Investigation of Fish Kills

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Overview

- Fish are the public's bio indicator
- PLANNING
- INTERPRETING THE SCENE
- TOXIC SUBSTANCES
- NATURAL CAUSES
- ROLE OF INFECTIOUS AGENTS
- DOCUMENTATION
- SAMPLING
- ANALYSES
- CASES

Investigation and Monetary Values of Fish and Freshwater Mollusk Kills

Robert I. Southwick and Andrew J. Loftus, editors

American Fisheries Society
Special Publication 35

Fish are high profile bio indicators

Fish kills are real evidence of a serious problem in a waterway. The general public viewpoint is that any loss of fish, whether a result of natural or other causes, can be an early warning of impending environmental problems. In addition to harm to natural resources, if related to toxins, there may also be human health concerns.

- Determination of the cause of fish kills relies on quick response (requiring preparedness) and knowledge of what to look for, what information to record, and what and how to collect samples.

- Because fish kills can be caused by a wide array of factors, determination of the cause can sometimes be very difficult.

- Information will be given on types of fish kills and on clues to watch for, data to collect, how to handle samples properly.

PLANNING

(What you need and who you need)

- Check list
- Coordination
- News Release

PLANNING

- PA conservation agencies are responsible for more than 85,000 miles of streams and rivers, along with 4,000 inland lakes and ponds covering 160,000 acres, plus 470,000 acres of Lake Erie.
- Most fish kills are first reported and observed by the public.
- An observant and educated general public, and state agencies with a prepared plan and infrastructure in place to investigate fish kills will greatly increase the chances of determining the cause of a fish kill

Planning - Checklist

- Educated Staff
- Procedure in place to investigate and document fish kills
- Well maintained equipment for sample collection and analysis
- Forms to track samples and investigations
- Access to appropriate toxicology and diagnostic laboratories
  - Should be specific to fish and aquatic ecosystems (certified if possible)
- Proper PPE (Toxic spills)

Planning - Coordination

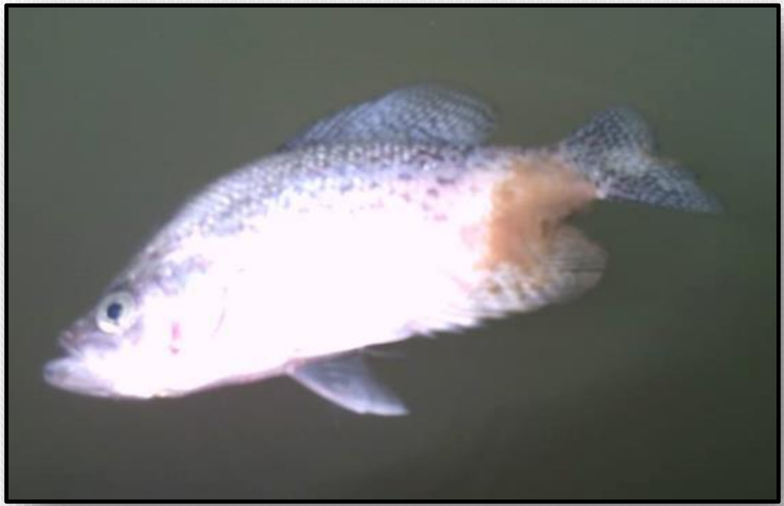
General Public

- Report to <u>responsible</u> State and Federal Agencies
  - PA Fish and Boat Commission (1 800 Fish Kill)
  - PA DEP, DCNR, USFWS
  - PFBC normally will not investigate mortality events on waterbodies not open to the public
- Report right away
  - Specimens need to be collected alive if possible
  - Don't assume others have reported it
  - Posting on face book is not reporting
  - All reports are important
- Need to describe what is observed
- Take Pictures

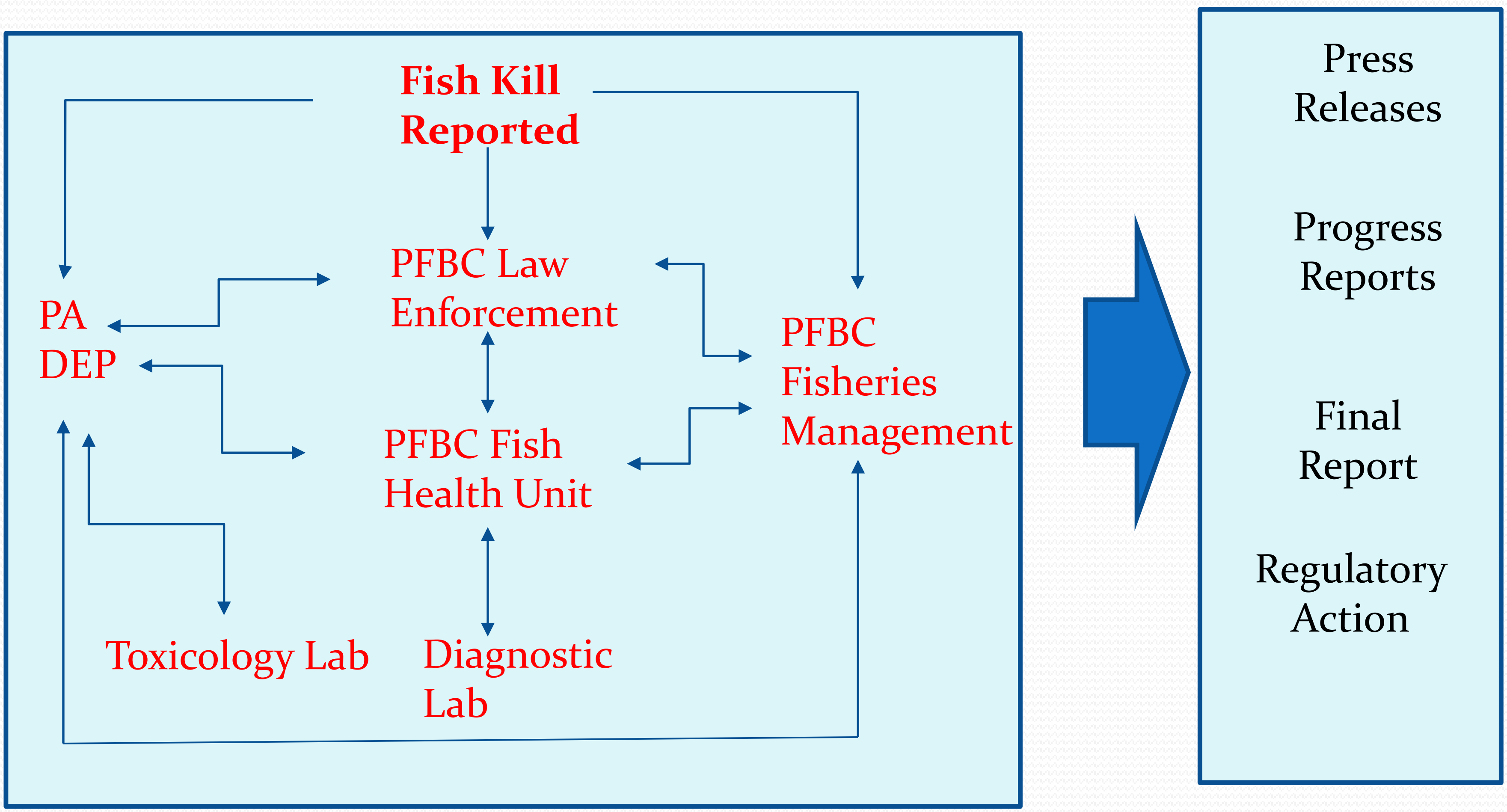
Planning - Coordination

Investigators (State and Federal Agencies)

- Determine Point of Contact
- Coordination between agencies (who to contact)
- Standardized protocols
- Press releases
  - Are conditions dangerous to Human Health?
  - Do restrictions need to be put in place on fishing or transporting fish?
- How to report



How Fish Kill Investigations are supposed to work in PA



Press Release/Interviews

- A single point of contact should be assigned
- Should be a coordinated effort between all investigating parties
- Talking points should be developed and updated as new information becomes available
- Include all groups involved in investigation (Agencies, Labs)
- All information provided should be based on what is actually known and not speculation
- It may take several weeks to get results.

Press Release/Interviews (FAQ's)

Q. Can I drink or swim in the water?

A. Depends on the event, it can usually be determined quickly if there is a risk to human health

Q. Can I eat the fish?

A. Depends on the event
   - Contamination or toxic spill
   - Pathogen- Very few fish pathogens can affect humans (When in doubt throw it out)

Interpreting the scene

- Fish kill investigations are often reactive, and environmental conditions have often changed or in the process of changing
- It is essential to collect data and specimens as soon as possible
- Following an established protocol for documenting a fish kill is essential to efficiently determining the cause
- Data may not seem important, but may be an important factor in establishing spatial and temporal patterns
- Preliminary assessment should be considered exploratory

Interpreting the scene (Types of Data)

Spatial Data

- What is specific about the location
- Compare data from similar locations.
- Requires collecting data from all locations.
- Need to look at everything

Temporal Data

- When did it start/stop
- Compare baseline (historical) data to current data and look for significant differences.

Interpreting the scene
(What to look for and Record)

- Record date and time of investigation
- Contact information of person(s) who reported the fish kill
  - Often reported by multiple parties
- Location (GPS, River mile)
  - Need to determine the geographical magnitude of the fish kill
  - Is it localized or wide spread?
- Identifying landmarks
  - Discharge pipes
  - Tributaries

Interpreting the scene

(What to look for and Record)

- When the fish kill was reported
- Estimated time when the fish kill began (rate of mortality)
  - **Acute** morality <24 hours may indicate environmental conditions are responsible
  - **Chronic** mortality may indicate pathogen involvement or low level environmental stressor.
- Weather conditions
  - Current and prior to report
- Recent changes or activities in the immediate area
  - Pesticide/herbicide treatments
  - Construction or changes in habitat

Interpreting the scene

(What to look for and Record)

- Water quality
  - Characteristics observed by investigator and reporter (Temp, color, smell…)
  - Chemical Parameters (DO, PH, Conductivity….)
  - Sediment
  - Changes in water quality can increase stress in fish or increase virulence of a pathogen

- Species Composition
  - Some species more more sensitive to changes in water quality
  - Some pathogens are species or taxonomic family specific (Viruses)

- Age and or size of affected species
  - Large Fish (May be oxygen related)
  - Small fish (May be toxicant related)
  - YOY only
  - Everything

Interpreting the scene

(What to look for and Record)

- Condition of affected species (Live, Dead, Moribund, Decaying)

- Physical appearance affected species
  - Color, Flared gills, excessive mucus, lesions

- Unusual behavior of affected species (all animals and plants)
  - At the surface
  - Loss of equilibrium
  - Avoiding specific areas

Interpreting the scene

(What to look for and Record)

- Need to determine the number of fish effected

- Need to determine significance of the fish kill

  - Magnitude of mortality is not always correlated with significance of mortality
    - 10,000 Gizzard shad in Lake Erie vs 10 adult brook trout in a first order stream

  - Species composition and number may dictate regulatory actions
    - Endangered or threatened species

Toxic Substances

- Highly Toxic substances act quickly – acute mortalities
- Some compounds kill both plants and animals and affects can be severe and dramatic
- Toxic substances may enter the ecosystem at sublethal levels over an extended time, environmental effects are more subtle.
    - Kills may occur at unexpected times of year
    - Or long after the discharge has ended.

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.

Toxic Substances

- https://www.youtube.com/watch?v=JjqJjoiBjLo

Toxic Substances

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).

Toxic Substances

- Biological responses
  - If the substance kills plants, the picture becomes confused by misleading indicators: low O_2, low pH, high CO_2, and dying algae.
- Fish
  - Rate of mortality
  - Size of fish affected
  - Species affected
  - Behavior

Toxic Substances – behaviors & observations

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

Toxic Substances

- Fish
- INVERTEBRATES
  - Zooplankton, snails
  - Crabs, crayfish, sandworms
- OTHER ANIMALS
  - Frogs, snakes, turtles
- ALGAE
  - Alive and normal, or
  - Absent or dead

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.

Toxic Substances

Toxicity is a function of concentration and duration of exposure
A toxic substance may not change water chem, but may leave residues

- WATER
  - Comparison with a reference site
- SEDIMENT
  - Compare with reference,
  - Above, at, and below site
- TISSUES
  - Compare with fish from reference site

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.

Toxic Substances

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

A Bighorn River brown trout shows evidence of gas bubble trauma on its gill plate.

David Palmer

Natural Causes

- Oxygen depletion
- Gas supersaturation
- Toxic algal blooms
- Turnovers
- Sudden or excessive temperature changes
- Lightning
- Infectious agents

Natural Causes

Most common natural cause of fish kills is oxygen depletion associated environmental evidence may include:

- Abruptly in the morning- 2:00 am to sunrise
- Large fish die first
- Species selectivity is evident
- pH between 6 and 7
- Water color change from light green to pea-soup, brown, gray or black
- Bad smell – rotting vegetation
- Zooplankters are dead/dying

Natural Causes

- Toxic Algal Blooms
  - Natural
  - Single species becomes dominant
  - Nutrients used up, levels of toxin rise
  - Example: Red Tides in marine waters
    - *Gymnodium brevis*
  - *Pfiesteria piscicida* case
    - 1997 Chesapeake Bay
    - Fish dying, people ill
    - "Pfiesteria hysteria"

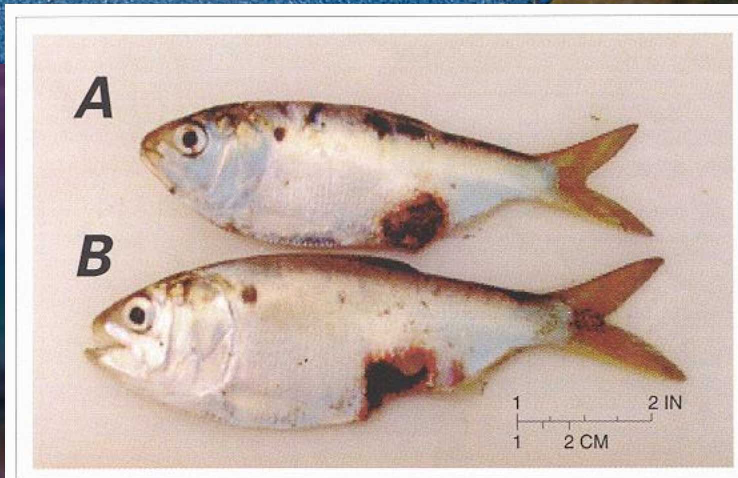
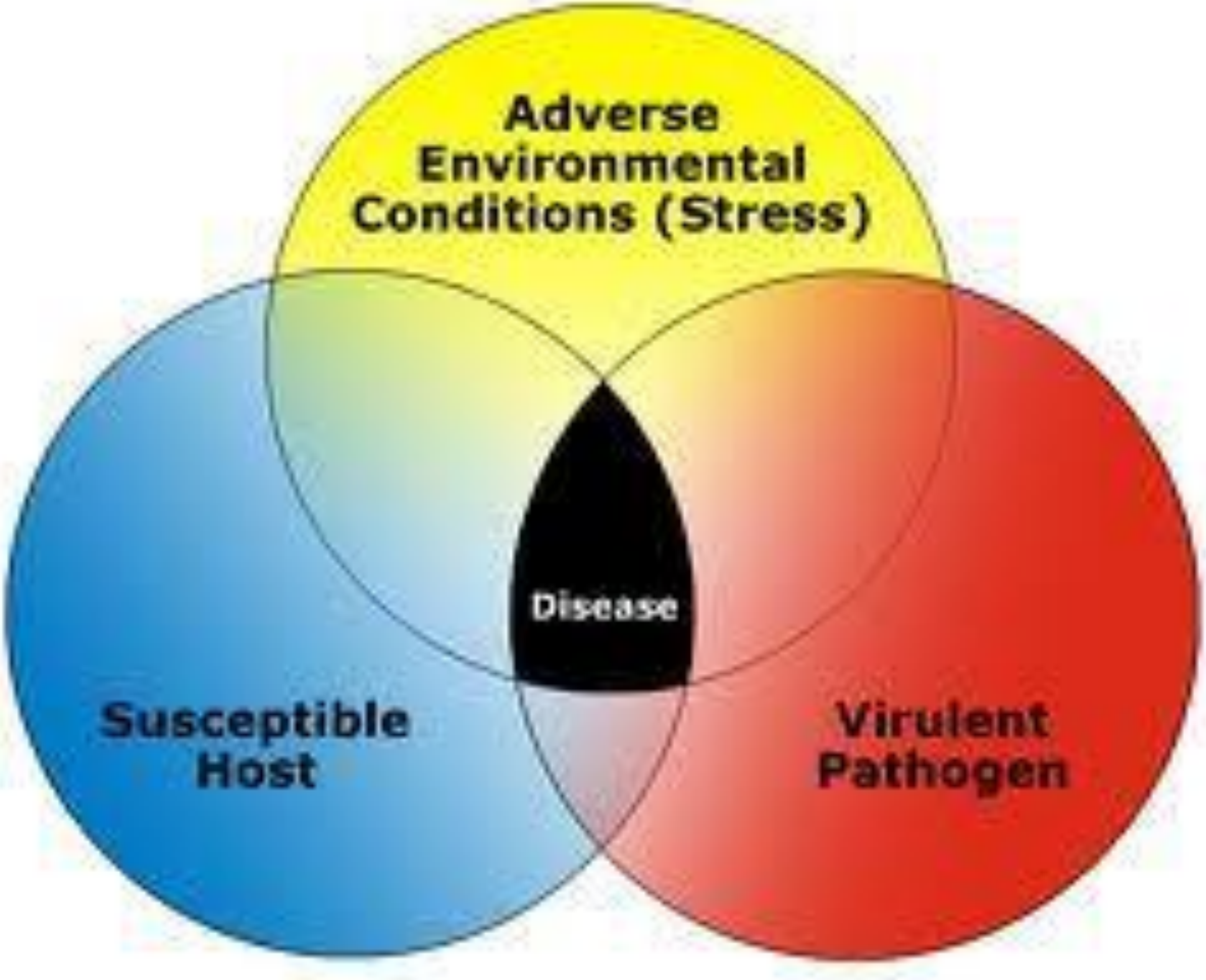


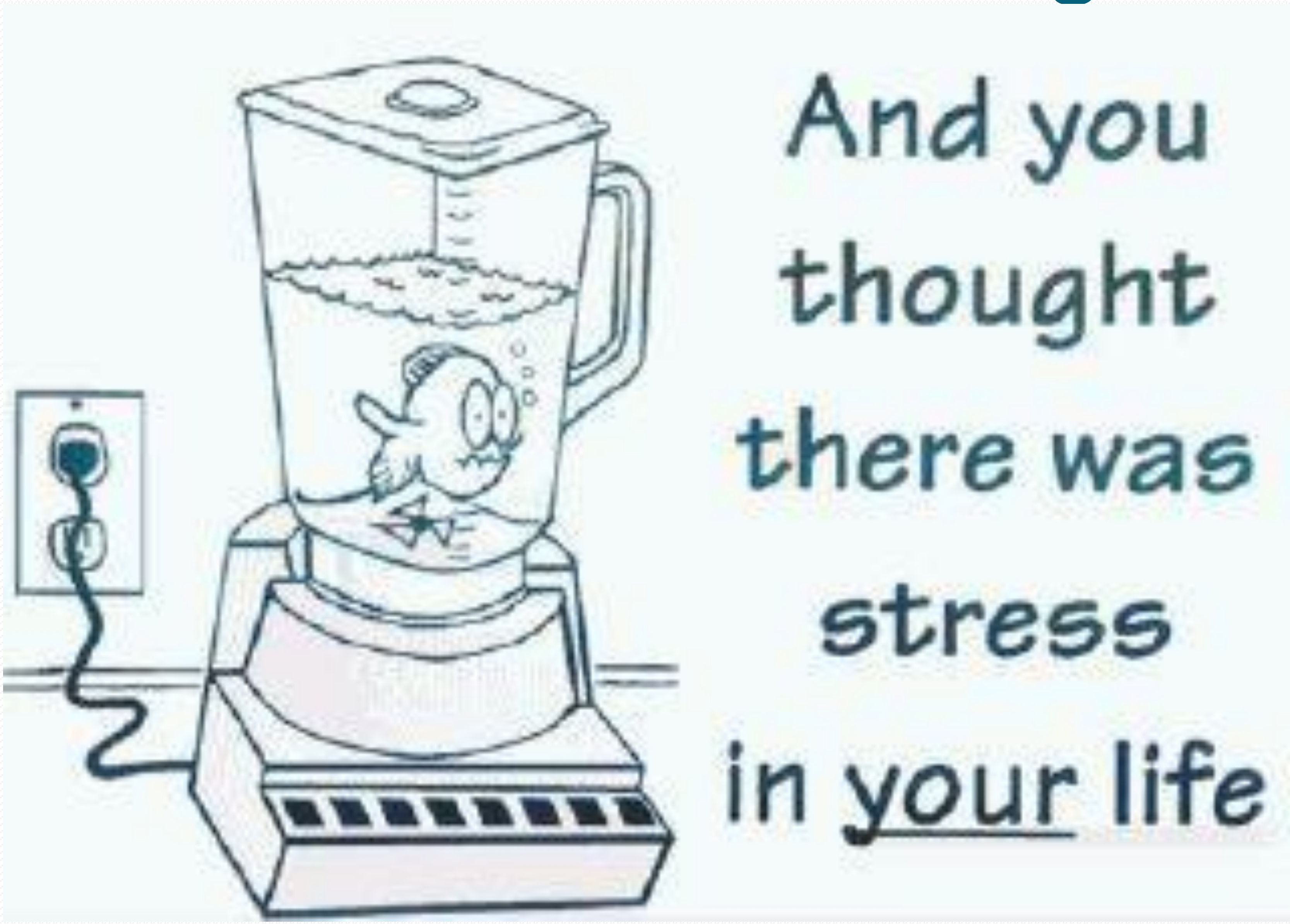
Figure 2. Lesions of menhaden that contain the fungal pathogen: ***A*** early raised lesion; ***B*** advanced ulcerated lesion.

Fig. 1 - Image of a "red tide" algal bloom from San Diego County, CA. Image source - NOAA.

Fish Kills from Infectious Agents



Fish Kills from Infectious Agents



Fish Kills from Infectious Agents

- Viruses, Bacteria, Fungi, Parasites
- Mass mortalities in natural waters from disease are associated with STRESSFUL ENVIRONMENTAL CHANGES, HIGH POPULATION DENSITIES, and/or SHORTAGES OF FOOD.\
- Losses are seldom abrupt

Fish Kills from Infectious Agents

- VIRUS OUTBREAKS

Fish Kills from Infectious Agents

- BACTERIAL DISEASE OUTBREAKS

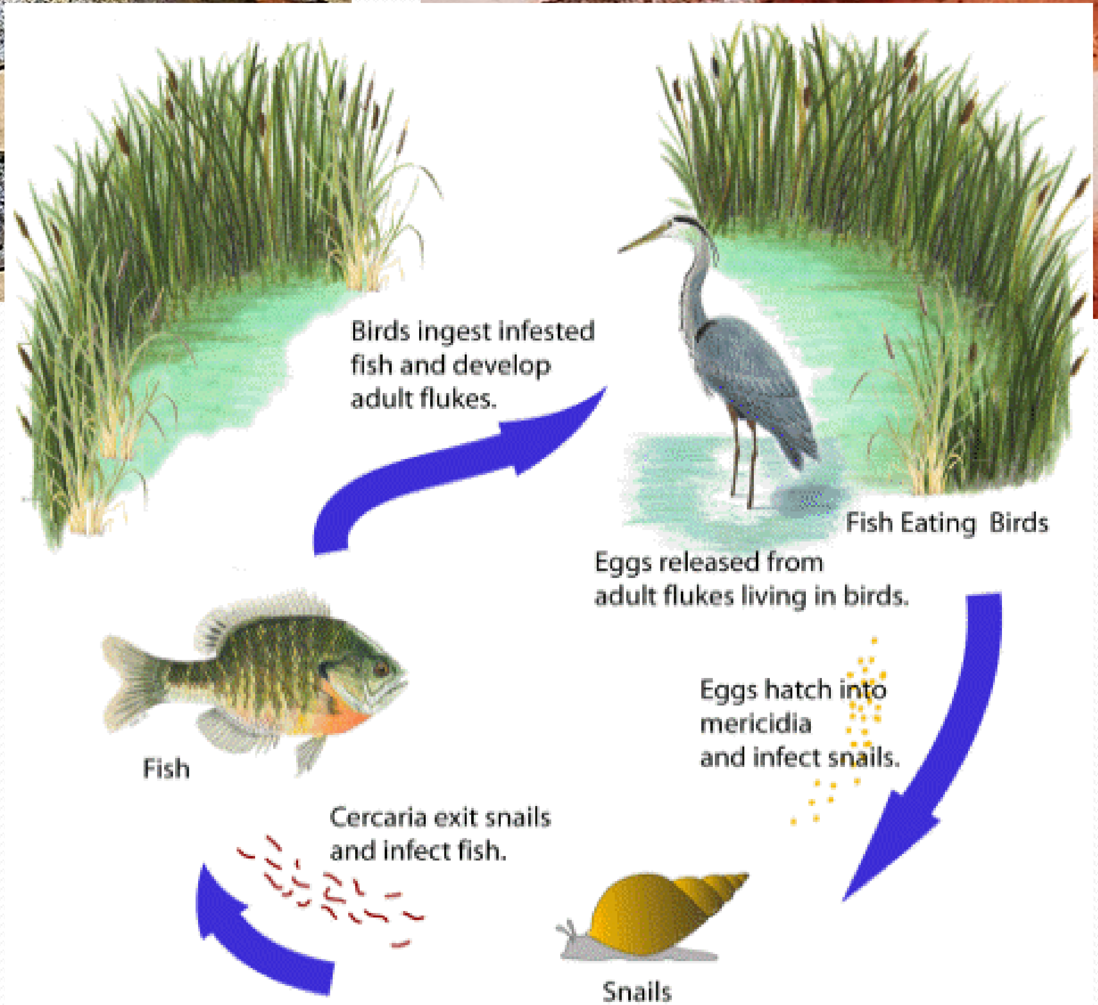
Mostly STRESS Related

Winter kills of gizzard shad -*Aeromonas hydrophila*

Migrating Pacific salmon – *Flavobacter columnaris*

Fish Kills from Infectious Agents

FUNGAL and PARASITIC DISEASE

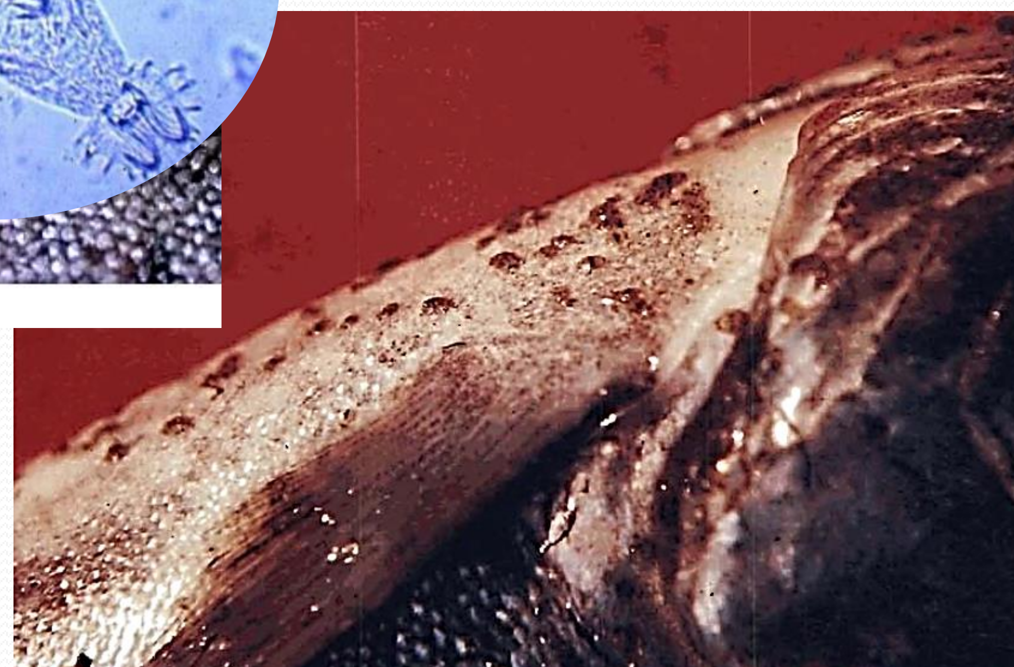


Fish Kills from Infectious Agents

- FUNGAL and PARASITIC DISEASE

Smc

o: Jarl Koksvik



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

Fish Kills from Infectious Agents

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 describing how the fish were sufficiently stressed to allow the infection to progress to an acute disease state.

Documentation

- Every fish kill investigation should be conducted with the expectation that it could result in regulatory action
- Consistent and accepted methods of assessing monetary value of the fish kill need to be used when documenting the magnitude and severity of the kill
- Factors that need to be considered are the monetary value of the fish and other aquatic organisms, remediation cost and cost associated with staff time and analysis

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Documentation

Observations and sample collection

- Photograph and video documentation should be obtained from scene
- Dates and locations should be indicated in photos
- All evidence/samples should be properly documented labeled/tagged and by collector before being sent to outside labs for analysis
- Chain of custody forms should be developed
- Calibrations and maintenance records should be kept for all equipment used to collect evidence in field

PFBC Fish Health Lab

PFBC Fish Health Lab

Documentation

(Investigation, Forms, Protocols)

FISH KILL NOTIFICATION

STATE: ______

DATE OF KILL: ______

DATE REPORTED: ______ TIME REPORTED: ______

NAME OF REPORTER: ______

ADDRESS: ______ PHONE: ______

ORGANIZATION: ______

WATER(s) INVOLVED: ______ COUNTY: ______

SPECIFIC LOCATION OF KILL (bridge, highway or state road, industry, landmark, mountain, park,etc.): ______

Township: ______ Range: ______ Section: ______ 1/4 Section: ______

SUSPECTED REASON FOR FISH KILL: ______

LOCATION OF SOURCE: ______

NAME OF ALLEGED POLLUTER: ______

ADDRESS: ______ PHONE: ______

SPECIES INVOLVED: ______

NUMBER: ______ FISH STILL DYING? ______

PERSONS AND AGENCIES NOTIFIED:

	NAME	TIME	PHONE
1.			
2.			
3.			

ADDITIONAL COMMENTS: ______

REPORT RECEIVED BY

CHAIN OF CUSTODY FOR FISH KILL SAMPLES

COLLECTOR'S NAME AND AFFILIATION (PLEASE PRINT)	DESCRIPTION OF SHIPMENT		PROJECT OR MAJOR FIELD CODE
	NUMBER OF SAMPLES		
	NUMBER OF CONTAINERS		
	HOW SEALED		

SAMPLE NUMBER	SAMPLE DESCRIPTION	COLLECTED DATE	COLLECTED TIME	ANALYSES REQUESTED FROM LABORATORY	FIELD ANALYSES						SITE CODE

CHAIN OF CUSTODY RECORD				IF SHIPPED	DELIVERED		PICKED UP	
COLLECTOR (SIGNATURE) ☐ SEALED ☐ SHIPPED	RECEIVED BY ☐ SEALED	DATE	TIME	CARRIER	DATE	TIME	DATE	TIME
COLLECTOR (SIGNATURE) ☐ SEALED ☐ SHIPPED	RECEIVED BY ☐ SEALED			CARRIER				
COLLECTOR (SIGNATURE) ☐ SEALED ☐ SHIPPED	RECEIVED BY ☐ SEALED			CARRIER				

FRONT

DATE COLLECTED ______ TIME ______ SAMPLE NUMBER ______

COUNTY ______

TYPE OF SAMPLE: WATER__ FISH__ SEDIMENT__ OTHER__

SAMPLE LOCATION ______

SAMPLE PRESERVED WITH ______ __NONE

OTHER INFORMATION ______

COLLECTED BY: ______

BACK

SAMPLE TRANSFER RECORD - SIGNATURES REQUIRED

DATE/TIME

FROM ______ TO ______

FROM ______ TO ______

FROM ______ TO ______

FROM ______ TO ______

FROM ______ TO ______

FROM ______ TO ______

Southwick, R. I., and A. J. Loftus, editors. 2017. Investigation and monetary values of fish and freshwater mollusk kills. American Fisheries Society, Special Publication 35, Bethesda, Maryland.

Documentation

(Fish/organism counts)

- Monetary loss of fish is dependent on number, species and size of organisms(species identification should be done by professional)
- Total counts of affected organisms are not always practical (not all dead fish float)
- Need to use accepted protocols for determining the magnitude of the event
- Need to chose protocol compatible with the affected area and nature of the fish kill
- Protocols should be chosen that will minimize the standard error when calculating the estimated loss

Documentation
(Fish/organism counts)

- Type of waterbody being sampled dictates sampling protocol
  - Small stream, Meandering stream, Large River, Lake, Pond

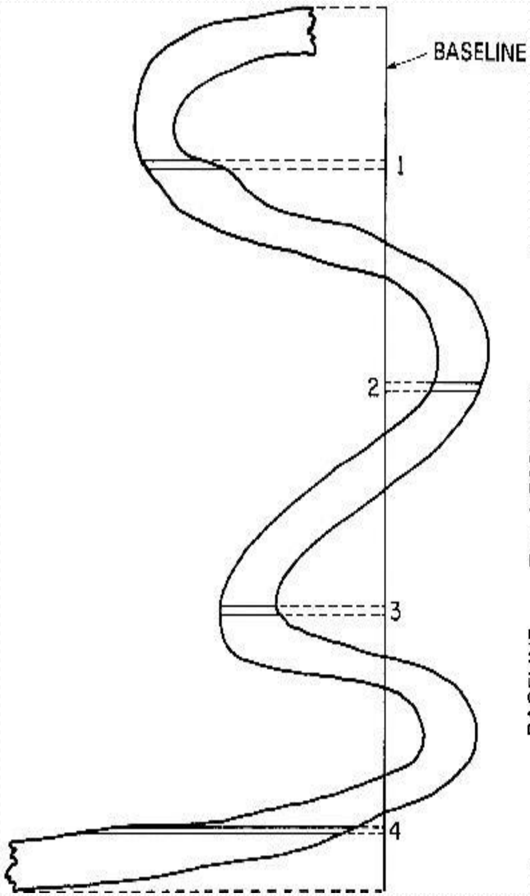
- May require subsampling and the use of transects, units or segments of the affected area

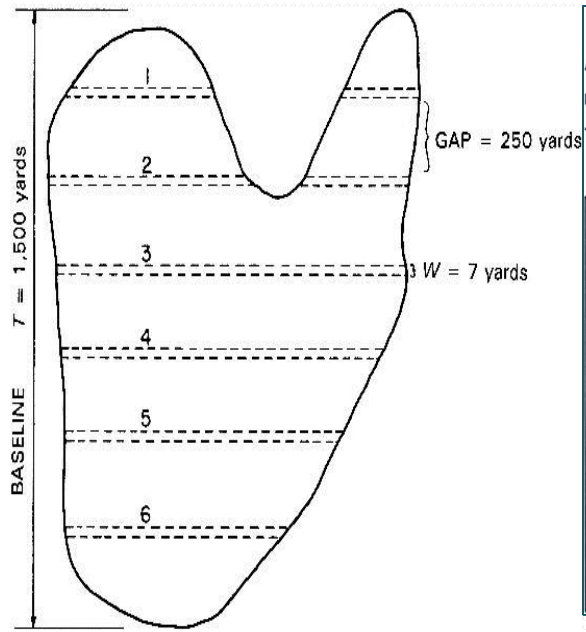
- Access to the waterbody may dictate sampling protocol

- Duration of the fish kill may require fish counts be conducted on over several days

Documentation

(Fish/organism counts)





FISH-COUNTING RECORD

AREA SAMPLED: Shoreline ____ Stream ____ Transect ____ Length or Area ____ Code Number ____ Date ________

LOCATION: ________ COUNTY: ________ INVESTIGATORS: ________

TIME: Start ________ Finish ________ NOTES: ________

INCH-CLASS

SPECIES	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

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Documentation
(Fish Kill Investigation Report)

- Final report needs to be accurate and complete
- Should be prepared with the expectation it will be evidence in a court
- Standardized forms reduce the chance of omissions and oversights that can result in the evidence being useless
- Diagnostic and analytical reports from secondary labs should be included

FISH KILL INVESTIGATION REPORT

Prepared and submitted by: ____________

Date: ____________

1. Kill reported to investigator by:

   Name ____________

   Address ____________

   Phone ________ Date ________ Time ________

2. Investigator reported kill to:

   Name ____________

   Address ____________

   Phone ________ Date ________ Time ________

   Name ____________

   Address ____________

   Phone ________ Date ________ Time ________

3. Body of water ____________
4. Nearest town ____________ County ____________
5. Time and date of kill ____________
6. Time and date of investigation ____________
7. Suspected cause of kill, or describe how kill occurred ____________
8. Alleged contamination source ____________
9. Duration of kill ____________
10. Extent of kill: Stream miles ________ Lake acres ________
11. Estimated total number of fish killed (from #24) ________ Value $________
12. Species of fish killed ____________

- 1 -

13. Condition of fish found (check one or more):

    Dead for several days ________ Dead for a short period ________

    Dying and in distress ________

    Other (write in) ____________

14. Symptoms of distressed fish ____________
15. Abnormal appearance of the water ____________
16. Investigation personnel (give name and organization represented):
17. Other persons and organizations involved:

    Name and title | Organization and address

    Remarks ____________

    Remarks ____________

    Remarks ____________

18. Topographic view or map of the affected area:

- 2 -

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19. Chemical data:

Sample location & tag number	Sample depth	Date	Time	D.O.	pH	Temp.	Other field tests	Water samples

Chemical data prepared by: ____________________

Title: ____________________ Date: ____________

20. Discussion:

21. Recommendations of corrective action needed:

22. Projected loss of fisheries:

Water body ____________________

Number of sample areas ________ Length ________ Surface acres ________

Total length of stream affected ____________

SPECIES

____

Length	Total number	Total weight	Value per fish	Total value	Length	Total number	Total weight	Value per fish	Total value
1"					11"				
2"					12"				
3"					13"				
4"					14"				
5"					15"				
6"					16"				
7"									
8"									
9"									
10"					Subtotal				

____

Length	Total number	Total weight	Value per fish	Total value	Length	Total number	Total weight	Value per fish	Total value
1"					11"				
2"					12"				
3"					13"				
4"					14"				
5"					15"				
6"					16"				
7"									
8"									
9"									
10"					Subtotal				

Total: ____________

Prepared by: ____________________

Title: ____________________ Date: ____________

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23. Field investigation expenses:

Agency ______________________________

Name	Hours	Salary	Miles driven	Milage alloca-tion	Boat opera-tion	Film	Other (itemize)
TOTALS							

Prepared by: ______________________________

Title: ____________________ Date: ______________

24. Totals:

Total number of fish lost ______________

Total fishery value lost $ ______________

Total expenses $ ______________

GRAND TOTAL $ ______________

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Sampling
(Supplies and equipment)

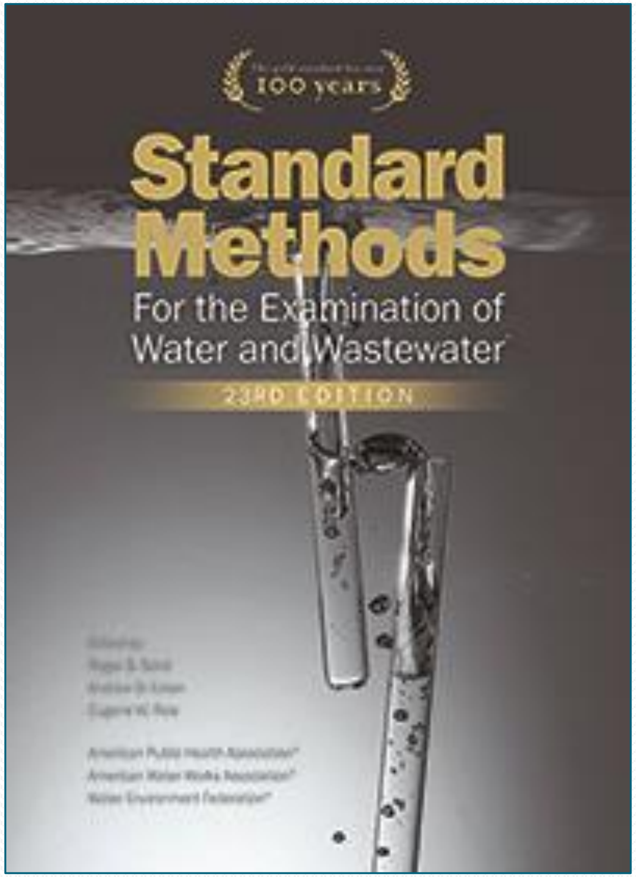
- Nature of the fish kill will dictate what type of analysis is needed
- Need to develop a checklist of equipment needed for a fish kill investigation
  - Waterproof forms, extra batteries, contact information, preservatives, PPE/first aid, extra memory cards for cameras, GPS unit, Nets, plastic bags, cooler and ice, shipping containers and shipping forms

Sampling
(Supplies and equipment)

- All water test meters and kits should be calibrated and reagents should be checked for expiration dates
- Labs should be contacted prior to collection to ensure correct protocol is followed and to determine the logistical needs
- When possible samples should be hand delivered

Sampling (water)

- Water Samples should be completed prior to fish
- Samples should be collected and preserved following the latest edition of *Standard Methods For the Examination of Water and Wastewater* or the protocols approved by the US Environmental Protection Agency
- Samples should be collected in a way that minimizes sediment contamination
- Water Quality parameters usually measured in the field include <u>dissolved oxygen</u>, <u>pH,</u> <u>total ammonia,</u> and <u>conductivity</u>



Sampling
(water)

- Always contact the lab and let them know you will be collecting and shipping samples
  - Depending on analysis samples may need a preservative added directly after collection or refrigerated
  - Specific container types are needed for certain analysis
  - Analysis require specific reagents may need to be made by the lab
  - Analysis have specific time restraints or requirements that may require the lab alter staff schedule

Sampling
(water)

- Lab will usually provide containers in advance
- Samples should be delivered or shipped to lab as quickly as possible
- In situations where it is suspected that a pollution event has occurred collect control samples (Upstream from discharge)

USGS

Sampling
(Sediment)

- Contact lab prior to collection, depending on analysis needed protocols and storage may vary.
- Samples should be taken from the same sites as water samples
- If pollution is suspected samples should be taken upstream of discharge.
- Samples will need to be kept cool (4°C), and may need to be frozen for preservation (20 °C).

Sampling

Collection, Preservation, and Shipment

- Delivery of live fish or fresh dead directly to the lab is optimal
- Contact lab prior to collection, depending on analysis needed protocols and storage may vary.
  - Bacteriology and cell culture need live fish or fresh dead
  - Histological samples need preserved
    - 10 % Neutral Buffered Formalin (NBF), 95 % ETOH, Z-fix
  - Genetic analysis may require other preservatives
    - 95% ETOH, Z-fix, RNA Latter
  - Toxicological samples may need to be frozen or may target specific tissue/organs

Sampling

(Fish)

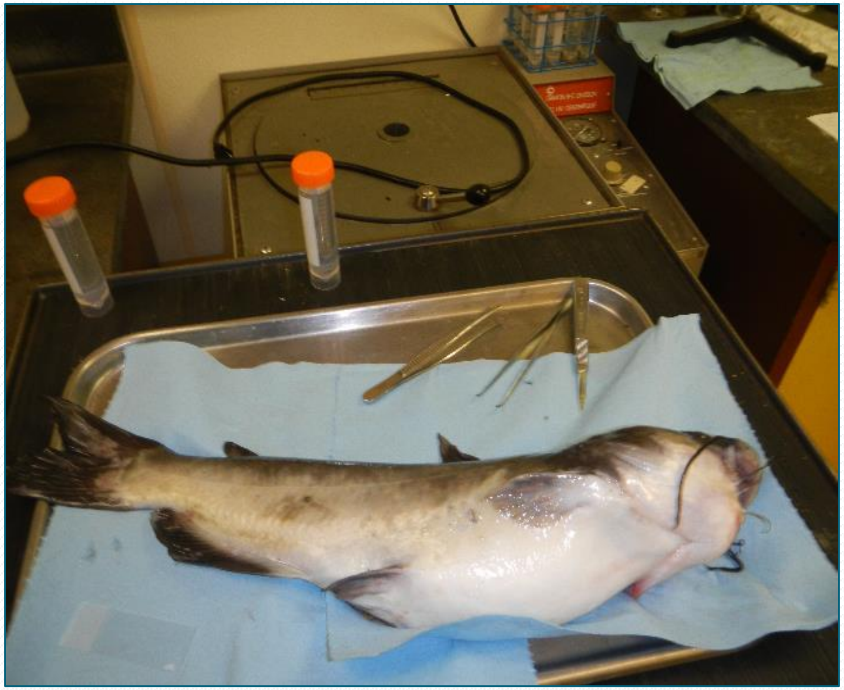
- Fish to collect
  - Moribund – Struggling
  - Fresh dead – clear eyes
- Tissue breaks down after death
- Representative sample of fish (species, size, location)

Sampling
(Fish)

- Representative sample of observed behavior
- If possible similar samples should be collected from an unaffected geographical area
- If possible collect samples for all available analysis, negative results are just as important as positive

Collecting fish and tissue samples for suspected toxic substances

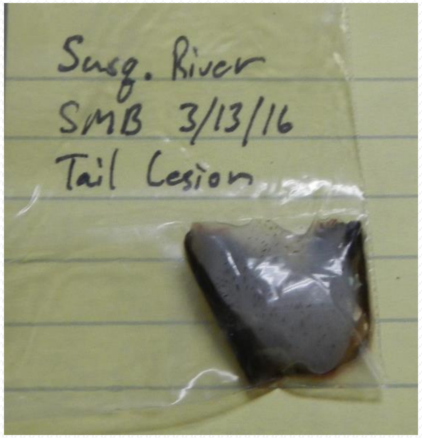
- For whole fish analysis for pesticides and other organic substances
  - Rinse with clean water
  - Wrap in aluminum foil, dull side contacting fish
  - Freeze immediately



- Sub sampled tissue and blood
  - Place tissue in Polyethylene bags frozen
  - May need placed glass containers and frozen if indicated by lab.

Samples Fish for Histological Analysis

- Whole small fish, opening needs to be made so preservative can enter visceral cavity
- Tissue samples should be places in preservative (1 part tissue/fish to 10 parts preservative)
- 10% Neutral Buffered Formalin, 70% ETOH, Z-fix, or other provided by the lab
- When collecting tissue samples if possible collect a sample that has affected and non-affected tissue.
- Do not freeze samples



Samples Fish for Histological Analysis

Collecting/transportation fish for Examination of pathogens

Live Fish

- Transport in a container with aeration or in a sealed plastic bag in a sealed container
  - Bag should be filled with oxygen
- If ice is needed to ensure constant water temperature the ice should be placed in a sealed plastic bag and placed in water or around bag
  - Ice can contain additives that may be harmful to fish
- Check fish during transport if fish, fish may need to be euthanized.

Collecting fish for Examination of pathogens

<u>Dead Fish</u> on ice within (24 hours)

- Fish should be placed in individual plastic bags and separated by Species.

- Drain all water/slime from bag with fish

- Fish should **<u>not</u>** be placed directly on ice, frozen tissue can not be processed for most pathogens. There should be a layer of insulation (Newspaper cardboard between ice and fish)

Sample collection, preservation, and shipment

- Have a network of laboratories in place, and be familiar with their specific protocols
- Familiarize yourself with types of analysis that may be needed and the collection and shipment protocols associated with the analysis
- Collect samples from non affected and affected areas
- Have shipping labels and containers ready
- Presence of contaminants and or pathogens does not always correlate with a mortality event

ANALYSES

- Observations
- Physical environmental parameters
- Fish Diseases

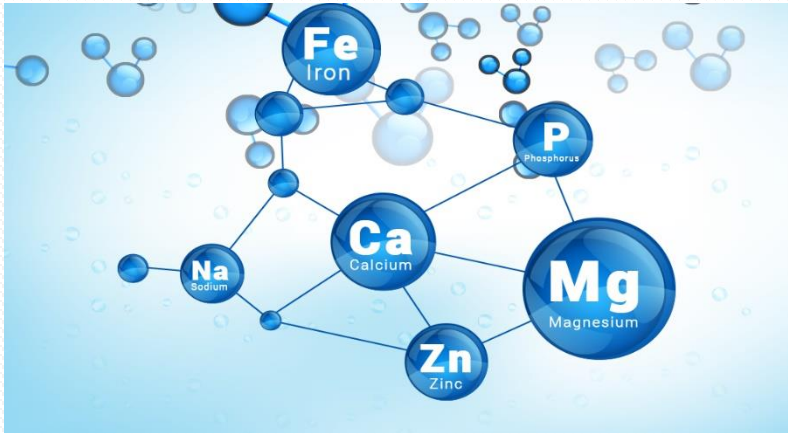
- Analytical labs –
  - Animal tissues (whole bodies or organs), Plants, Sediment/Soil, Water
    - perform inorganic analyses (primarily metals)
    - determination of organic compounds

ANALYSES

INORGANICS

Arsenic	Boron	Iron	Sodium
Selenium	Cadmium	Lead	Strontium
Mercury	Calcium	Magnesium	Thallium
Aluminum	Chromium	Manganese	Vanadium
Barium	Cobalt	Molybdenum	Zinc
Beryllium	Copper	Nickel	

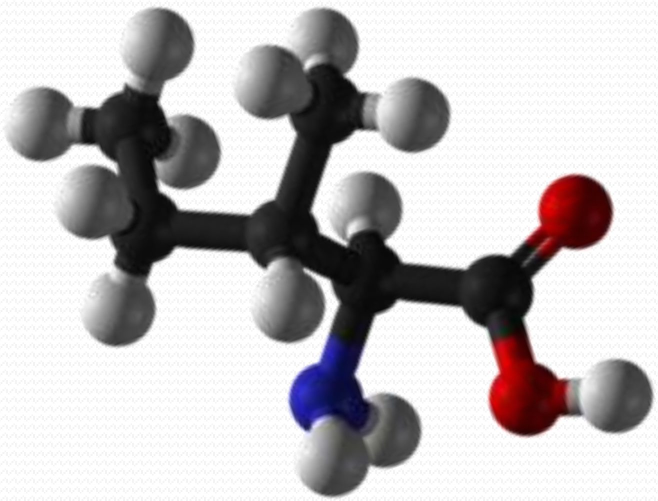
Phosphorous	Sulfite	Neutron Activation	Radiochemistry
Nitrogen	Sulfate	Chromium (III), (VI)	Graphite Furnace
Nitrate	Acid Volatile Sulfides	Arsenic Speciation	Hydride Generation
Nitrite	AVS/SEM	Selenite Methylmercury	
Chloride	Organ-tins	Selenate	



ANALYSES

ORGANICS

Single Component Organochlorines/PCB's
Multiple Component Organochlorines/PCB's
PCB Congeners
Aliphatic Hydrocarbons
Aromatic Hydrocarbons
Organophosphate/
carbamate pesticides
2,3,7,8-TCDD and TCDF
Other Dioxins and Furans
Polybrominated Diphenyl Ethers



ANALYSES

U.S. Fish and Wildlife Service

Environmental Contaminants Data Management System (ECDMS)

Contract Lab Name & Address	Lab ID	Primary Contact	Phone Number, Email Address, Web Site
Inorganic / Organic Labs			
Alpha Analytical 320 Forbes Boulevard Mansfield, MA 02048	AWH	Elizabeth Porta	508-844-4124 eporta@alphalab.com Web Site: alphalab.com
Columbia Analytical Services (now ALS Global) 1317 South 13th Avenue Kelso, WA 98626	CAS (ALS)	Inorganics Jeff Coronado	360-501-3310 jeff.coronado@alsglobal.com Web Site: caslab.com
		Organics Shar Samy	360-501-3293 Shar.Samy@ALSGlobal.com Web Site: caslab.com
Inorganic Labs			
Applied Speciation & Consulting 18804 Northcreek Parkway Bothell, Washington 98011	ASC	Russell Gerads	425-483-3300 russ@appliedspeciation.com Web Site: appliedspeciation.com
Brooks Rand Labs 3958 6th Ave NW Seattle, WA 98107	BRL	Amanda Royal	206-753-6111 amanda@brooksrand.com Web Site: brooksrand.com
Envirosystems Incorporated 1 Lafayette Road Hampton, NH 03843-0778	ESI	Russ Foster	603-926-3345 rfoster@envirosystems.com Web Site: envirosystems.com
Trace Element Research Laboratory College of Veterinary Medicine Dept. of Veterinary Anatomy and Public Health Texas A&M University VMA Bldg., Room 107, Highway 60 College Station, TX 77843-4458	TERL	Bob Taylor	979-458-0035 rtaylor@cvm.tamu.edu Web Site: vetmed.tamu.edu/terl

Organic Labs			
AXYS Analytical Services 2045 Mills Road West Sidney, BC Canada V8L 5X2	AXYS	Nicholas Corso	250-655-5800 ncorso@axys.com Web Site: axysanalytical.com
GEL Laboratories 2040 Savage Road Charleston, SC 29407	GEL	Valerie Davis	843-769-7391 vsd@gel.com Web Site: www.gel.com
Geochemical & Environmental Research Group 833 Graham Road College Station, TX 77845	GERG	Dr. Terry Wade	979-862-2323 x134 terry@gerg.tamu.edu Web Site: gerg.tamu.edu
Pace Analytical Services 2190 Technology Drive Schenectady, NY 12308	PACE	Kelly Miller	518 346 4592 x23 Kelly.Miller@pacelabs.com Web Site:pacelabs.com
TDI-Brooks International 14379B South Dowling Road College Station, TX 77845	TDI	Juan Ramiez	979 693 3446 juanramirez@tdi-bi.com Web Site: tdi-bi.com

egis.umn.edu/fishkillmap/s2.html?app=apps2/fishkillmap

Fish Kill Reporting Map

MWFWC2018_Phelps.pdf

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Why investigate fish kills?

1. Prepare, identify and respond to new threats
2. Cost effective "syndromic surveillance"
3. Public concern
4. Advance the science of fish health and fisheries management
5. Identify trends overtime

Fish Kills Surfacing Around MN's Lakes

The Minnesota Department of Natural Resources says high temperatures may be contributing to fish kills in lakes around the state.

WCCO


egis.umn.edu/fishkillmap/s2.html?app=apps2/fishkillmap
Fish Kill Reporting Map
MWFWC2018_Phelps.pdf
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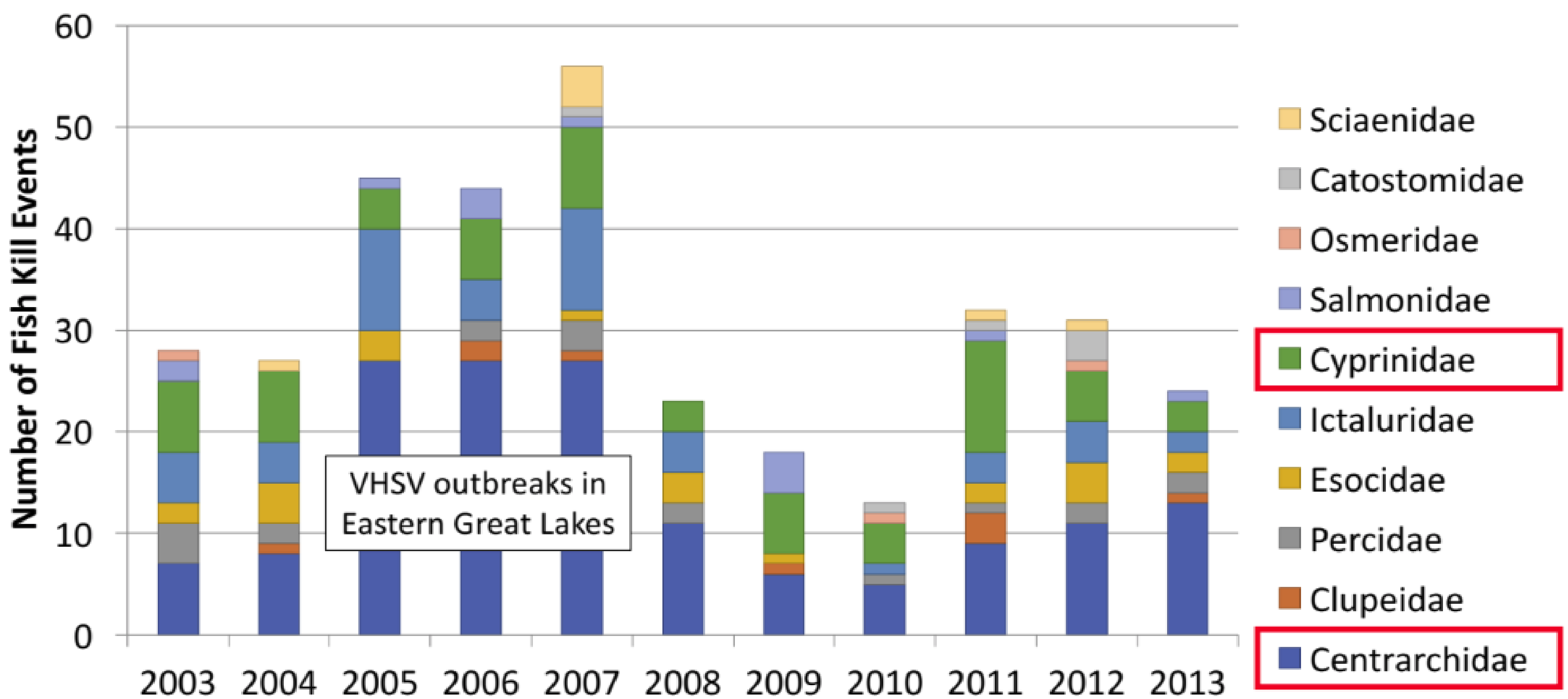
Fish kills 2003-2013:
Spills and Kills:
237 reported fish kills
Pathology Lab:
104 investigated fish kills
255 reported fish kill events from 2003-2013
≠
Consensus poll of DNR biologists = ~500 fish kills per year

Fish kills 2003-2013:



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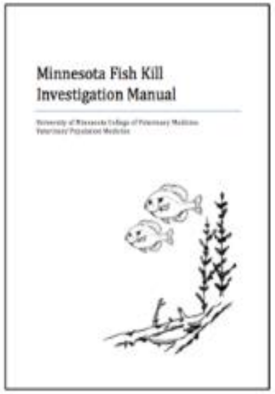
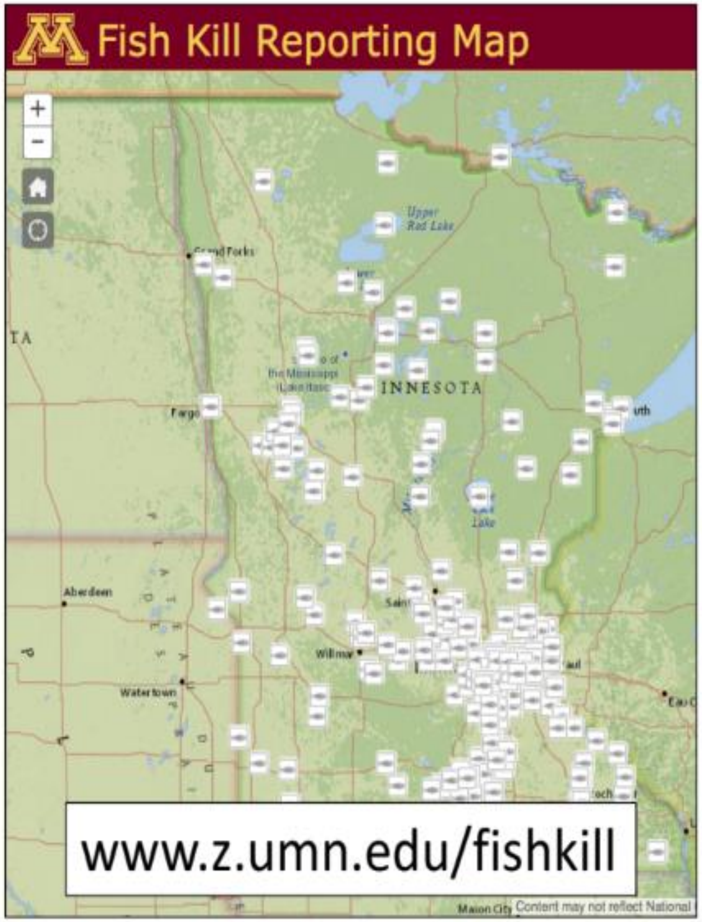
Improved response

- Online user-friendly reporting
- Standardized investigation manual
- Trained network of investigators
- Created outreach material





egis.umn.edu/fishkillmap/s2.html?app=apps2/fishkillmap

Fish Kill Reporting Map

MWFWC2018_Phelps.pdf

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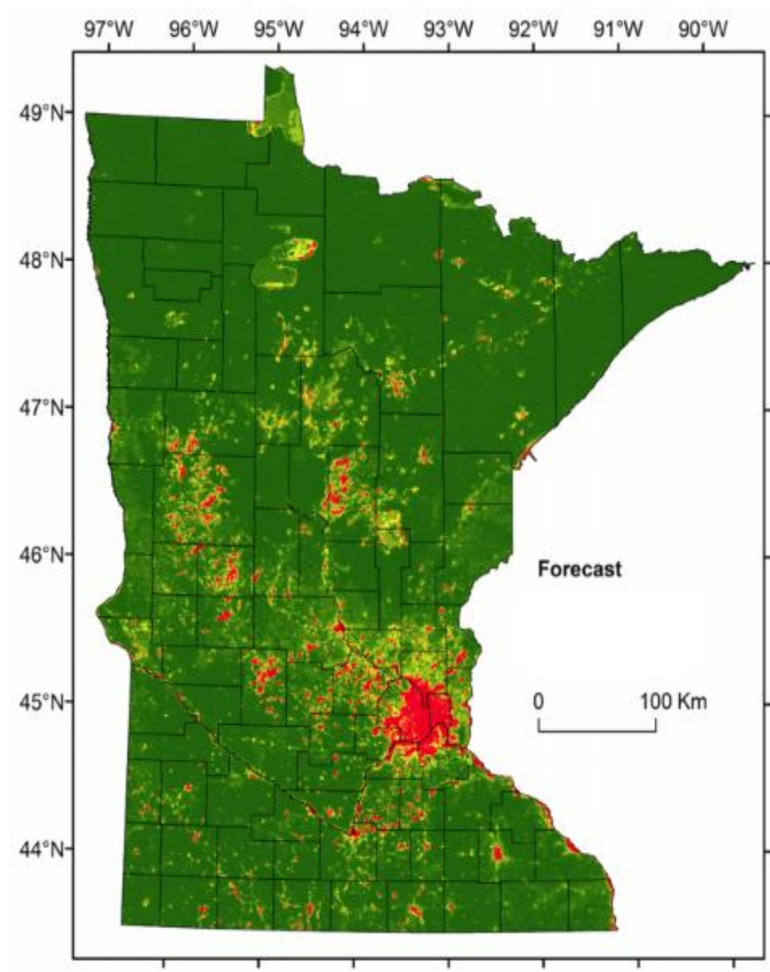
Predicting fish kills

- Primary factors:
    - Maximum overnight temp
    - Percent cropland cover
    - Nighttime light index
    - Population density
- Not perfect… but starting point
    - Update as data improves
- Monitor for trends overtime



CASES

The Case of the BOTCHED BATCH

Commercial fishermen report many dead fish in their nets
Recent rise in water level after a prolonged drought
No dead fish noted before or during the rise, but seen shortly after
You observe many small and some large dead fish
Some large fish listless, but if disturbed show convulsions
Wide array of species affected
pH = 7.5; DO= 7.0 ppm
Temp 27 C ; hardness 230 ppm

Water shows abundance of live algae of many species
Absence of benthic organisms, and many dead crayfish

Trib in area: many benthic organisms, live crayfish, algae of many species
pH 7.5; Do 7.0; 25 C and hardness 218

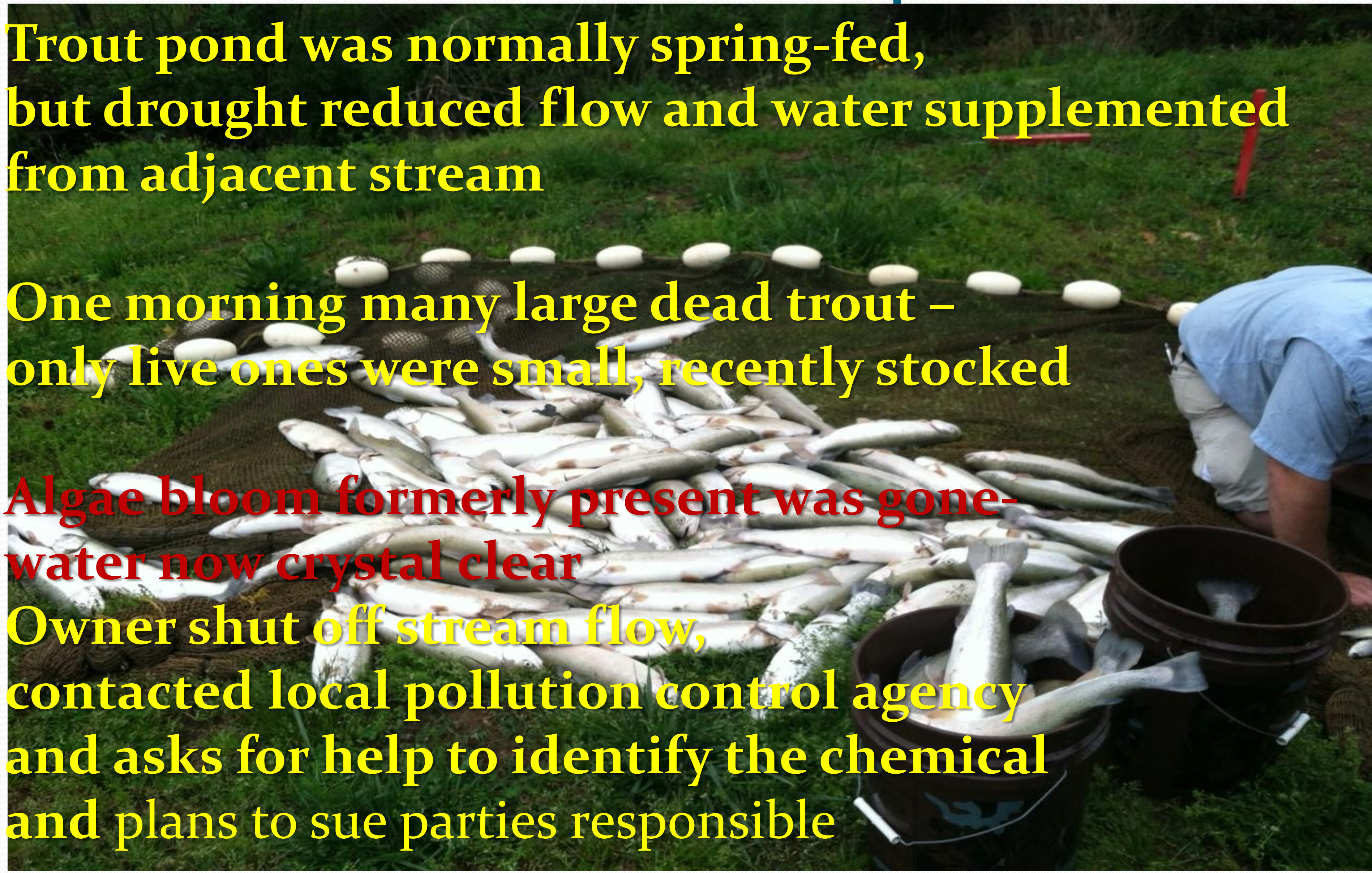
Botched Batch

- Preliminary Conclusions?
  - Source of problem upriver
  - Oxygen depletion not a cause
  - Too many different organisms for an infectious disease
  - Suspect a toxin – wide array of affected and small died 1st
  - Toxin not a herbicide: killed fish, benthos, crustaceans, not algae
- What next?
  - Survey upstream to a point where things are normal
  - Collect water samples to test for pesticides
  - Collect moribund fish – blood brain liver
  - Check all tribs and point sources – sediment, water, fish
  - Collect similar samples from normal trib

Botched Batch

- Information
  - Fish kill traced upstream to a single affected tributary
  - Kill zone ends at a landfill site adjacent to that tributary
  - Recent high water had eroded the landfill/stream bank
  - Dark viscous layer on top of landfill
  - Lab analysis on dark substance = endrin insecticide
  - Water upstream of trib no endrin, significant amounts downstream
  - Sediment in trib upstream of landfill no endrin, high conc. at & below
  - Whole fish and blood, brain, and liver showed many pesticides, including endrin – at 0.15 – 0.22 mg/L
  - Toxicity info on endrin says lethal to fish at 0.19 mg/L
  - Several industries in area, one produces endrin
  - Plant Manager denies any releases through any plant drains
  - It becomes revealed that a production run of endrin had gone bad, was terminated, and dumped into the landfill.
- FINAL CONCLUSION
  - Endrin poisoning resulted from illegal dumping

the case of the Clear Creek Caper



Trout pond was normally spring-fed,
but drought reduced flow and water supplemented from adjacent stream

One morning many large dead trout –
only live ones were small, recently stocked

Algae bloom formerly present was gone-
water now crystal clear
Owner shut off stream flow,
contacted local pollution control agency
and asks for help to identify the chemical
and plans to sue parties responsible

Clear Creek Caper

- Preliminary Conclusions
  - Large fish dead, small alive –not toxicity, suggests O2
  - Disappearance of plankton bloom and clarity of water suggests herbicide rather than eutrophication

- What Next ?
  - Check DO in pond and creek
    - Immediately; late afternoon and again at daybreak
  - Check on plant and aquatic life in stream above and below pond outlet
  - Investigate water chemistry – stream & pond
  - Check on herbicide uses the day before in surrounding area

Clear Creek Caper

- Information Found
  - DO in pond was 4ppm at 2Pm & 4PM, 3ppm at daybreak
  - DO in stream was a constant 8.0 ppm
  - All plants and biota upstream and below pond were thriving
  - Water chemistries in stream & pond were essentially the same
  - No herbicide was applied in the watershed of the stream or on the shore of the pond
  - Pond owner's worked commented that the pond had been treated the previous day with 2 mg/L Cutrine to control protozoan parasites- also had been used three other times in the last 9 months without a problem.
- FINAL CONCLUSION
  - Cause of the kill- oxygen depletion triggered by algicidal action of Cutrine
    - Loss of photosynthesis & decay of algae reduced O2 below lethal limit for large trout – small fish able to obtain enough from spring and stream flow.
    - Other times spring flow was enough to provide adequate O2

the case of The Black Lagoon

Long shallow (av depth 1.8 m) municipal lake fed by a trib stream
with a steep graded watershed at upper end.

Large feed mill and several retail farm & garden chemical stores at lower end.

In August a partial kill of fish occurred – investigators arrived at 11:00 am – many bullheads seen swimming at surface, all other fish seen were dead.

Water had been dark green earlier in week – now very dark & odorous

Preceding day heavy rain, with hail, had fallen in area.

City concerned that at toxic substance may have washed into lake from chemical companies or feed mill-
and want to know what samples should be taken to identify

At 1:00 pm DO was 2 ppm, lake rose about 0.3 m in 6 hours after the rain event

Strong odor of hydrogen sulfide and methane

the case of The Black Lagoon

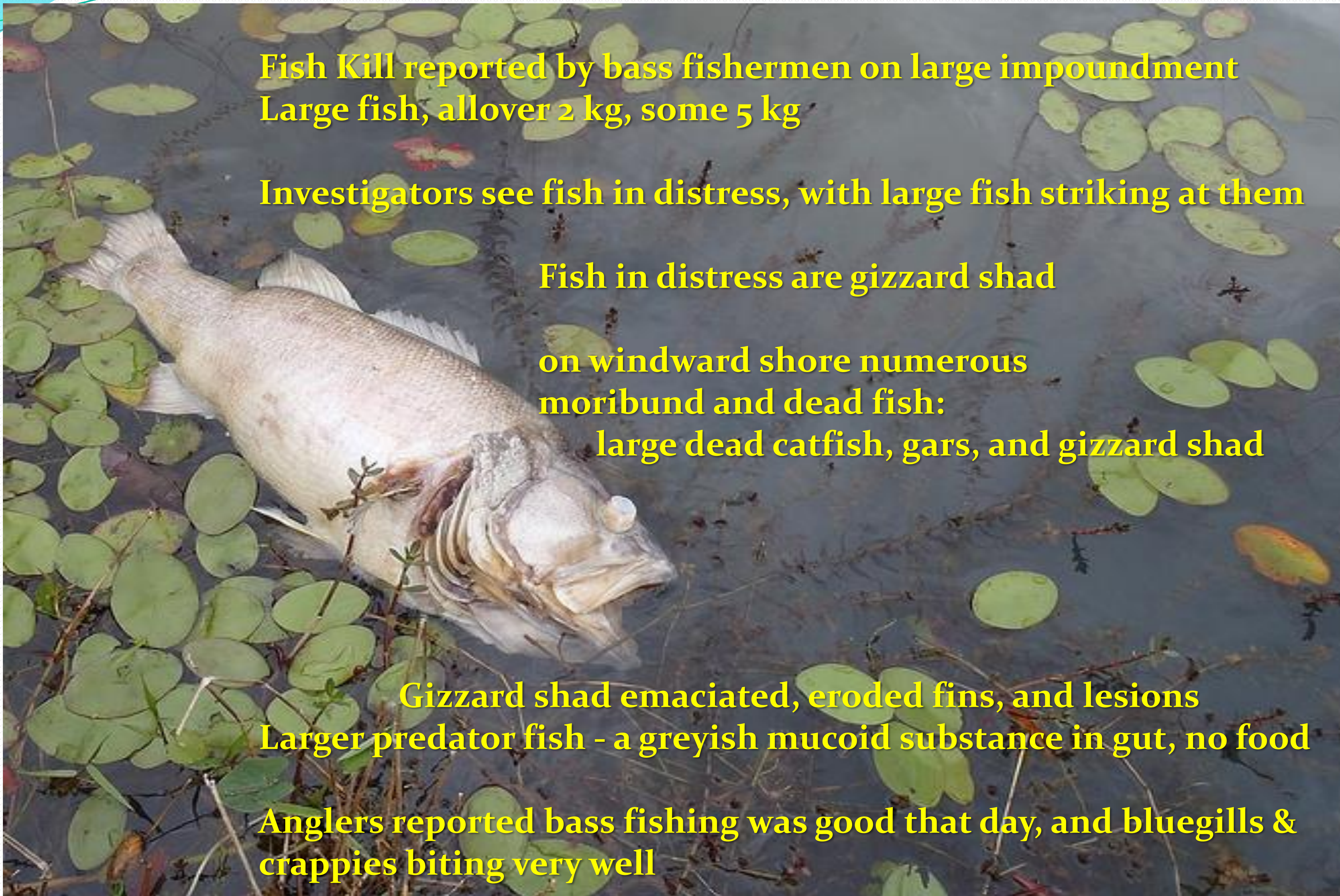
- Preliminary Conclusions
  - Oxygen depletion is suggested –but rain should have oxygenated the water
  - Bullheads are among the most resistant species to toxic substances, all other fish are dead, thus a toxic substance san not be ruled out
- What Next?
  - Check DO and pH on site immediately
  - Collect water samples
    - Intervals along length of lake
    - At surface, mid-depth, and near bottom
  - Collect samples of whole fish, livers, gills, and blood for pesticides and other lab analyses
  - Obtain hydrological & limnologic data on the lake

the case of The Black Lagoon

- Information Found
  - When bullheads collected bled freely from gills
    - Gills had many aneurysms
    - Blood was dark brown rather than bright red
  - Water had black detritus and dead algal cells
  - Water tests showed high hydrogen sulfide, high CO_2, and nitrites and nitrates
  - Acidification of blood samples released hydrogen sulfide odor
  - Other fish and water tests revealed numerous compounds, but none of concentrations that would be toxic
  - Normally the lake is stratified with temps differing by 11C from top to bottom –On date of investigation the temperature was the same from top to bottom.

- FINAL CONCLUSION
  - Loss of fish due was due to combination of low O_2 and hydrogen sulfide poisoning. Heavy cold rain induced a turnover having the anoxic bottom water mix with surface water.

the case of The Lethal Lunch



Fish Kill reported by bass fishermen on large impoundment
Large fish, allover 2 kg, some 5 kg

Investigators see fish in distress, with large fish striking at them

Fish in distress are gizzard shad

on windward shore numerous
moribund and dead fish:
large dead catfish, gars, and gizzard shad

Gizzard shad emaciated, eroded fins, and lesions
Larger predator fish - a greyish mucoid substance in gut, no food

Anglers reported bass fishing was good that day, and bluegills & crappies biting very well

the case of The Lethal Lunch

- Preliminary Conclusions
  - Water quality not a problem – good fishing
  - Toxic substance(s) probably not involved –some fish thriving and only large fish dying
  - Affected fish: gizzard shad and large predators –relationship?

- What Next?
  - Collect moribund fish of all species for parasite and bacterial testing
  - Gross microscopy on fish – especially lesions and gray substance in gut
  - Inoculate bacterial culture media from lesions, kidney, and gut

the case of The Lethal Lunch

- Information Found
  - No parasite common to all affected fish
  - Microscope exam of gizzard shad lesions showed bacteria resembling columnaris disease
  - Microscope exam of gray material from predator guts showed bacteria resembling columnaris disease
  - Bacterial cultures were identified as *Flavobacter columnaris*, the cause of columnaris disease
  - Gizzard shad are all of one year class and in poor condition
- FINAL CONCLUSION
  - Cause is columnaris disease. Disease originatedin the over-abundant year class of gizzard shad, because of their age, poor condition, and other stressors. Fish large enough to feed on the moribund shad contracted the disease and died from a systemic infection – fish too small to eat the shad were unaffected.

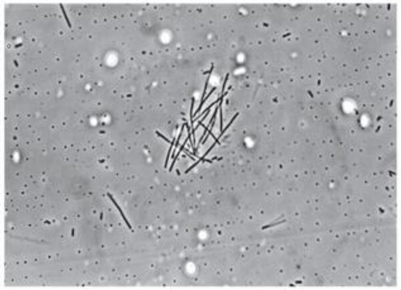


Figure 2. Wet mount strains (100x) of long bacilli *Flavobacterium columnare* isolates.

the case of The ACID RAIN

Hatchery uses city water as its water supply

Has a system to remove chlorine

One day all fish on station died

Fish tried to get out of water,
looked bleached,
died with gills flared

In ponds water cleared and plants turned brown or white

Hatchery contacted Water Department – water supply is a reservoir fed by streams from forested mountains

Rain had fallen recently and they suspect that runoff had transported pesticides into the reservoir

the case of The ACID RAIN

- Preliminary Conclusions
  - Incident is a catastrophic environmental event – something highly toxic to fish and plants

- What Next?
  - Check water at hatchery for presence of chlorine
  - Visit Water Department to determine what was done differently in the last 24 hours
  - Have complete water analyses run on water from fish tanks
  - Collect and freeze fish samples for possible future analyses
  - Check reservoir and collect water for analyses

the case of The ACID RAIN

- Information Found
  - No residual chlorine in hatchery water –removal system working properly
  - Water Department states water treatment has been normal
  - Visit to reservoir – water had been low from prolonged drought; recent heavy rains raised level by 3.5 m; reservoir got turbid- red clay; fisherman reported angling had been fair; vegetation appeared normal
  - Hatchery water analyses:

    hardness 30 ppm, DO 8.0; pH 3.0; alkalinity 0 ppm; TSS 5 mg/L
  - Reservoir water analyses:

    hardness 35 ppm; DO8.0; pH 7.1; alkalinity 27 ppm; TSS 500 mg/L
  - Water treatment plant – because of high total solids, aluminum sulfate was added prior to sand filter, changing the charge on the clay particles to cause them to participate. Sand filter removed the clay –a standard treatment
  - pH of water ahead of and behind sand filter showed no change; however sample taken after chlorination showed a drop from 7.1 to 2.5
  - Discussion with a chemist revealed that chlorination of water containing dissolved aluminum sulfate results in formation of sulfuric acid

- FINAL CONCLUSION
  - Fish killed by low pH caused by sulfuric acid formation. Fish signs consistent with low PH toxicity.

Thank You

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