Die-offs, also referred to as fish kills, occur in both natural and cultured populations of fish and shellfish. Sometimes die-offs are caused by applications or spills of chemicals, agricultural waste, or sewage, or by pump, aerator, or well failures or power outages. But more often they are caused by natural events such as heat, drought, turnover, disease, parasites, or low dissolved oxygen.

When fish die-offs or disease outbreaks occur at an aquaculture facility, it is usually an emergency situation. The same may or may not be true for non-commercial ponds and lakes. A fish die-off in a private foodfish pond managed for family sustenance is likely to be considered an emergency, but in some instances a die-off in a recreational angling pond may be viewed as a natural correction that restores populations to levels sustainable with limiting water quality thresholds. No matter the scenario, producers and private pond and lake owners should be prepared in advance to respond to a die-off. That means having an emergency plan and knowing who to contact for assistance.

Preparation for a fish die-off or disease problem

To minimize fish losses, the following preparations should be made:

- Monitor fish populations frequently and note unusual behavior or conditions.
- Purchase a water quality test kit and/or meters and learn how to use them.
- Have an emergency aeration plan and equipment.
- Contact the fish disease diagnostic laboratory in your state from the list on page 6. If no diagnostic lab is listed for your state, contact your county Extension agent or Extension aquaculture or fisheries specialist to determine the labs that will accept samples from your state. Establish communication with a lab before a die-off occurs, and post the telephone number and address of the lab in a prominent place.
- Call the laboratory and ask how samples should be collected, handled, and shipped. Also ask what days and times samples may be delivered.
- Determine whether the diagnostic laboratory has any special terms or exceptions (e.g., call before submitting samples, in-state only, no shellfish, fee-based only).
- Have on hand the type of container(s) needed to ship samples.
- Determine the best method of transportation (personal delivery, air freight, overnight express) and the schedule that will ensure prompt delivery.
- Have a designated veterinarian who has inspected the facility, is familiar with the species cultured, and can write prescriptions for any necessary treatments.
- Determine which feed mills can manufacture medicated feeds and how fast they can be delivered to you.
- Maintain stocks of common treatment chemicals, such as salt, potassium permanganate, formalin, or hydrogen peroxide, when feasible.
Determining a water quality problem

The term water quality really refers to water chemistry, which includes gas solubility and the concentration of many substances in the water, such as carbonate, bicarbonate, calcium and magnesium metal ions, ammonium/ammonia, nitrite, nitrate, salts, and dissolved carbon dioxide and oxygen. A person cannot typically see water quality, although it may be possible to smell or taste certain substances that contribute to water quality. For more information on acceptable and preferred water quality parameters, see SRAC Publication No. 4606, Interpretation of Water Analysis Reports for Fish Culture.

Poor water quality alone can cause massive fish die-offs. Low dissolved oxygen, a critical water quality parameter, is the largest cause of fish die-offs in recreational angling ponds and lakes in the southern region. Low dissolved oxygen die-offs are less common in cultured fish because of intense management and the greater availability of emergency aeration devices, but other water quality issues such as high ammonia or nitrite concentrations may be more problematic with high stocking densities. Water quality can be a major factor contributing to viral and bacterial infections and parasite infestations in fish and shellfish.

Water quality does not remain constant, but fluctuates daily, seasonally, and when certain events take place (e.g., rainfall, fertilizer application, fish stocking, etc.). In ponds, water quality can change dramatically over a few hours. An example would be substantial decreases in pH, dissolved oxygen, and alkalinity concentrations at night when plants are not photosynthesizing (SRAC Publication No. 464, Interactions of pH, Carbon Dioxide, Alkalinity and Hardness in Fish Ponds). Even water from deep wells and springs can change over time as compounds are depleted from or dispersed into the groundwater.

Because water quality is dynamic, commercial fish and shellfish producers should not rely on previous diagnostic laboratory results to identify water quality problems. It is extremely important to have a water quality test kit, know how to use it, and be able to interpret the results (see SRAC Publication No. 4606, Interpretation of Water Analysis Reports for Fish Culture). Quick water quality test strips can determine a starting point for titration testing or provide a quick indication of water quality in an emergency, but they should never be relied upon as an accurate or sufficient testing method. Whenever possible, chemical titration or high quality digital meters and thermometers should be used to accurately determine water quality.

Water quality should be monitored routinely to identify problems before fish die-offs occur (see SRAC Publication Nos. 462, 463, 464, 468, 4601, 4603, and 4604). Also, any time fish or shellfish appear stressed or mortalities are observed, water quality should be evaluated immediately for temperature, dissolved oxygen, total ammonia, pH, and nitrite. It may be several hours from discovery of dead or stressed fish before a water sample can be taken and analyzed by an Extension specialist, veterinarian, or certified fish health specialist, and the water quality often changes considerably during that time. Other tests may be appropriate depending on the results of the initial screen.

Testing for toxicity from pesticides, hydrocarbons, or other chemical residues

Fish disease diagnostic laboratories will test water samples to determine if there is a basic water quality issue, but most do not test for pesticides, hydrocarbons, or other chemical residues in water or tissue samples. Most laboratories do not have the capacity to do such testing. Aquatic animal pathology and chemical residue laboratories are highly specialized in terms of equipment and methodology, so it is not common to find a laboratory that does all the necessary testing that may be required to determine the cause of a fish kill.

If there is evidence that a fish die-off was caused by a toxic chemical or a toxic algae bloom (see SRAC Publication No. 4605, Algal Toxins in Pond Aquaculture), water and tissue samples should be submitted immediately to a laboratory that tests for pesticides, organic chemicals, and algae. Do not delay, as many chemicals deteriorate quickly in water or the presence of sunlight, while others are quickly bound by pond sediment. If a sample cannot be sent immediately to a laboratory, freeze it immediately to slow any chemical breakdown that could occur.

Not every state has a pesticide residue testing laboratory, so it may be necessary to ship a sample out of state. Contact your state Department of Agriculture or aquaculture or fisheries Extension specialist for assistance in finding labs that may accept samples from your area. Ask the laboratories how to submit samples and what types of samples should be submitted.

Special sampling and handling procedures may need to be used if a toxic substance is suspected in a fish die-off (see SRAC Publication No. 4600, Toxicities of Agricultural Pesticides to Selected Aquatic Organisms). In many states, very specific instructions must be followed if any legal action is to be taken. These steps are often referred to as chain of custody. Become familiar with your state’s chain of custody requirements by contacting the state Depart-
ment of Agriculture or animal health regulatory agency (often referred to as state veterinarians). Ask the lab about approved procedures before collecting samples.

The chain of custody must be maintained on all samples considered potential evidence to be sure it will be admissible in court. Chain of custody is of utmost importance in situations such as environmental spills or herbicide drift/overspray, where a criminal or civil case may be pursued. (Chain of custody is not necessary in the case of disease or parasite outbreaks.) It may be necessary to prove that the evidence presented in court is the same as that collected at the scene of the die-off. As potential evidence is transferred from one person to another for storage, shipping, or testing, a chain of custody form must be signed and dated by each person involved. Each transfer must be clearly noted on the form and every person who handled the sample may be required to testify in court before sample results are admitted as evidence.

**Submitting samples for toxicology diagnosis**

Live, sick fish usually are not required to test for a toxin. Instead, freeze several recently deceased fish or shellfish (as long as bloating or decomposition has not begun) and a water sample and submit those immediately. Clinical signs associated with toxicosis in fish, and common causes, are shown in the table below:

<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>Possible causative agent</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>White film on gills, skin, and mouth</td>
<td>Acids, heavy metals, trinitrophenols, wood preservatives, inorganic insecticides, herbicides</td>
<td>Fertilizers, wood preservatives, phospholipid, biological waste, galvanization compounds, herbicides, solvents, mining refinement</td>
</tr>
<tr>
<td>Sloughing of gill epithelium (outer layer or lining)</td>
<td>Copper, zinc, lead, ammonia, alkylbenzenesulfonates, quinoline</td>
<td>Organic and inorganic debris, naturally occurring mineral found in some deep well water sources</td>
</tr>
<tr>
<td>Clogged gills</td>
<td>Turbidity, ferric hydroxide</td>
<td>Herbicides, plastic and resin manufacturing, paint strippers, cosmetics, sunscreen, hair dyes, insecticides, surfactants, plasticizers, dyes, leather tanning agents, dispersants in lead-acid batteries</td>
</tr>
<tr>
<td>Inflamed mouth, esophagus, and gills</td>
<td>Arsenic compounds</td>
<td>Insecticides, wood preservatives, herbicides</td>
</tr>
<tr>
<td>Extremely bright red gills</td>
<td>Cyanide compounds</td>
<td>Fertilizers, biological waste, herbicides, plastic and resin manufacturing, paint strippers, cosmetics, sunscreen, hair dyes, insecticides, surfactants, plasticizers, dyes, leather tanning agents, dispersants in lead-acid batteries</td>
</tr>
<tr>
<td>Dark gills</td>
<td>Phenol, naphthalene, nitrite, hydrogen sulfide, low dissolved oxygen</td>
<td>Insecticides, insect repellents, polyurethanes, wood preservatives, paint preservatives, cosmetic preservatives</td>
</tr>
<tr>
<td>Hemorrhagic gills</td>
<td>Alkylbenzenesulfonates</td>
<td>Detergents</td>
</tr>
<tr>
<td>Distended opercles</td>
<td>Phenol, cresols, ammonia, cyanide</td>
<td>Fertilizers, mining waste, stainless steel, tool steel, cast iron, high-temperature superalloy manufacturing</td>
</tr>
<tr>
<td>Blue stomach</td>
<td>Molybdenum</td>
<td>Toxic cyanobacteria bloom</td>
</tr>
<tr>
<td>Pectoral fins locked in extreme forward position</td>
<td>Organophosphates, carbamates</td>
<td>Toxic golden algae (Prymnesium parvum) bloom</td>
</tr>
<tr>
<td>Piping, redness, or hemorrhaging at base of fins, around mouth, under chin,</td>
<td>Prymnesins, ichthyotoxin, cytotoxin, hemolysin</td>
<td>Toxic cyanobacteria bloom</td>
</tr>
<tr>
<td>Respiratory distress, rapid opercular beats, disorientation, intestinal tract</td>
<td>Cyanotoxins, neurotoxins, cytoxins, endotoxins, hepatotoxins</td>
<td>Toxic cyanobacteria bloom</td>
</tr>
<tr>
<td>Gas bubble (fins, eyes, skin)</td>
<td>Supersaturation of gases</td>
<td>Supersaturation of water under high pressure or from deep wells</td>
</tr>
</tbody>
</table>
Submitting a sample for disease or parasite diagnosis

In most instances live, sick fish and a water sample are required for the diagnosis of diseases or parasites. Fish in the samples should exhibit obvious physical signs of disease or parasites, such as:

- visible external or internal parasites
- open sores, hemorrhaging, pustules
- internal fluid accumulation (ascites) leading to a swollen abdomen
- bulging or distended eyes, referred to as popeye (exophthalmia)
- small red and white ulcers and pinpoint red spots (petechial hemorrhage)
- raised or eroded red ulcers protruding through the top of the skull
- yellowish or light-colored, slightly eroded areas on the body, fins, or gills, or in the mouth
- swollen, fused, clubbed, or red and white mottled gills
- eroded or bloody fins
- excessive mucous (slime coat) on the body or gills
- gulping water/air at the surface despite sufficient dissolved oxygen

An excellent sample would also include several fish exhibiting abnormal or unusual behavior, such as lying listlessly in shallow water or at the water surface, inability to maintain equilibrium (turning on side or upside down), erratic darting while rubbing against objects (flashing), gathering around incoming water sources, or swimming erratically or in circles.

The best method of collecting sick fish is to walk the pond bank with a dip net or cast net to selectively remove fish that are at the surface, at the water’s edge, or otherwise appear abnormal. It may take extra effort to find and catch sick fish in this manner, but the quality of the resulting information will be well worth it. Taking a random sample of fish from a seine has a poor probability of identifying the cause of the fish loss because many of the fish in the pond may be healthy. The worst way to collect sick fish is by hook and line. Sick fish usually do not eat, but the healthiest fish in the pond will still be actively feeding, so catching fish with a rod and reel will result in a sample of little or no diagnostic value.

If live, sick fish are unavailable, recently deceased fish (generally less than 20 minutes in warm water, less than 1 hour in cold water) can be a fair sample. Recently deceased fish used as samples should have gills, eyes, color, and mucus that still appear similar to live fish, although the likelihood of isolating pathogens or external parasites may be greatly diminished. As a general rule, if the gills are still bright red, a dead fish may be a valid sample for submission, but it should be placed into well drained ice immediately to preserve the integrity of the sample. If the gills are bright pink, the sample quality has deteriorated substantially, but there is still a small chance that pathogens or parasites may be isolated from the sample. If the gills are light pink, white, or grey, the fish is useless for diagnostic testing. Dead fish that have floated to the surface of a pond are also useless for diagnostic purposes. In both these instances, it is impossible to tell whether isolated bacteria found on or in the dead fish were responsible for its disease, and most external parasites will have fled the dead host or died themselves. NEVER freeze a sample that is to be submitted for disease or parasite diagnostic testing. Instead, ship it in direct contact with dry ice, as freezing renders the sample useless for pathology.

Ideally, a minimum of three to six sick fish with obvious signs of disease or parasites and three to six fish exhibiting abnormal or unusual behavior should be submitted for examination. If only a single fish is submitted, the diagnosis may be inaccurate or incomplete. A single fish is not entirely representative of a population. Most fish disease outbreaks involve more than one problem. Therefore, a representative sample containing several fish is essential for making good management decisions.

In ponds larger than 1 surface acre, a minimum of two 1-pint water samples should be collected from opposite ends of the pond for analysis. Dissolved oxygen should be checked by the property owner at the pond or lake bank using a dissolved oxygen meter or lower cost titration kit. Dissolved oxygen cannot be accurately measured at the laboratory because of air trapped in the sample bottle and the biological processes of bacteria and plankton within the sample bottle. Do not combine fish and the water in the same container, but their separate containers may be shipped in the same package.

A water sample without a fish or shellfish sample is generally of little value in determining the pathogen or parasite responsible for a die-off. However, fish produce waste products such as ammonia and nitrite that are toxic to the fish themselves can weaken the animal’s immune system, make it more vulnerable to pathogens and parasites, and can sometimes be the direct cause of death. Therefore, submitting a water sample along with fish or shellfish samples is critical, as the cause of toxicity is not easily determined from the animal sample alone.

Some chemicals such as ammonium/ammonia, nitrite, nitrate, salts, and lime can sometimes enter ponds from
outside sources and may be the cause of fish die-offs. Therefore, a water sample should be submitted to the diagnostic laboratory along with fish samples even though toxicity may not be suspected. Freeze an additional water sample to send to a toxicity testing laboratory in case no pathogens or parasites are detected and potential toxins cannot be detected from standard water quality testing.

**Diagnosing diseases in aquarium or ornamental pond fish**

Because live fish do not survive the testing process, it may be necessary to sacrifice one or more of these fish for disease diagnostics in order to save the rest of the fish in the aquarium or pond. There are a few private practice veterinarians who specialize in diagnosing diseases and parasites in live ornamental fish, but the fish usually must be delivered in person to the veterinary facility and diagnosis can be extremely expensive compared to a disease diagnostic laboratory.

**Shipping samples to a disease diagnostic laboratory**

Ideally, sick fish should be transported live to the diagnostic laboratory. If the lab is less than an hour away, sick fish can be transported in a container of water with a lid. Sick fish also can be shipped live in a plastic bag with water and pure oxygen. The bag should be sealed and placed in an insulated shipping container (large, hard-sided coolers work well) with ice to maintain a cool water temperature. This will keep sick fish alive for several hours.

For longer shipping durations, sick or newly deceased fish should be wrapped in moist paper towels, placed in a plastic bag (without water), and transported on crushed ice in a hard-sided cooler or high-density, Styrofoam-lined shipping box. Dry ice may be used in extremely warm conditions, but the sample must be packed so that it does not come into direct contact with the dry ice. Follow the methods for overnight or air shipment samples as well. A sample handled in this manner should be of some diagnostic value for up to 48 hours, although the likelihood of isolating only the bacterial pathogen gradually declines over time.

Water samples can be collected in any clean glass or plastic containers. A water sample of at least 1 pint should be shipped on ice with the fish sample. As previously mentioned, special instructions must be followed if a toxic substance might be involved that could result in criminal or civil litigation.

**Information to include with sample shipments**

The following information should be included with each sample submitted to a fish disease laboratory.

1. Name, address, phone number, and e-mail address of the fish owner.
2. Physical location of the pond or tank containing the sick fish. These may be required for toxicology testing if litigation could result from the test results. Include the facility name, physical address, and GPS coordinates, if possible. Make sure to note this on the chain of custody form as well.
3. Name or designation of the pond or tank from which fish were removed. (Note: Fish collected from different ponds or tanks should be labeled and shipped in separate, labeled containers and accompanied by a water sample from each unit.)
4. Dimensions of the pond or tank, including average and maximum depths.
5. Species, number, and average size of fish stocked.
6. Statement indicating whether multiple species are sick or dead.
7. Date when fish were last stocked (include number, species, and size stocked).
8. Amount fed per day. (Are fish still eating? If not, when did they stop eating?)
9. Date when mortalities were first noticed.
10. Number of fish that have died per day since mortalities were first noticed.
11. The most recent treatment used, including treatment date and amount of chemical used.
12. Condition of the plankton bloom, as determined by the maximum depth that a Secchi disk (can substitute a 1-gallon paint can lid or pie plate that is painted white) is still visible.
13. Any water quality data collected immediately before or within 1 hour of discovery of the sick fish.
14. Unusual behavior (e.g., flashing, inability to maintain equilibrium, piping) noticed before or at the time of the discovery of sick or dead fish.
15. Physical symptoms (e.g., lesions, hemorrhaging, scale loss) noted before or at the time of the discovery of sick or dead fish.
16. Weather conditions for 3 to 5 days before the discovery of the sick or dead fish.
17. The presence of aquatic vegetation and approximate area coverage.
Awaiting test results

Individual laboratories vary in the time required to process samples and communicate the results to fish or shellfish owners. In most cases, water quality data and results of the necropsy and parasitology examination should be available within 24 hours of receipt of samples, but labs may differ in their policies pertaining to releasing partial exam results or waiting until the full diagnostic work-up is completed. Depending on circumstances and the rate of fish loss, a presumptive diagnosis may be made immediately after the completion of water quality, necropsy, and parasitology examination.

Microbiology (bacterial isolation and antibiotic sensitivity testing), virology (molecular identification of virus), and histopathology (microscopic examination of specially prepared tissues) take more time. These tests are usually complete within 48 to 120 hours of sample submission, but virology may take 2 weeks or more for completion.

The best ways to save remaining fish or shellfish while awaiting diagnostic results are to improve water quality and reduce animal density. Increasing aeration and filtration, adding small quantities of salt (1 to 3 parts per thousand [g/L]) for freshwater animals or slightly reducing salinity for marine species, and flushing fully or partially with fresh water can alleviate many problems. Reducing animal density can improve water quality, reduce pathogen or parasite density, and decrease the likelihood of transmission from one fish to another. However, stress induced by capture and transport can worsen the effect or spread of illness, so reducing animal density should be attempted only when it can be done quickly with minimal disturbance. Reducing fish density can be accomplished quickly in many tank or raceway systems, but is not recommended in pond systems unless the fish density can be reduced passively by allowing the fish to swim from one pond to another through piping or a levee opening.

Conclusions

Die-offs, also referred to as fish kills, occur in both natural and cultured populations of fish and shellfish. In aquaculture facilities, best management practices (see SRAC Publication Nos. 474, 4703, 4707, 4708, and 4712) and proper nutrition (see SRAC Publication Nos. 4711 and 5003) can help prevent fish or shellfish die-offs. It is extremely important to have a response plan in place before a die-off occurs. When die-offs do occur on a fish farm or private pond or lake, it is usually an emergency situation. To optimize their response, producers and recreational pond owners must be prepared to quickly check water quality parameters, obtain proper fish and water samples while following chain of custody regulations, and transport samples using appropriate methods as quickly as possible to a predetermined diagnostic laboratory. While waiting for diagnostic results, the best approach to saving any remaining fish is to improve water quality by increasing aeration and flushing with fresh water.

Fish Diagnostic Laboratories

Alabama
Southeastern Fish Disease Diagnostic Laboratory
(Auburn University)
Department of Fisheries & Allied Aquacultures
203 Swingle Hall
Auburn University, AL 36849
Diagnostic services: (334) 844-9220
Swingle main office: (334) 844-4786
Note: must call to make arrangements before submitting a sample

UAPB Fish Health Laboratory
Alabama Fish Farming Center
529 South Centerville Street
Greensboro, AL 36744
Phone: (334) 624-4016
Note: must call to make arrangements before submitting a sample

Arkansas
UAPB Fish Health Laboratory
Aquaculture/Fisheries Center
Mail Slot 4912
University of Arkansas at Pine Bluff
1200 N. University Drive
Pine Bluff, AR 71601
Phone: (870) 575-8137
Note: must call to make arrangements before submitting a sample

UAPB Fish Health Laboratory
Lonoke Agriculture Center
P.O. Box 357
2001 Hwy. 70 East
Lonoke, AR 72086
Phone: (501) 676-3124
Note: must call to make arrangements before submitting a sample

UAPB Fish Health Laboratory
1652 C Hwy. 65 & 82 South
Lake Village, AR 71653
Phone: (870) 265-5440
Note: must call to make arrangements before submitting a sample
Florida
Fish Disease Diagnostic Laboratory
UF/IFAS Tropical Aquaculture Laboratory
1408 24th St. SE
Ruskin, FL 33570
Phone: (813) 671-5230 Ext. 0
Note: must call to make arrangements before submitting a sample
Fish Health Laboratory
University of Florida
IFAS Extension Veterinarian for Aquaculture
Aquatic Animal Health Program in Fisheries and Aquatic Sciences
7922 NW 71st Street
Gainesville, FL 32606
Phone: (352) 273-3613
Note: must call to make arrangements before submitting a sample
North Florida Aquatic Veterinary Services
945 SW Pathfinder Glen
Fort White, FL 32038
E-mail: drpetty@nflaquavetsrvc.com
Note: must email to make arrangements before submitting a sample
Aquatic Animal Health Laboratory, HBOI-FAU
Susan Laramore
5600 US 1 North
Fort Pierce, FL 34946
Phone: (772) 242-2525
E-mail: slaramol@fau.edu
Note: specializes in shellfish
Bronson Animal Disease Diagnostic Laboratory
Bureau of Diagnostic Laboratories, Division of Animal Industry
Florida Department of Agriculture and Consumer Services
2700 John Young Parkway
Kissimmee, FL 34741
Phone: (321) 697-1400

Georgia
Georgia Public Fish Diagnostic Services
University of Georgia Aquaculture Specialists
Gary Burtle
2360 Rainwater Road
Tifton, GA 31793
Phone: (229) 386-3364
Email: gburtle@uga.edu

Kentucky
KSU Fish Disease Diagnostic Laboratory Aquaculture Research Center
103 Athletic Road
Kentucky State University
Frankfort, KY 40601
Phone: (502) 597-6581
Fax: (502) 597-8118

Louisiana
Louisiana Aquatic Diagnostic Laboratory
Louisiana State University School of Veterinary Medicine
Baton Rouge, LA 70894
Phone 1: (225) 578-9777
Phone 2: (225) 578-9705

Mississippi
Mississippi State University
College of Veterinary Medicine
Thad Cochran National Warmwater Aquaculture Center
Aquatic Research & Diagnostic Laboratory
127 Experiment Station Road
Stoneville, MS 38776
Phone: (662) 686-3302
Notes: must call to make arrangements before submitting a sample; specializes primarily in catfish, no mollusks or shellfish
Fish Diagnostic Laboratory, College of Veterinary Medicine
Mississippi State University
240 Wise Center Drive
Mississippi State, MS 39762
Phone: (662) 325-3432
SRAC fact sheets are reviewed annually by the Publications, Videos and Computer Software Steering Committee. Fact sheets are revised as new knowledge becomes available. Fact sheets that have not been revised are considered to reflect the current state of knowledge.

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