights (6 volt)
Stainless steel 3-gallon* buckets
Galvanized tubs
Measuring boards
Scales
Long-handled dip nets
Minnow seines
Drift net sampler
Kick nets
Dissolved oxygen meter
pH meter and standards
Thermometer (certified)
Salinity and conductivity meters
Water test kit
Secchi disk
Turbidimeter
Saturometer
Ekman dredge
Surber sampler
Sieves
Wisconsin plankton net or equivalent
Rake
Plant hook
Four-tined fork
Compound microscope (×10, ×40, ×440)
Microscope slides and cover slips
Lens paper
Dissecting kit
Dissecting pans
Washbasin, plastic (10 in. × 14 in.*)
Widemouthed glass jars (4, 8, and 16 oz*) with screw caps
Glass vials (28 × 70 mm and 200 cc) with screw caps
Sample collection containers provided by the analytical laboratory, and fixatives (acids)
Ziploc plastic bags (8 × 5 in., 12 × 9 in., and 18 × 9 in.*)
Large garbage bags
Printed blank sample labels
Printed blank sample tags
Chain-of-custody forms
Laboratory marking pen to mark on glass, plastic, and paper
Roll of evidence tape
Roll of masking tape
Roll of aluminum foil
Normal saline solution (1 liter)
Propanol—70% (1 liter)
Buffered formalin—10% (4 liters)
Roccal solution—10% (1 liter)
Lugol's solution (250 mL)
Ice chests or insulated coolers
Wet ice or blue ice
Note: Dry ice may be needed later for shipping frozen samples
Ball of heavy cord or twine
Shipping boxes
Insulated shipping containers
Packing material such as bubble wrap and foam inserts
Shipping labels

*The units of measurement shown are most commonly used for commercially available items.

Specialized Supplies and Equipment Needed for Investigating Fish Kills

Water

Kemmerer sampler
Sample bottles (1 liter)
- Plastic—polyethylene or equivalent; acid rinsed
- Glass—acid rinsed; organic solvent rinsed

Preservatives
- Acids—H_2SO_4, HNO_3
- Bases—NaOH
- Zinc acetate
- Sodium thiosulfate—$Na_2S_2O_3$

Plankton and Macrophytes

Preservatives
- Phytoplankton—neutralized formalin or Lugol's solution
- Zooplankton—5% neutral formalin, 70% propanol

Sediments—for Organic Substances or Metals

Core sampler
Widemouthed (acid cleaned) glass jars (4, 8, 16, and 32 oz*)
Teflon-lined lids (tight fitting) for above jars
Note: If Teflon-lined lids are not available, use hexane-rinsed aluminum foil for the lining
Assorted vials (acid washed) with Teflon-lined lids

Bacteriological

Capped test tube slants with brain heart infusion agar or Trypticase soy agar for routine isolation and culture of most fish pathogens. If fish involved are marine or brackish water species (cases where *Vibrio* might be suspected), add 1% NaCl (sodium chloride)
Capped test tube slants with tryptone yeast extract agar for isolation and culture of *Flexibacter* sp.
Capped test tube slants with blood agar for isolation of fastidious bacteria
Sterile loops (prepackaged disposable or reusable metal type)
Methyl alcohol for disinfecting instruments
Cotton balls or swabs
Propane burner

*The units of measurement shown are most commonly used for commercially available items.

CHAPTER 13

Test Your Skill

Fred P. Meyer

Introduction

Approaches, procedures, documentation, and many other aspects of properly conducting a fish kill investigation have been discussed in the previous chapters. However, no opportunity was provided for readers to review the details of actual kills and attempt to ferret out the causes. That opportunity is given here: seven case histories are described that illustrate some of the problems an investigator is likely to encounter.

Each case history is presented in four parts: a description of the site and of the kill, the procedure followed during the investigation, the results of laboratory and field work, and the assignment of the cause. At the end of each part, you are urged to write down any conclusions you might have reached before proceeding to the next part. You may wish to refer to the dichotomous key in Chapter 3 for general guidance in attempting to determine the presumptive causes. The final determination is provided at the end of each case history.

The Case of The Botched Batch

A. Narrative

Commercial fishermen on a large river reported that many dead fish were appearing in their nets. There had been a recent rise in water level after a prolonged drought. No dead fish were noted before or during the rise, but they were seen shortly afterward. Upon visiting the site, the observer noticed many small and some large dead fish, and some large fish that were listless and lethargic. If disturbed, some affected fish showed convulsions. A wide variety of species was affected. A check of the water revealed the following characteristics: pH, 7.5; dissolved oxygen, 7 ppm; temperature, 27° C; and hardness, 230 ppm (as $CaCO_3$). The river water seemed normal and was otherwise unaffected. Samples collected from the river revealed an abundance of live algae of many species, an absence of live benthic organisms, and many dead crayfish. A check of a tributary in the area indicated many live fish of all sizes, many benthic organisms, live crayfish, and algae of many species. Physicochemical characteristics of the tributary were as follows: pH, 7.5; dissolved oxygen, 7 ppm; water temperature, 25° C; and hardness, 218 ppm.

B. What preliminary conclusions can be reached on the basis of the above information?

1. The source of the problem was upriver.
2. The cause was not related to an oxygen depletion.
3. The cause was not an infectious disease because so many different kinds of organisms were affected.
4. The cause must have been a toxic substance because a wide array of fish species were affected, and small fish died first.
5. The toxic substance was something other than a herbicide. It might have been an insecticide, pesticide, or other chemical because it killed fish, benthos, and large crustaceans, but not algae.

C. How should the investigator proceed?

1. Conduct a survey of the river upstream from the initial site to a point where the fish are alive and the biota normal.
2. Collect water samples to be analyzed for pesticides.
3. Select moribund (not dead) fish; collect tissue samples of blood, brain, and liver, as well as whole fish. Freeze samples and send them to an analytical laboratory along with the water samples.
4. Check all tributaries and potential point sources in the vicinity just downstream from where normal biota are observed. Collect samples of sediment, water, and moribund fish as in (2) and (3) for chemical analysis.

5. Collect similar samples from the tributary where aquatic life was not affected.
6. When the suspected cause has tentatively been identified, contact individuals or companies in the area that may be possible sources. If necessary, obtain warrants to enter and inspect the property of suspect parties.

D. Information derived from laboratory and field work

1. The survey of the river indicated that the fish kill could be traced to a single tributary on which several industrial plants were located. The affected portion of the system extended more than 1.6 km upstream from any of the plants.
2. The kill zone ended at a landfill site adjacent to the stream.
3. A check of the site showed that the recent high water had eroded the bank between the stream and the landfill and that dumped material was now in contact with the streamflow.
4. A large quantity of a dark, viscous substance covered a large area of the landfill.
5. A laboratory analysis revealed that the dark substance contained a very high concentration of endrin.
6. Water samples taken upriver from the mouth of the stream contained no endrin, but samples immediately below the mouth contained significant amounts.
7. Sediment samples taken from sites upstream from the landfill contained no endrin, but high concentrations were present in the immediate area where the landfill abutted the stream.
8. One of the industrial plants was known to produce pesticides, but it was not known whether it produced endrin. Sediment samples collected at the plant outfall contained only traces of endrin.
9. Whole-body analyses of fish from the initial kill site revealed moderately high concentrations of several pesticides from fish in both the main stream and the tributary.
10. Blood, brain, and liver showed low levels of many pesticides and 0.15 to 0.22 mg/L of endrin.
11. A check of toxicity information on endrin revealed that concentrations of 0.19 mg/L or more in the brain induce convulsions and narcolepsy and are lethal to fish.
12. A check of the point discharges of the various plants revealed that one plant produced endrin, but the plant manager denied releasing any through the plant drains.
13. Sediment and water samples taken below the discharge point sources of the several industrial plants revealed that endrin concentrations in water and sediments were virtually equal at all of the plants.
14. A recheck of the landfill site revealed evidence identifying the pesticide company as the source of the dark substance.
15. Detailed discussions with plant personnel at the pesticide company revealed that a production run of endrin had "gone bad," was terminated, and was dumped into the landfill.

E. Final conclusion

Endrin poisoning resulted from illegal dumping.

The Case of The Clear Creek Caper

A. Narrative

A trout pond adjacent to a trout stream was normally spring-fed, but because of a prolonged drought, the flow had been low and supplemental water was being pumped from the stream to maintain pond levels. One morning the owner of the trout pond was confronted by the sight of many large dead trout. The only live fish were small, recently stocked trout. In addition, a bloom of algae formerly present was gone, and the water had become crystal clear. The owner shut off the pump from the stream and called the local pollution control agency to ask for help in identifying the chemical that might have flowed down the creek and killed his fish. He suspected that someone had dumped a toxic substance into the stream, and planned to sue the person responsible.

B. What preliminary conclusions can be reached on the basis of the above information?

1. The presence of large dead fish, but live small fish ruled out acute toxicity and suggested possible oxygen depletion.
2. The disappearance of the plankton bloom and

the clarity of the water suggested that a herbicidal compound rather than eutrophication was the cause of the problem.

C. How should the investigator proceed?

1. Check the dissolved oxygen in the pond and creek (immediately, at 4:00 p.m., and again at daybreak).
2. Check to see if there has been any effect on plants or other aquatic life in the stream above and below the pond outlet.
3. Check the water chemistry of the stream water and of the pond.
4. Check to see if any herbicidal substances were used the day before in the area surrounding the creek or pond.

D. Information derived from laboratory and field work.

1. The dissolved oxygen level in the pond was 4 ppm during bright sunshine at 2:00 p.m., and also at 4:00 p.m.; at daybreak, it was 3 ppm. In the stream, it was a constant 8.0 ppm.
2. All plants and biota upstream from and below the pond were alive and thriving.
3. The water chemistries in the stream and pond were essentially the same.
4. No herbicide had been applied in the watershed of the stream or on the shore around the pond.
5. A member of the owner's staff commented that the pond had been treated the previous day with 2 mg/L Cutrine as a prophylactic measure to control protozoan parasites. However, this concentration had been used at least three times in the past 9 months without problems.

E. Final conclusion

The cause of the kill was oxygen depletion, triggered by the algicidal action of Cutrine. Loss of photosynthetic activity coupled with decay of algae reduced the dissolved oxygen below the lethal limit for large trout. Small fish were able to obtain enough oxygen in the spring flow or at the pump discharge to survive. During cool seasons when the flow from the spring was normal, there would have been adequate oxygen to offset the effects of Cutrine. But under the circumstances in this case, the dissolved oxygen was too low to counteract the effects of Cutrine.

The Case of The Black Lagoon

A. Narrative

A long, shallow municipal lake (average depth, 1.8 m) had a tributary stream with a steeply graded watershed at the upper end, and a large feed mill and several retail farm and garden chemical stores at the lower end. In August, there was a partial kill of fish in the lake. When investigators arrived at 11:00 a.m., many bullheads were seen swimming about at the surface, but all other fish seen were dead. The water had been dark green earlier in the week, but had suddenly turned dark and odorous. On the preceding day, a heavy rain, accompanied by hail, had fallen in the area. The city was concerned that a toxic substance might have washed into the lake from the chemical companies or the feed mill and wanted to know what kind of samples should be taken to identify the compound. At 1:00 p.m., the dissolved oxygen was 2 ppm. The lake level rose about 0.3 m in 6 hours after the rain, and there was a strong odor of hydrogen sulfide and methane.

B. What preliminary conclusions can be reached on the basis of the above information?

1. An oxygen depletion is suggested, but the rain tends to confuse the situation because runoff should have added oxygenated water.
2. Black bullheads are among the most resistant species to toxic substances; fish of all other species were dead. Thus a toxic substance cannot be ruled out.

C. How should the investigator proceed?

1. Check dissolved oxygen and pH on site immediately.
2. Collect water samples at several intervals along the length of the lake at surface, middepth, and near bottom. Have part of each sample analyzed for water chemistry characteristics and part for pesticides.
3. Collect samples of whole fish, livers, gills, and blood for laboratory analysis.
4. Obtain hydrological and limnological data on the lake.

D. Information derived from laboratory and field work

1. When the bullheads were picked up, blood spilled from the gills. Upon closer examina-

tion, many aneurysms were apparent on the gill lamellae. In many fish, the gills were brownish rather than bright red. The blood was chocolate brown rather than the normal red color.
2. Examination of the water samples revealed an abundance of dark black detritus and dead algal cells.
3. The water samples showed high levels of hydrogen sulfide, high CO_2, and high nitrites and nitrates.
4. Acidification of blood samples released an odor of hydrogen sulfide.
5. Analyses of fish and water samples showed low levels of numerous compounds, but none at concentrations that would be toxic to fish.
6. Normally, this lake stratified rigidly each summer, surface and bottom water temperatures commonly differed by 11° C. On the date of the investigation, the lake water temperature was the same from top to bottom.

E. Final conclusion

Loss of the fish was due to a combination of low oxygen and hydrogen sulfide poisoning. The uniform temperature from top to bottom and the presence of black detritus throughout the water column indicated that the lake was no longer stratified. Thus, it is likely that the heavy cold rain induced a turnover or so thoroughly roiled the lake that anoxic bottom water became mixed with the surface water. This resulted in releases of hydrogen sulfide and methane and caused a drastic drop in dissolved oxygen. The combination was seemingly lethal to all fish except the resistant bullheads, which showed signs of sulfhemoglobin (brown blood) and hydrogen sulfide poisoning (aneurysms) in the gills.

The Case of One Too Many for the Road

A. Narrative

When a commercial fisherman on a large river downstream from a major urban area lifted his nets on a Saturday morning after an overnight set, he found them filled with dead fish of a number of species. Although most of the dead fish were less than 15 cm long, many large fish were also present. He found no live fish. All of the dead fish had gaping mouths and flared gills, and seemed to have died in agony or rigor. Except for the dead fish, the river appeared normal. The fisherman called the State Department of Natural Resources and requested assistance in determining the cause of the kill. A preliminary survey revealed no dead fish at a point 4 km upstream from the fisherman's nets (a site above the city's industrial district). A drainage ditch littered with dead fish entered the river at this point. Passage up the ditch ended at a chain link fence and a sign "No trespassing—U.S. Government property"; a 30-cm pipe protruded from the bank about 75 m beyond the fence.

B. What preliminary conclusions can be reached on the basis of the above information?

1. Whatever killed the fish was a sudden, catastrophic event in the ecosystem since the fish died overnight.
2. The kill affected many species and sizes of fish—suggesting that the cause was a toxic substance.
3. The source was clearly upstream from the fisherman's nets and may have originated in the drainage ditch.

C. How should the investigator proceed?

1. Collect samples of fish gills, livers, and blood to submit for laboratory analyses. At this point, the general identity of the possibly toxic substance is unknown.
2. Thoroughly survey the area upstream from the fisherman's nets, paying particular attention to slack-water areas, shallows, and shorelines when looking for dead fish. Note all plant discharges as travel proceeds up the river to the drainage ditch noted in the preliminary survey.
3. Collect sediment samples in the river 100 m above the mouth of the drainage ditch, at its mouth, 100 m downstream from the mouth, and 50 m up the ditch.
4. Contact the local police to determine the name of the plant and the name and telephone number of the responsible management official.
5. Contact the management official and request permission to enter the property. Be prepared to contact a Federal judge, if necessary, to obtain a warrant to inspect the property and to collect needed samples. [In this example, per-

mission was freely granted.] The fenced area proved to be a Federal munitions manufacturing site. Because the plant did not operate during weekends, only custodial and maintenance personnel were present. When shown the dead fish in the ditch, the manager was at a loss to explain their presence. He claimed that the drainpipe to the ditch was essentially a storm sewer, but acknowledged that floor drains in the building were connected to it. However, he said that all of the plant's toxic wastes were stored in 55-gallon drums that were emptied every Friday after work by an approved disposal company. No waste was disposed of in any other way. The presence of 25 empty waste containers attested to the fact that the disposal company had picked up the waste.

6. Collect fish gills, livers, and blood, as well as water and sediment samples, immediately below the drainpipe. Send them to the analytical laboratory.
7. Collect samples of whatever substances remain in several of the drums and send them to the analytical laboratory. Ask for analyses related to substances used in the manufacture of munitions.

D. Information derived from laboratory and field work

1. Analyses of the fish samples collected at the fisherman's nets showed no significant levels of pesticides.
2. Analyses of samples collected at the mouth of the ditch, 100 m downstream, in the ditch, and at the outfall of the storm sewer showed high levels of a toxic substance used in the manufacture of munitions.
3. Analyses of samples taken from the toxic waste drums contained high concentrations of the same toxic substance.
4. Reanalysis of fish samples collected from the fisherman's nets revealed high concentrations of the same substance.
5. A check with industrial plants located on or discharging into streams leading to the river revealed that the munitions plant was the only user of the suspect chemical.

E. Final conclusion

The fish were killed by the indicated toxic substance, that originated at the munitions plant. The manager of the facility steadfastly maintained that his plant was innocent and that his personnel were under strict instructions prohibiting the dumping of any chemicals. However, during an in-depth investigation at the plant, it was learned that on the date in question the waste disposal contractor sent two trucks to pick up the chemicals, each with a capacity for the contents of 12 drums. Rather than call for an additional truck to pick up the contents of the remaining drum, the drivers moved it to the floor drain and dumped the contents. A custodian observed the dumping but thought the drivers were merely washing out the container.

The Case of The Lethal Lunch

A. Narrative

A fish kill was reported by bass fishermen on a large impoundment. They reported that only largemouth bass over 2 kg were affected, including some fish up to 5 kg. The investigator from the Department of Natural Resources arrived at the marina to meet one of the anglers who reported the unusual kill. A tour of the lakeshore began, but because the wind was blowing outward, a decision was made to look for dead fish on the far shore. On the way across the lake, fish were seen in distress at the surface, and large fish were seen striking at the affected fish. The fish in distress proved to be gizzard shad 15 to 20 cm long. Reconnaissance along the windward shore revealed dead or dying large bass, but there were also numerous moribund and dead large catfish, gars, and gizzard shad. The gizzard shad were emaciated, had eroded fins, and extensive necrotic areas over the body. Some of the lesions had fungal growths. Inspection of the large predator fish revealed that their stomachs and intestines contained a grayish, mucoid substance, but no food. Anglers in the area on the day of the inspection reported that bass fishing was very good and that bluegills and crappies were biting well.

B. What preliminary conclusions can be reached on the basis of the above information?

1. Deterioration of water quality was not the cause of the problem, as evidenced by the presence of many healthy fish.

2. No toxic substances could have been involved because small fish were thriving and only large fish were dying.
3. The cause could not be oxygen depletion because angling was very successful.
4. All affected species other than gizzard shad were large predators. Some factor related to both was suggested.

C. How should the investigator proceed?

1. Collect moribund fish of all affected species for bacteriological study and check for parasites. Place the fish in individual plastic bags on wet ice.
2. Examine moribund fish with a microscope—be sure to check the lesions on the gizzard shad and the unusual gray substance in the intestines of the predators.
3. Inoculate bacteriological culture media from lesions, kidney, and intestine.

D. Information derived from laboratory and field work

1. No parasite common to all of the affected species was found.
2. Microscopic examination of the lesions on the gizzard shad revealed heavy infections of myxobacteria typical of those that cause columnaris disease.
3. Microscopic examination of the peculiar grayish substance in the intestine of the predator fishes revealed the presence of massive numbers of myxobacteria.
4. Cultures of bacteria derived from the grayish material were identified as *Flexibacter columnaris*, the cause of columnaris disease.
5. Cultures for other bacterial pathogens were negative.
6. The gizzard shad were all of one year class and in poor physical condition.

E. Final conclusion

The cause of the fish kill was columnaris disease. The disease originated in the collapse of an overabundant year class of gizzard shad, probably because of their age, poor physical condition, and other stressors. Predators that were large enough to feed on the moribund gizzard shad contracted the disease and died when systemic infections developed. Fish too small to eat gizzard shad 15 cm long were unaffected.

The Case of The Capricious Cotton Gin

A. Narrative

In a southern State, a lake in a cotton-producing area had an abundant fish population, but a fish kill developed in December. Catfish, gars, largemouth bass, and large crappies were affected; however, live forage fish were seen along the shore in seemingly good health. The water temperature was 3° C; pH, 8.0; dissolved oxygen, 8 ppm; and total hardness, 375 ppm (as $CaCO_3$). A slight green bloom was present. No city or industry was in the area. A cotton gin on the edge of the lake discharged lint toward the lake. Employees at the gin said they frequently saw small fish actively feeding on particulate matter discharged by the gin, but had never seen small dead fish. However, they reported having seen large dead fish in December of past years. No ginning had been done during the last 5 weeks. Aerial spraying of insecticides and defoliants was done on adjacent fields, but not during the last 2 months. No fish kill was noted during the spraying season.

B. What preliminary conclusions can be reached on the basis of the above information?

1. The kill was not related to a chemical spill or unexpected release of a toxic substance because small fish were still alive.
2. No herbicide was involved because a phytoplankton bloom was still present.
3. The cause was not oxygen depletion, as evidenced by the high dissolved oxygen, relatively low water temperature, and phytoplankton bloom.
4. An infectious agent was unlikely because many species were affected.

C. How should the investigator proceed?

1. Check the fish population to determine its species and size composition.
2. Check for benthos and zooplankton.
3. Collect water and sediment samples along the length of the lake and near the cotton gin. Send them to be analyzed for cotton pesticides.
4. Collect samples of liver, brain, and blood, and whole dead and live fish. Send these to be

analyzed for cotton pesticides.
5. Check weather data for the past 2 weeks.
6. Inoculate bacteriological media to check for pathogenic organisms.
7. Check for signs of parasitic infestations.

D. Information derived from laboratory and field work

1. The structure of the fish population was very unusual. Forage and herbivorous species and young-of-the-year fish of predator species were abundant. However, there were few predator species more than 3 years old, even though nonpredator species of age groups 0 to 10 were common.
2. Water and sediment samples showed trace levels of cotton pesticides throughout the lake and significant residue levels in sediments downwind from the cotton gin.
3. Benthic organisms were scarce; live zooplankton was present.
4. Whole fish residues showed that moderate to low concentrations of a number of cotton pesticides were present in forage and herbivorous fishes and young of the year. Whole-body levels in live fish of predator species were much higher. In many, the levels were above the LC50 for the chemical and fish species concerned.
5. Liver samples from live fish indicated moderate to low concentrations of a number of cotton pesticides; in samples from dead fish, the levels were high.
6. Blood and brain samples showed moderate to high concentrations of cotton pesticides in the dead predators. In live predators, the levels were lower, but still much higher than in live nonpredators.
7. A comparison of concentrations in blood and brain with published values in the literature indicated that the levels were above the lethal limit in the dead fish. In the living predators, the concentrations were elevated, but not to toxic or lethal limits.
8. Checks for parasites were negative, and no pathogens were observed in the bacteriological cultures.
9. Weather conditions had changed drastically during the preceding 10 days. The temperature dropped rapidly about 1 week before the kill and had not recovered since then.

E. Final conclusion

The kill was an indirect result of chronic releases of cotton pesticides into the lake. Although the amount entering at any one time was probably well below toxic levels, bioaccumulation and biomagnification in the food chain resulted in significant residues in the fish. Because large predators are at the top of the food chain, stored residues were highest in them. As winter approached, the fish began to use stored fat. The recent cold front probably caused increased mobilization of the fat, along with stored pesticide residues. Analyses showed that levels in the blood and brain were at or above lethal limits in the dead fish. This conclusion was supported by the residue levels in the various components of the food chain and by the unusual population structure of the fish community.

The Case of The Acid Rain

A. Narrative

A fish hatchery on the outskirts of a large city used city water as its water supply. It had done so for many years and had a system to remove the chlorine. One day, all fish on the station suddenly died. The fish in hatchery tanks attempted to jump out, were bleached, and died with gills flared and mouths agape. In the ponds, the water cleared markedly and plants in the water turned brown or white. The hatchery manager contacted the city Water Department to ask what happened. The water supply was drawn from a local reservoir fed by streams from forested mountains in the watershed. The Water Department called the State Department of Natural Resources for assistance in determining what pesticides might have been sprayed on the forests that could have gotten into the water supply and killed the fish. Rain had fallen recently and they suspected that runoff had transported pesticides into the reservoir.

B. What preliminary conclusions can be reached on the basis of the above information?

1. The incident was the result of a catastrophic environmental problem caused by a substance that was highly toxic to fish and plants.

C. How should the investigator proceed?
   1. Check the water at the hatchery for the presence of chlorine.
   2. Visit the city Water Department to determine what might have been done differently in the last 24 hours.
   3. Have complete chemical analyses run on a water sample from the fish tanks at the hatchery.
   4. Collect and freeze fish samples from the hatchery. Hold for possible future analyses.
   5. Check the reservoir used as the water source. Collect water samples at the reservoir for analysis.

D. Information derived from laboratory and field work
   1. There was no residual chlorine in the water at the hatchery. The chlorine removal system was functioning normally.
   2. The city Water Department claimed that water treatment had been normal.
   3. A visit to the reservoir revealed that the water level had been very low because of a prolonged drought. However, torrential rains over the mountains in the watershed over the preceding 48 hours had raised the water level by 3.5 m. The reservoir was filled with turbid red water because of clay siltation. Fishermen reported that angling was fair on the day of the visit, and that they had seen no dead fish in the reservoir. Vegetation along the shoreline was normal.
      Note: At this point, the evidence indicated that the source of the problem was not related to the reservoir. Whatever the cause, it had developed in the water system between the reservoir and the fish hatchery.
   4. Water chemistry analyses from samples at the fish hatchery revealed the following characteristics: hardness, 30 ppm ($CaCO_3$); dissolved oxygen, 8.0 ppm; pH, 3.0; total alkalinity, zero; and total suspended solids, 5 mg/L.
   5. Water chemistry analyses of samples from the reservoir indicated the following characteristics: hardness, 35 ppm ($CaCO_3$); dissolved oxygen, 8.0 ppm; pH, 7.1; total alkalinity, 27 ppm; and total suspended solids, 500 mg/L.
   6. A return visit to the water treatment plant was required for further investigation.
   7. The manager of the water treatment plant said that his crew followed a routine step-by-step procedure:
      a. Filtration through a sand filter to remove particulate matter
      b. Filtration through activated charcoal to remove taste and odor
      c. Chlorination to destroy bacteria in the water
      d. Storage in an elevated water tower for distribution through the city water mains
   8. It was obvious that there were two major differences in water from the reservoir and water from the fish hatchery—in pH and total suspended solids. Something had been done to the water that caused the changes. Further discussions with the water treatment plant manager revealed that, because of the high total suspended solids (colloidal clay), aluminum sulfate was added to the water before it entered the sand filter. He indicated that this merely involved changing the charge on the clay particles to cause them to precipitate. The sand filter then removed the clay. This was standard water treatment procedure.
   9. Water samples drawn ahead of the sand filter and after filtration showed no change in pH. A sample drawn past the charcoal filter gave the same result. However, a sample taken from the line after chlorination showed a drop from pH 7.1 to 2.5.
   10. Discussions with a chemist revealed that chlorination of water containing dissolved aluminum sulfate would result in the formation of sulfuric acid.

E. Final conclusion

   The fish were killed by low pH caused by sulfuric acid that resulted from the combination of treatments applied by the city Water Department. Clinical signs shown by the dying and dead fish were consistent with low pH toxicity.

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Suggested Additional Reading

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Appendix A. Metric to English Conversions

(modified from Moore and Mitchell 1987)

Measurement or temperature[a]

Metric	English	Celsius (°C)	Fahrenheit (°F)
Centimeter (cm)	0.394 in.	−20	−4
Cubic centimeter (cc or cm^3)	0.061 $in.^3$	−10	14
Gram (g)	0.0353 oz	0	32
Kilogram (kg)	2.2046 lb	3	38
Kilometer (km)	0.621 mi	4	39
Liter (L)	0.264 gal	4.4	40
Meter (m)	3.281 ft or 1.094 yd	10	50
Micrograms per liter (μg/L)	parts per billion	11	52
Milligrams per liter (mg/L)	parts per million	25	77
Milliliter (mL)	0.0338 fl oz	27	80
Millimeter (mm)	0.0394 in.		

[a] Formula for conversion of temperatures:

$°F = (°C \times 9/5) + 32$

$°C = (°F - 32) \times 5/9$

Appendix B. Water Quality Standards Chart
(modified from EPA 1986)

Compound or factor	Priority pollutant[a]	Suspected carcinogen[a]	Acute and chronic toxicity to aquatic life (concentrations in μg/L)			
			Fresh acute criteria	Fresh chronic criteria	Marine acute criteria	Marine chronic criteria
Acenapthene	Y	N	1,700[b]	520[b]	970[b]	710[b]
Acrolein	Y	N	68[b]	21[b]	55[b]	—[b]
Acrylonitrile	Y	Y	7,550[b]	2,600[b]	—	—
Aldrin	Y	Y	3.0	—	1.3	—
Alkalinity	N	N	—	20,000	—	—
Ammonia	N	N	Criteria are pH and temperature dependent—see document			
Antimony	Y	N	9,000[b]	1,600[b]	—	—
Arsenic (pent)	Y	Y	850[b]	48[b]	2,319[b]	13[b]
Arsenic (tri)	Y	Y	360	190	69	36
Bacteria	N	N	For primary recreation and shellfish uses—see document			
Barium	N	N	NA	NA	NA	NA
Benzene	Y	Y	5,300[b]	—	5,100[b]	700[b]
Benzidine	Y	Y	2,500[b]	—	—	—
Beryllium	Y	Y	130[b]	5.3[b]	—	—
BHC	Y	N	100[b]	—	0.34[b]	—
Cadmium	Y	N	3.9[c]	1.1[c]	43	9.3
Carbon tetrachloride	Y	Y	35,200[b]	—	50,000[b]	—
Chlordane	Y	Y	2.4	0.0043	0.09	0.004
Chlorinated benzenes	Y	Y	250[b]	50[b]	160[b]	129[b]
Chlorinated naphthalenes	Y	N	1,600[b]	—	7.5[b]	—
Chlorine	N	N	19	11	13	7.5
Chloroalkyl ethers	Y	N	238,000[b]	—	—	—
Chloroform	Y	Y	28,900[b]	1,240[b]	—	—
Chlorophenol 2	Y	N	4,300[b]	2,000[b]	—	—
Chlorophenol 4	N	N	—	—	29,700[b]	—
Chlorpyrifos	N	N	0.083	0.041	0.011	0.0056
Chloro-4-methyl-3-phenol	N	N	30[b]	—	—	—
Chromium (hex)	Y	N	16	11	1,100	50
Chromium (tri)	N	N	1,700[c]	210[c]	10,300[b]	—
Color	N	N	Narrative statement—see document			
Copper	Y	N	18[c]	12[c]	2.9	2.9
Cyanide	Y	N	22	5.2	1	1
DDT	Y	Y	1.1	0.001	0.13	0.001
DDT metabolite (DDE)	Y	Y	1,050[b]	—	14[b]	—
DDT metabolite (TDE)	Y	Y	0.06[b]	—	3.6[b]	—
Demeton	Y	N	—	0.1	—	0.1
Dichlorobenzenes	Y	N	1,120[b]	763[b]	1,970[b]	—
Dichloroethane 1,2	Y	Y	118,000[b]	20,000[b]	113,000[b]	—
Dichloroethylenes	Y	Y	11,600[b]	—	224,000[b]	—
Dichlorophenol 2,4	N	N	2,020[b]	365[b]	—	—

Appendix B. *Continued.*

Compound or factor	Priority pollutant[a]	Suspected carcinogen[a]	Acute and chronic toxicity to aquatic life (concentrations in μg/L)			
			Fresh acute criteria	Fresh chronic criteria	Marine acute criteria	Marine chronic criteria
Dichloropropane	Y	N	23,000[b]	5,700[b]	10,300[b]	3,040[b]
Dichloropropene	Y	N	6,060[b]	244[b]	790[b]	—
Dieldrin	Y	Y	2.5	0.0019	0.71	0.0019
Dimethylphenol 2,4	Y	N	2,120[b]	—	—	—
Dinitrotoluene	N	Y	330[b]	230[b]	590[b]	370[b]
Dioxin (2,3,7,8-TCDD)	Y	Y	0.01[b]	0.00001	—	—
Diphenylhydrazine 1,2	Y	N	270[b]	—	—	—
Endosulfan	Y	N	0.22	0.056	0.034	0.0067
Endrin	Y	N	0.18	0.0023	0.037	0.0023
Ethylbenzene	Y	N	32,000[b]	—	430[b]	—
Fluoranthene	Y	N	3,960[b]	—	40[b]	16[b]
Gases, total dissolved	N	N	Narrative statement—see document			
Guthion	N	N	—	0.01	—	0.01
Haloethers	Y	N	380[b]	122[b]	—	—
Halomethanes	Y	Y	11,000[b]	—	12,000[b]	6,400[b]
Heptachlor	Y	Y	0.52	0.0038	0.053	0.0036
Hexachloroethane	N	Y	980[b]	540[b]	940[b]	—
Hexachlorobutadiene	Y	Y	90[b]	9.3[b]	32[b]	—
Hexachlorocyclohexane (Lindane)	Y	Y	2.0	0.08	0.16	—
Hexachlorocyclopentadiene	Y	N	7[b]	5.2[b]	7[b]	—
Iron	N	N	—	1,000	—	—
Isophorone	Y	N	117,000[b]	—	12,900[b]	—
Lead	Y	N	82[c]	3.2[c]	140	5.6
Malathion	N	N	—	0.1	—	0.1
Manganese	N	N	NA	NA	NA	NA
Mercury	Y	N	2.4	0.012	2.1	0.025
Methoxychlor	N	N	—	0.03	—	0.03
Mirex	N	N	—	0.001	—	0.001
Naphthalene	Y	N	2,300[b]	620[b]	2,350[b]	—
Nickel	Y	N	1,400[c]	160[c]	75	8.3
Nitrate/Nitrite	N	N	NA	NA	NA	NA
Nitrobenzene	Y	N	27,000[b]	—	6,680[b]	—
Nitrophenols	Y	N	230[b]	150[b]	4,850[b]	—
Nitrosamines	Y	Y	5,850[b]	—	3,300,000[b]	—
Oil and grease	N	N	Narrative statement—see document			
Oxygen dissolved	N	N	Warmwater and coldwater criteria matrix—see document			
Parathion	N	N	0.065	0.013	—	—
PCB's	Y	Y	2.0	0.014	10	0.03
Pentachlorinated ethanes	N	N	7,240[b]	1,100[b]	390[b]	281[b]
Pentachlorophenol	Y	N	20[d]	13[d]	13	7.9[b]
pH	N	N	—	6.5–9	—	6.5–8.5
Phenol	Y	N	10,200[b]	2,560[b]	5,800[b]	—
Phosphorus elemental	N	N	—	—	—	0.1
Phthalate esters	Y	N	940[b]	3[b]	2,944[b]	3.4[b]
Polynuclear aromatic hydrocarbons	Y	Y	—	—	300[b]	—
Selenium	Y	N	260	35	410	54

Appendix B. *Continued.*

Compound or factor	Priority pollutant[a]	Suspected carcinogen[a]	Acute and chronic toxicity to aquatic life (concentrations in μg/L)			
			Fresh acute criteria	Fresh chronic criteria	Marine acute criteria	Marine chronic criteria
Silver	Y	N	4.1[c]	0.12	2.3	—
Solids suspended and turbidity	N	N	Narrative statement—see document			
Sulfide-hydrogen sulfide	N	N	—	2	—	2
Temperature	N	N	Species dependent criteria—see document			
Tetrachlorinated ethanes	Y	N	9,320[b]	—	—	—
Tetrachloroethane 1,1,2,2	Y	Y	—	2,400[b]	9,020[b]	—
Tetrachloroethanes	Y	N	9,320[b]	—	—	—
Tetrachloroethylene	Y	Y	5,280[b]	840[b]	10,200[b]	450[b]
Tetrachlorophenol 2,3,5,6	Y	N	—	—	—	440[b]
Thallium	Y	N	1,400[b]	40[b]	2,130[b]	—
Toluene	Y	N	17,500[b]	—	6,300[b]	5,000[b]
Toxaphene	Y	Y	0.73	0.0002	0.21	0.0002
Trichlorinated ethanes	Y	Y	18,000[b]	—	—	—
Trichloroethane 1,1,1	Y	N	—	—	31,200[b]	—
Trichloroethane 1,1,2	Y	Y	—	9,400[b]	—	—
Trichloroethylene	Y	Y	45,000[b]	21,900[b]	2,000[b]	—
Trichlorophenol 2,4,6	Y	Y	—	970[b]	—	—
Zinc	Y	N	120[c]	110[c]	95	86

[a]N = no; Y = yes; NA = not applicable; — = no data available.
[b]Insufficient data to develop criteria. Value presented is the Lowest Observed Effect Level—LOEL.
[c]Hardness-dependent criteria (100 ppm used).
[d]pH-dependent criteria (7.8 pH used).

Appendix C. Order Numbers for Water Quality Criteria Documents[a]

Standard	NTIS order number	Standard	NTIS order number
Acenaphthene	PB81-117269	2,4,-Dimethylphenol	PB81-117558
Acrolein	PB81-117277	Dinitrotoluene	PB81-117566
Acrylonitrile	PB81-117285	Diphenylhydrazine	PB81-117731
Aesthetics	PB-263943	Endosulfan	PB81-117574
Aldrin and Dieldrin	PB81-117301	Endrin	PB81-117582
Alkalinity	PB-263943	Ethylbenzene	PB81-117590
Ammonia	PB81-227114	Fluoranthene	PB81-117608
Antimony	PB81-117319	Gases, total dissolved	PB-263943
Arsenic	PB85-227445	Guthion	PB-263943
Asbestos	PB81-117335	Haloethers	PB81-117616
Bacteria	PB-263943	Halomethanes	PB81-117624
Bacteria	PB86-158-045	Hardness	PB-263943
Barium	PB-263943	Heptachlor	PB81-117632
Benzene	PB81-117293	Hexachlorobutadiene	PB81-117640
Benzidine	PB81-117343	Hexachlorocyclohexane	PB81-117657
Beryllium	PB81-117350	Hexachlorocyclopentadiene	PB81-117665
Boron	PB-263943	Iron	PB-263943
Cadmium	PB85-227031	Isophorone	PB81-117673
Carbon tetrachloride	PB81-117376	Lead	PB85-227437
Chlordane	PB81-117384	Malathion	PB-263943
Chlorinated benzenes	PB81-117392	Manganese	PB-263943
Chlorinated ethanes	PB81-117400	Mercury	PB85-227437
Chlorinated phenols	PB81-117434	Methoxychlor	PB-263943
Chlorinated naphthalenes	PB81-117426	Mirex	PB-263943
Chlorine	PB85-227429	Naphthalene	PB81-117707
Chloroalkyl ethers	PB81-117418	Nickel	PB87-105359
Chloroform	PB81-117442	Nitrates, nitrites	PB-263943
2-Chlorophenol	PB81-117459	Nitrobenzene	PB81-117723
Chlorpyrifos	PB87-105359	Nitrophenols	PB81-117749
Chromium	PB85-227478	Nitrosamines	PB81-117756
Color	PB-263943	Oil and grease	PB-263943
Copper	PB85-227023	Oxygen, dissolved	PB86-208253
Cyanide	PB85-227460	Parathion	PB87-105383
DDT and metabolites	PB81-117491	Pentachlorophenol	PB87-105391
Demeton	PB-263943	pH	PB-263943
Dichlorobenzenes	PB81-117509	Phenol	PB81-117772
Dichlorobenzidine	PB81-117517	Phosphorus	PB-263943
Dichloroethylenes	PB81-117525	Phthalate esters	PB81-117780
2,4,-Dichlorophenol	PB81-117533	Polychlorinated biphenyls	PB81-117798
Dichloropropanes and Dichloropropenes	PB81-117541	Polynuclear aromatic hydrocarbons	PB81-117806

Appendix C. *Continued.*

Standard	NTIS order number	Standard	NTIS order number
Selenium	PB81-117814	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	EPA 440/5-84-007
Silver	PB81-117822		
Solids (dissolved) and salinity	PB-263943	Tetrachloroethylene	PB81-117830
		Thallium	PB81-117848
Solids (suspended) and turbidity	PB-263943	Toluene	PB81-117855
		Toxaphene	PB87-105375
Sulfides, hydrogen sulfide	PB-263943	Trichloroethylene	PB81-117871
Tainting substances	PB-263943	Vinyl chloride	PB81-117889
Temperature	PB-263943	Zinc	PB87-153581

[a] Cited by the U.S. Environmental Protection Agency (1986) and available through the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, Virginia 22161. Telephone (703) 487-4650.

Appendix D. Water Quality Standards: State and Federal Criteria

Standard	NTIS order number	Standard	NTIS order number
Acidity–alkalinity (pH)	PB89-141527	Iron	PB89-141543
Antidegradation	PB89-141600	Lead	PB89-141626
Arsenic	PB89-141501	Mercury	PB89-141378
Bacteria	PB89-141394	Mixing zones	PB89-141477
Cadmium	PB89-141469	Nitrogen, Ammonia, Nitrite, and Nitrate	PB89-141618
Chromium	PB89-141584		
Copper	PB89-141592	Organics	PB89-141386
Cyanide	PB89-141485	Other elements	PB89-141436
Definitions	PB89-141493	Pesticides	PB89-141535
Designated uses	PB89-141402	Phosphorus	PB89-141444
Dissolved oxygen	PB89-141568	Temperature	PB89-141550
Dissolved solids	PB89-141576	Turbidity	PB89-141451
General provisions	PB89-141428	Zinc	PB89-141519
Intermittent streams	PB89-141410		

Appendix E. Formulae for Solutions Used in Fish Kill Investigations

I. Preservatives

A. Algae

1. Lugol's solution—dissolve 20 g of potassium iodide (KI) and 10 g of iodine crystals in 200 mL of distilled water containing 20 mL of glacial acetic acid.
2. Formalin (buffered)—37% formaldehyde neutralized with sodium tetraborate (pH 7.0 to 7.3).
3. For other acceptable preservatives, see APHA et al. (1985), p. 1048.

B. Fish tissue

1. Bouin's solution—dissolve 21 g of picric acid in 1,000 mL of distilled water. Mix 1,500 mL saturated picric acid solution, 900 mL of formaldehyde (37% solution), and 100 mL of glacial acetic acid.

   Caution: Picric acid must not be allowed to dry out because it can be a dangerous explosive.
2. Buffered neutral formalin—mix 100 mL of formaldehyde (37% solution), 900 mL of distilled water, 4 g of sodium phosphate, monobasic monohydrate, and 6.5 g of sodium phosphate, dibasic, and anhydrous.
3. Dietrich's fixative—mix 1,500 mL of distilled water, 750 mL of 95% ethanol, 250 mL of formaldehyde (37% solution), and 50 mL of glacial acetic acid.

Appendix F. Example of Habitat Assessment Form

Fish Kill Investigation

Case number: _____ Date: _____
Reported by _____ Investigator's name _____
Address _____ Address _____
_____ _____
_____ _____
Telephone _____ Telephone _____

Location of Die-off

State: _____ County: _____
Description (attach map):

Environmental Factors

1. Weather (include temperature, cloud cover, wind speed, direction, and water flow):
2. Human activities:
3. Limnological:

Time of measurement	pH	Dissolved oxygen	Turbidity	Conductivity	Water temperature	Other

Fish Mortality

1. Extent in time and space:
2. Species affected:
3. Sizes affected:
4. Numbers affected:
5. Condition of fish:
6. Behavior, lesions, or clinical signs:
7. Comments:

Characteristics of Other Biota (species, alive, dead, present, absent)

1. Algae
2. Macrophytes
3. Zooplankton
4. Insects
5. Other vertebrates

Witnesses or Providers of Information

Name: _____ **Information:**
Address: _____
_____
_____
Telephone: _____

Name: _____ **Information:**
Address _____
_____
_____
Telephone: _____

Appendix G. Specimen Synopsis and Catalog Samples

Catalog title for sample:

Submitter: _____ Die-off Location (State and County):
Address: _____
_____
Telephone: _____

Case Number: Catalog Number:

Background: (Describe the general habitat type in which the kill occurred and significant environmental, anthropogenic, and limnological factors. Summarize extent, sequence, size of fish kill, and other biotic changes.)

Instructions to laboratory: Summarize the number of samples and the specific services requested; their priority in cases of restricted sample or funding availability should be noted.

Special needs: That is, sensitivity requirements for pesticide analysis, etc. Quality assurance needs not routinely performed in the laboratory should be identified.

Sample results shipped to:
Name: _____
Address: _____
_____
Telephone: _____

Sample remainders shipped to:
Name: _____
Address: _____
_____
Telephone: _____

Comments:

Chain of Custody: _____ Yes _____ No

Catalog: Attached; list total number of samples, their type, and what services were requested.

Date of submission: Results needed by (date): _____

Submitter: _____
Signature

Appendix G. *Continued.*

CATALOG OF SAMPLES

No: Title:

Sample no.	Sample description	Collector	Date of collection	Collection location	Sample weight	Preservative used	Analysis requested

Signed by: _____

Title: _____

Appendix H. Regional Offices of the U.S. Fish and Wildlife Service

Regions	Address
Region 1—Pacific Region California, Hawaii, Idaho, Nevada, Oregon, Washington, Guam, and American Samoa	Assistant Regional Director—Law Enforcement U.S. Fish and Wildlife Service Eastside Federal Complex 911 N.E. 11th Avenue Portland, Oregon 97232 Telephone: (503) 231-6125
Region 2—Southwest Region Arizona, New Mexico, Oklahoma, and Texas	Assistant Regional Director—Law Enforcement U.S. Fish and Wildlife Service Box 1306 Albuquerque, New Mexico 87103 Telephone: (505) 766-2091
Region 3—North Central Region Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Ohio, and Wisconsin	Assistant Regional Director—Law Enforcement U.S. Fish and Wildlife Service Federal Building, Fort Snelling Twin Cities, Minnesota 55111 Telephone: (612) 725-3530
Region 4—Southeast Region Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee	Assistant Regional Director—Law Enforcement U.S. Fish and Wildlife Service Richard B. Russell Federal Building 75 Spring Street, S.W. Atlanta, Georgia 30303 Telephone: (404) 331-5872
Region 5—Northeast Region Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, and West Virginia	Assistant Regional Director—Law Enforcement U.S. Fish and Wildlife Service One Gateway Center, Suite 700 Newton Corner, Massachusetts 02158 Telephone: (617) 965-2298
Region 6—Rocky Mountain-Prairie Region Colorado, Kansas, Montana, Nebraska, North Dakota, South Dakota, Utah, and Wyoming	Assistant Regional Director—Law Enforcement U.S. Fish and Wildlife Service Box 25486 Denver Federal Center Denver, Colorado 80225 Telephone: (303) 236-7540
Region 7—Alaska Region Alaska	Assistant Regional Director—Law Enforcement U.S. Fish and Wildlife Service 1011 East Tudor Road Anchorage, Alaska 99503 Telephone: (907) 786-3311

Appendix I. Examples of Chain-of-custody Records

DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE
DIVISION OF LAW ENFORCEMENT

CHAIN OF CUSTODY RECORD

FILE NO. INV.

DATE AND TIME OF SEIZURE:	DISTRICT:	EVIDENCE/PROPERTY SEIZED BY:
4/20/88; @ 5:00 PM	PT-1	L.N. Owens, Special Agent

SOURCE OF EVIDENCE/PROPERTY (person and/or location):
☒ TAKEN FROM: P.W.I., six inch steel pipe on
☐ RECEIVED FROM: ponds 21 and 23
☐ FOUND AT:

CASE TITLE AND REMARKS:
Petroleum Waste Inc. (PWI)
Larry Moxley, President
Eric Almberg, Director of Technical Services
Wendell Fells, Site Manager

ITEM NO.	DESCRIPTION OF EVIDENCE/PROPERTY (include Seizure Tag Numbers and any serial numbers):
1	One (1) San Joaquin Kit Fox pup-- tag number 536610.
2	One (1) adult male San Joaquin Kit Fox-- tag number 536611.

ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE	DELIVERED VIA:
1&2	L.N. Owens, Special Agent U.S. Fish and Wildlife Serv. Division of Law Enforce.	L.N. Owens	4/20/88	☐ U.S. MAIL ☐ IN PERSON
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE	☒ OTHER: air freight
	~~Dr. Nancy Thomas~~ B. Roubicek U.S. Fish and Wildlife Serv. Nat'l. Wildlife Health Lab.	Bernard Roubicek	4/21/88	
ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE	DELIVERED VIA:
1 & 2	~~Dr. Nancy Thomas~~ B. Roubicek U.S. Fish and Wildl. Serv. Nat'l. Wildlife Health Lab.	Bernard Roubicek	4/21/88	☐ U.S. MAIL ☒ IN PERSON
	TO: (PRINT NAME, AGENCY) ~~Dr. Thomas~~ ~~L.N. Owens, Special Agent~~ U.S. Fish and Wildl. Serv. NWHRC ~~Division of Law Enforce.~~	RECEIPT SIGNATURE:	RECEIPT DATE	☐ OTHER:
		Nancy J. Thomas	4/21/88	
ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE	DELIVERED VIA:
2	Nancy J. Thomas USFWS - NWHRC	Nancy J. Thomas	7/12/88	☐ U.S. MAIL ☐ IN PERSON
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE	☒ OTHER: Federal Express
	Martha Gay USFWS - PWRC	Martha L. Gay	7/13/88	

☐ ADDITIONAL TRANSFERS ON REVERSE SIDE

FORM 3-2063 (9/81)

DEPARTMENT OF THE INTERIOR
U. S. FISH AND WILDLIFE SERVICE
DIVISION OF LAW ENFORCEMENT

CHAIN OF CUSTODY RECORD

FILE NO.
INV.

DATE AND TIME OF SEIZURE:	DISTRICT:	EVIDENCE/PROPERTY SEIZED BY:

SOURCE OF EVIDENCE/PROPERTY (person and/or location):	CASE TITLE AND REMARKS:
☐ TAKEN FROM: ☐ RECEIVED FROM: ☐ FOUND AT:	

ITEM NO.	DESCRIPTION OF EVIDENCE/PROPERTY (include Seizure Tag Numbers and any serial numbers):

ITEM NO.				
	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE	DELIVERED VIA: ☐ U.S. MAIL ☐ IN PERSON ☐ OTHER:
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE	
ITEM NO.				
	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE	DELIVERED VIA: ☐ U.S. MAIL ☐ IN PERSON ☐ OTHER:
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE	
ITEM NO.				
	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE	DELIVERED VIA: ☐ U.S. MAIL ☐ IN PERSON ☐ OTHER:
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE	

☐ ADDITIONAL TRANSFERS ON REVERSE SIDE

FORM 3-2063 (9/81)

FILE NO.

INV.

CHAIN OF CUSTODY RECORD (continued)

ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE	DELIVERED VIA: ☐ U.S. MAIL ☐ IN PERSON ☐ OTHER:
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE	
ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE	DELIVERED VIA: ☐ U.S. MAIL ☐ IN PERSON ☐ OTHER:
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE	
ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE	DELIVERED VIA: ☐ U.S. MAIL ☐ IN PERSON ☐ OTHER:
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE	
ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE	DELIVERED VIA: ☐ U.S. MAIL ☐ IN PERSON ☐ OTHER:
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE	
ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE	DELIVERED VIA: ☐ U.S. MAIL ☐ IN PERSON ☐ OTHER:
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE	
ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE	DELIVERED VIA: ☐ U.S. MAIL ☐ IN PERSON ☐ OTHER:
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE	
ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE	DELIVERED VIA: ☐ U.S. MAIL ☐ IN PERSON ☐ OTHER:
	TO: (PRINT NAME, AGENCY)	RECEIPT SIGNATURE:	RECEIPT DATE	

Appendix J. Available Fish Disease Diagnostic Services (modified from Honer 1988)

Alabama
Bill Hemstreet, Disease Specialist
Alabama Fish Farming Center
P.O. Box 487
Greensboro, Alabama 36744
(205) 624-4016

Southeastern Cooperative Fish Disease Laboratory
Department of Fisheries and Allied Aquaculture
Auburn University
Auburn, Alabama 36849
(205) 826-4786

Arkansas
John K. Beadles
Arkansas State University
Box 60
State University, Arkansas 72467
(501) 972-3029

Andrew J. Mitchell, Fish Pathologist
U.S. Fish and Wildlife Service
Fish Farming Experimental Laboratory
P.O. Box 860
Stuttgart, Arkansas 72160
(501) 673-4483

California
Ron Hedrick
Department of Medicine
School of Veterinary Medicine
University of California
Davis, California 95616
(916) 752-3411

Robert Toth
Fish Disease Laboratory
California Department of Fish and Game
407 West Line Street
Bishop, California 93514
(714) 872-2791

Colorado
Dennis Anderson
Fish Disease Control Center
U.S. Fish and Wildlife Service
1100 E. Burlington Avenue
P.O. Box 917
Fort Morgan, Colorado 80701
(303) 867-9474

Florida
Florida Department of Agriculture
Animal Disease Diagnostic Laboratory
P.O. Box 460
2700 N. Bermuda Avenue
Kissimmee, Florida 32742
(407) 847-3185

Live Oak Diagnostic Laboratory
912 Nobles Ferry Road
P.O. Drawer "O"
Live Oak, Florida 32060
(904) 362-1216

Shamrock Fisheries
P.O. Box 1620, Hwy. C 351A
Shamrock, Florida 32628
(904) 498-5293

Georgia
Athens Diagnostic Laboratory
College of Veterinary Medicine
University of Georgia
Athens, Georgia 30602

Gary Burtle, Assistant Professor
Coastal Plain Experiment Station
University of Georgia
P.O. Box 748
Tifton, Georgia 31793
(912) 386-3364

Howard M. Jackson
Warm Springs Fish Health Center
Route 1, Box 105A
Warm Springs, Georgia 31830
(404) 655-3620

Hawaii
James A. Brock
Anuenue Fisheries
Area 4, Sand Island
Honolulu, Hawaii 96819

Idaho
B. F. Grant, Director
Rangen Aquaculture Research Center
Route 1, Box 264
Hagerman, Idaho 83332
(208) 837-6191

A. K. Hauck
Idaho Department of Fish and Game
Route 1, Trout Road
Fish Health Laboratory
Eagle, Idaho 83616

Joe C. Lientz
Dworshak National Fish Health Center
Box 18
Ahsahka, Idaho 83520
(208) 476-4591

Illinois
Roy Heidinger
Fisheries Research Laboratory
Southern Illinois University
Carbondale, Illinois 62901
(618) 536-7761

Rodney W. Horner, Fish Pathologist and Aquaculture Coordinator
RR 4, Box 54
Manito, Illinois 61546
(309) 968-7531

Louisiana
Ronald Thune
Aquatic Animal Diagnostic Laboratory
School of Veterinary Medicine
Louisiana State University
Baton Rouge, Louisiana 70803
(504) 346-3281

Maine
Roger Dexter
AFS Fish Pathologist
East Orland, Maine 04430
(207) 469-2601, May–Nov.
(813) 343-5889, Dec.–Apr.

Maryland
Frank Hetrick
Bacterial and Viral Diseases
Fish Disease Laboratory
Department of Microbiology
University of Maryland
College Park, Maryland 20742
(301) 454-5411

Massachusetts
Eleanor Horwitz
Chief of Information and Education
Division of Fisheries and Wildlife
Field Headquarters
Westboro, Massachusetts 01581
(617) 727-2864

Michigan
John Hnath, Julia Zischke
Michigan Department of Natural Resources
Wolf Lake State Fish Hatchery
Fish Health Laboratory 34270 CR652
Mattawan, Michigan 49071
(616) 668-2132

Missouri
AquaScience Research Group, Inc.
1100 Gentry Street
North Kansas City, Missouri 64116
(816) 842-5936

Charlie Suppes
Missouri Department of Conservation
Blind Pony Hatchery
Route 2
Sweet Springs, Missouri 65351
(816) 335-4531

Montana
Charlie E. Smith
U.S. Fish and Wildlife Service
Fish Technology Center
4050 Bridger Canyon Road
Bozeman, Montana 59715
(406) 587-9265

New Hampshire
Jay Hendee
RD 10, Box 375
Concord, New Hampshire 03301
(603) 798-5474

New Jersey
Susan Ford
Rutgers University
P.O. Box 687
Port Norris, New Jersey 08204
(609) 785-0074

New York
Paul R. Bowser
Fish Diagnostic Laboratory
New York State College of Veterinary Medicine
Cornell University
Ithaca, New York 14853
(607) 253-3365

John Schachte, Jr.
Associate Fish Pathologist
Fish Disease Control Unit
8314 Fish Hatchery Road
Rome, New York 13440
(315) 337-0910

North Carolina
Edward J. Noga
Department of CASS
College of Veterinary Medicine
4700 Hillsborough Street
Raleigh, North Carolina 27606
(919) 829-4200, ext 236

Oklahoma
Lonnie Cook
J. A. Manning State Fish Hatchery
HC 32, Box 580
Lawton, Oklahoma 73501
(405) 529-2795

Jack Harper
Southeast Region
Route 1, Box 188
Caddo, Oklahoma 74729
(405) 924-4087

Conrad Kleinholz
Langston University
P.O. Box 730
Langston, Oklahoma 73050
(405) 466-3836

Oregon
J. L. Fryer
Professor
Department of Microbiology
Nash Hall
Oregon State University
Corvallis, Oregon 97331
(503) 754-4441

J. S. Rohovec
Department of Microbiology
Nash Hall 220
Oregon State University
Corvallis, Oregon 97331

Pennsylvania
John Thoesen, John Fletcher, and John Coll
Fish Health Unit
U.S. Fish and Wildlife Service
P.O. Box 155
Lamar, Pennsylvania 16848
(717) 726-6611

Rhode Island
R. E. Wolke
Comparative Aquatic Pathology Laboratory
University of Rhode Island
Kingston, Rhode Island 02881
(401) 792-2334

Texas
S. K. Johnson
Extension Fish Disease Diagnostic Laboratory
Texas A&M University
Department WFS, Nagle Hall
College Station, Texas 77843
(409) 845-7471
FAX: (409) 845-3786

B. J. Lee
AquaMed
P.O. Box 49160
Austin, Texas 78765
(512) 474-6225

Donald H. Lewis
Texas A&M University
Veterinary Microbiology and Parasitology
Room 119, VMS Building
College Station, Texas 77843
(409) 845-4270

Thomas G. Meade
Department of Life Sciences
Sam Houston State University
Huntsville, Texas 77341
(409) 294-1551

Utah
Richard Heckmann
153 WIDB, Zoology Department
Brigham Young University
Provo, Utah 84602
(801) 378-2495 or 2006

Virginia
Frank O. Perkins
Virginia Institute Marine Sciences
260 Cedarwood Way
Newport News, Virginia 23602
(804) 874-7784

Washington
Ray Brunson and John Morrison
Olympia Fish Health Center
2625 Parkmont Lane, Building A
Olympia, Washington 98502
(206) 753-9046

Steve Leek and Eric Pelton
Lower Columbia River Fish Health Center
MP 61.75R
Underwood, Washington 98651
(509) 493-3156

Steve Roberts
Washington Department of Wildlife
580 Nelson Place
E. Wenatchee, Washington 98802
(509) 884-0970

West Virginia
National Fish Health Research Laboratory
U.S. Fish and Wildlife Service
Box 700
Kearneysville, West Virginia 25430
(304) 725-8461

Wisconsin
Richard C. Nelson
Fish Disease Control Center
U.S. Fish and Wildlife Service
P.O. Box 1595
La Crosse, Wisconsin 54602
(608) 783-6451

Susan Marcquenski
Wisconsin Department of Natural Resources
Box 7921
101 S. Webster Street
Madison, Wisconsin 53707
(608) 266-2871

Wyoming
Douglas L. Mitchum
Game and Fish Laboratory
University of Wyoming
P.O. Box 3312
Laramie, Wyoming 82071
(307) 766-5618

Canada
Hilda Lei Ching
Parasitologist
Hydra Enterprises Ltd.
P.O. Box 2184
Vancouver, British Columbia V6B 3V7, Canada
(605) 736-0757

John Cornick
Fish Health Service Unit
Canada Department of Fisheries and Oceans
P.O. Box 550
Halifax, Nova Scotia B3J 2S7, Canada
(902) 426-8381

Fish Pathology Laboratory
Department of Pathology
Ontario Veterinary College
University of Guelph
Guelph, Ontario N1G 2W1, Canada
(519) 823-8800, ext. 4640

Jean-Louis Frechette
Department of Pathology and Microbiology
Faculty of Veterinary Medicine
University of Montreal
P.O. Box 5000
St. Hyacinthe, Quebec J2S 7C6, Canada
(514) 773-8521

Laurel Whistance-Smith
Extension Development Supervisor
Ministry of Natural Resources
Wildlife Branch
Whitney Block, Queen's Park
Toronto, Ontario M7A 1W3, Canada

Appendix K. How to Select and Ship Fish Samples (courtesy of T. L. Wellborn, Mississippi State University Cooperative Exension Service)

MCES COOPERATIVE EXTENSION SERVICE • MISSISSIPPI STATE UNIVERSITY

Selecting and Shipping Samples to Help Determine Cause of Fish Kills

You should send the best sample possible packed in the best manner possible to a disease specialist as soon as you can. It is a good policy to call the disease specialist before carrying him a sample so that he can be expecting you. The disease specialists are interested in the welfare of the fish farmers and will be more than happy to assist in identifying and eradicating problems.

Diagnostic Samples

Listed In Order Of Preference
From Excellent To Unusable

1. Fish exhibiting behavioral symptoms such as:
- lying lethargically in shallow water and not moving off rapidly when disturbed;
- hanging listlessly at the water's surface and not going down quickly when disturbed, or if they do go down, returning quickly to the surface;
- swimming rapidly in a circle or in an erratic manner.

Because these fish sometimes are hard to find and, more often than not, hard to catch, many farmers will make only a halfhearted effort to catch them. They then will resort to an easier-to-obtain, but less desirable sample. It is well worth your time, however, to obtain this type of sample since it offers the best chance of correctly identifying problems in the shortest time.

EXCELLENT SAMPLE: Probability of finding cause of death is high.

2. Live fish that exhibit overt physical symptoms such as:
- open sores;
- light-colored, slightly eroded areas in front of dorsal fins or other parts of the body;
- yellowish areas inside the mouth cavity;
- eroded and light areas on gills;
- swollen, fused, or clubbed gills;
- eroded and hemorrhagic fins.

There are other symptoms but these will give a general idea of what to look for.

EXCELLENT SAMPLE: Probability of finding cause of death is high.

3. Dead fish that still have red gills and somewhat normal amounts of mucus and color.

FAIR SAMPLE: Probability of identifying the cause of death depends on how long the fish has been dead. The longer the fish remains in the pond the poorer sample it makes. Tissues begin to break down and normal putrifying bacteria attack the fish almost immediately.

4. Several fish taken at random from the seine sample:

POOR SAMPLE: Probability of identifying the cause of the fish loss is low since a majority of the fish in the pond may be healthy. Sometimes you can get an indication of what could be causing the problem by the number of species of parasites found on the fish when examined.

5. Live fish caught by hook and line from several different areas of the pond:

POOR SAMPLE: Probability of finding out what is causing the fish loss in the pond is very low. Healthier fish usually bite more readily than weaker fish; thus, a fish caught by hook and line will likely be free of parasites and pathogenic organisms. Occasionally, as in Example 4, you can get an indication of the problem by the number and types of pathogenic organisms found. This is especially true if the fish examined show approximately the same level of infection.

6. Dead fish that have lost body color and mucous coat and have white, mushy gills:

   TOTALLY UNUSABLE SAMPLE: Save your time and the disease specialist's time by not bothering with this type of sample. It is good management, however, to remove all dead fish from your pond each day. By daily removing the dead fish, you will be able to determine how rapidly the loss is increasing. You also will have an accurate record of your mortality at the end of the production year.

7. Water sample from the pond containing diseased fish:

   TOTALLY UNUSABLE SAMPLE: A water sample is of little value as a diagnostic aid in determining the cause of a fish kill unless a toxic substance is suspected. In this case, collect a one-gallon water sample in a clean glass container and send it along with the fish samples to the disease specialist. If, however, you intend to treat the pond with a chemical whose toxicity varies with the water hardness, send a water sample with the fish sample to the specialist.

Determining Factors in Fish Kill

If possible, send this information (along with the fish samples) to the disease specialist:

1. Number of fish lost since the die-off started.
2. Approximate number of fish lost each day.
3. When the losses started:
   - date -
   - time of day -
4. Number of surface acres per pond (or exact dimensions of the vat or holding tank).
5. Average depth of the pond.
6. Number of fish stocked in the pond.
7. Condition of the bloom:
   Light – The pond has visibility of 18 inches or more and has no accumulation of algae in the corners or on the down-wind side.
   Moderate – The pond has a visibility of 12 to 15 inches and may have a moderate amount of algae accumulated in the corners or on the down-wind side.
   Heavy – The pond has a visibility of 12 inches or less.
8. The last time the pond was treated:
   - Why was it treated?
   - What chemical was used and how much?

Transporting and Shipping Samples

1. Place live fish in a plastic bag and seal. Then place the bag in an ice chest containing crushed ice.
2. If the fish are to be hauled for a short distance, you may place them in a container or ice chest containing well -oxygenated water. Add a few chunks of ice to keep the water cool.
3. Fish can be frozen for transport to the lab when there is **no other way** to keep them from spoiling. Frozen samples are hard to work with and should be avoided whenever possible. Frozen samples are acceptable if they are for pesticide analysis.
4. Ice down immediately all dead fish collected but which are still acceptable for examination (red gills, etc.) as in Example 1, to retard further tissue breakdown.

For assistance in determining the cause of fish kills, contact your county agent or **Dr. Thomas L. Wellborn, Jr.**, Extension Wildlife and Fisheries Specialist, Mississippi State University.

Information Sheet 667
Extension Service of Mississippi State University, cooperating with U.S. Department of Agriculture. Published in furtherance of Acts of Congress, May 8 and June 30, 1914. JAMES R. CARPENTER, Director (5M-4-85)

Meyer, Fred P., and Lee A. Barclay, editors. 1990. **Fish Manual for the Investigation of Fish Kills.** U.S. Fish Wildl. Serv., *Resour. Publ.* 177. 120 pp.

Federal and State agencies have expressed the need for a compendium of known and accepted methods and techniques that should be followed by anyone investigating a fish kill. This manual attempts to fill that need by addressing the many facets involved in a fish kill investigation and providing instruction, guidance, examples, and sample forms. The manual will prove to be useful for interpreting evidence at the site of a fish kill, gathering needed evidence and data, making the final decision of the cause and needed remedial and corrective actions, and preparing for appearance as a court witness.

Key words: Fish kills, contaminants, toxic substances, fish diseases, natural fish kills, environmental stressors.

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