Submission of Fish for Diagnostic Evaluation[1]

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Introduction

Determining the cause of a fish's illness and death can be challenging. Without a thorough diagnostic workup, disease problems are often misdiagnosed, which leads to fish receiving inappropriate treatment that may cause more harm than good. Such treatments are often costly and cause a delay in diagnosis, which can lead to secondary infections (caused by agents that invade fish tissue after the damage by the initial agent has occurred). Therefore, it is important to contact a veterinarian, fish health professional, or a fish disease diagnostic laboratory as soon as a disease problem is suspected, to prevent misdirection in your fish health management plan.

When fish become sick or die, the first response should be to find out why. The sooner the cause of illness is determined, the faster a response can be started to address the problem. Whether it is a pond, home aquarium, production farm, or a fish kill observed in the wild (see USDA SRAC No. 472), it is imperative to respond quickly and appropriately. The following outlines the proper procedures to submit fish and water samples to a diagnostic facility. Following these steps should enable a correct evaluation and the best recommendation for treatment.

The Importance of History and Records

When a client contacts a diagnostic laboratory, he/she will be asked a routine set of questions. Everyone involved should be knowledgeable about the aquatic system and animals that live there. Keeping records of water chemistry parameters, water change schedules, species in the system, and recent additions of animals and plants can accelerate the processing of samples and provide the needed recommendation. The following is a selection of questions a fish health specialist is likely to ask, depending on the individual's situation. The client should be prepared to provide information on the following:

General

- What is the size and design of the aquatic system involved?
- How old is the aquatic system? When was the last time it was taken apart and/or otherwise thoroughly cleaned?
- What is the water source and is the water treated prior to use?
- What are the species and numbers of each species in that system?
- What are their sizes and ages?
- Which species are sick or dying? Which are not?
- Have there been recent additions? Which species and when?
- Were the new additions quarantined before being added to the system?
- When was abnormal behavior or death first noticed?
- Number of total fish/by species? Number of sick fish per day? Number of mortalities per day?
- What weather changes were observed? (This is important for outdoor aquatic systems.)
- Are there any birds, turtles, or other animals in or around the water? (For outdoor aquatic systems.)
- Have there been problems in this aquatic system before?
- Have there been problems with this/these species before?

Behavioral Changes:

- What are the fish doing (e.g., are they flashing/rubbing against surfaces, swimming abnormally, is their breathing rate increased, are they lethargic/slow moving)?
- What are the positions of the fish in the water column (at the surface, vertical, lying on the bottom, near the aerator or pond edge)?
- Are the fish eating normally? If not, when did they stop?

Physical Changes:

- What is the fish's body condition (e.g., thin, bloated)?
- Are one or both eyes normal, sunken in, or protruding?
- Are the fins clamped down, frayed, or bloody?
- Are the gills discolored, bloody, or frayed?
- Are there lesions or growths on the fish?
- What else looks abnormal on the fish?

Routine Procedures:

- What type and size of feed is fed?

- How much is fed and how often per day?
- How is the feed stored?
- Has there been any change in feeding or system maintenance recently?
- When was the last water change? How much was changed?

Previous Treatments:

- What treatment(s) and dosage(s) have been attempted?
- When was the last treatment?
- Did the issue improve, worsen, or stay the same with treatment?

Submission of a Water Sample

Fish spend their entire life in water, which means when a fish gets sick, that environment must be tested. Many water quality issues that cause disease in fish, such as increased ammonia or nitrite, pH fluctuations, and low oxygen levels, are invisible to the human eye. For this reason, the best way to monitor water quality changes is to frequently test and keep records of your water quality parameters (e.g., temperature, pH, ammonia, nitrite, dissolved oxygen). The diagnostic lab may also ask you to submit a water sample for analysis along with any sick or dead fish.

When submitting a water sample for analysis, a few simple yet important procedures must be followed. First, water should be sampled as soon as the fish begin to act abnormally and before water changes are initiated. A clean container (approximately one quart), thoroughly rinsed of any foreign matter or soap residue, should be used. If the system in question is a pond, it is important to submerge the container under the water and place the cap on the container beneath the surface. This removes any air bubbles which could interfere with the dissolved oxygen measurement. (Ideally, dissolved oxygen and temperature should be measured at the pond, and if the pond is large, at multiple depths and locations.) If there are multiple systems involved, samples from each will be needed. In cage culture, water should be sampled inside the cage as well as outside the cage.

The water sample should be separate from the fish sample. The water that the fish are brought in will not correctly reflect what is occurring in your system. The chemistry of the water, which includes pH and ammonia, will change during transport. For example, while the fish is being transported to the diagnostic laboratory, its metabolic activities (i.e., respiration, waste excretion) will cause the pH to decrease and the ammonia to increase in the shipping water.

It is important to label all samples with pertinent information, such as client's name, sample location, depth, and the time of collection. Keep the water sample refrigerated and in a dark location once collected. When shipping water, place the sample on ice or ice packs. A Styrofoam® cooler in a cardboard shipping carton works well for shipping water overnight to a diagnostic facility.

Submission of a Fish Sample

The best fish samples for diagnostic evaluation are the fish that are near death (moribund) and/or showing signs of distress or illness that are common to many of the sick fish. Dead fish are rarely acceptable for diagnostic tests. However, if the fish are in good condition, that is, with eyes that are clear and gills still red, they may have some diagnostic value. If they are obviously decomposed or malodorous, do not submit them. It is also important to submit a representative number of each species involved. Three to five moribund fish will usually be sufficient. This ensures an accurate representation of the population as a whole. If the fish are alive and appear able to survive the trip to the laboratory, place them into well-aerated water in a heavy ply plastic bag (fish shipping bag or commercial freezer bag), and a Styrofoam® cooler to hold a steady temperature. If you have access to an oxygen tank (e.g., at a commercial facility) fill the bag with pure oxygen so that the sealed bag contains 1/3 volume of water and 2/3 volume of oxygen (or air). This can then be placed in a cardboard shipping carton and shipped overnight. For live fish, an ice or heat pack should be included in the cooler if the weather temperature is particularly hot or cold.

In some scenarios, live moribund fish may be too sick to survive transport to the diagnostic lab and should therefore be humanely killed prior to transport. Live fish can be euthanized in a number of ways, but under some circumstances, logistics may require humane killing using depopulation approaches. If working with an aquatic veterinarian, seek their assistance for more specifics or discuss with diagnostic laboratory personnel prior to preparation for shipment. Some suggested euthanasia options are available in the AVMA Guidelines for the Euthanasia of Animals, 2020 (https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf). If necessary, humane killing using depopulation methods can be found in the AVMA Guidelines for the Depopulation of Animals, 2019 (https://www.avma.org/sites/default/files/resources/AVMA-Guidelines-for-the-Depopulation-of-Animals.pdf).

If the fish are freshly dead, the carcasses should be kept moist with wet paper towels in an empty, heavy-ply plastic bag. Keep the sample cold packed with ice or ice packs in a Styrofoam® cooler and shipping carton. It is important not to freeze the sample, especially if tissues are to be submitted for histopathology (examination of tissues for disease processes at the microscopic level), as freezing will damage the tissues. The best way to avoid this problem is to never allow the fish to directly touch the ice or ice packs;

instead, wrap the plastic bag containing fish in a layer of bubble wrap or a few extra layers of paper towels to cushion them from freezing temperatures.

There are commercial overnight carriers that will ship live and dead fish, if they are properly packed as mentioned above. Ideally, overnight shipments should be verified for morning arrival. If fish arrive in the late afternoon they may not be processed the same day. Also, most diagnostic facilities require prior notification that a sample is being shipped to their laboratory. This ensures a contact person will be there to receive the shipment and be able to start work on it in an expedient manner. Listed in the appendix are a number of laboratories in Florida that are qualified to diagnose fish diseases. Contact the one closest to you for further information.

Samples that are hand delivered to a laboratory should also be properly transported. Laboratories will want information regarding the case prior to arrival and will often have case history or submission forms that need to be filled out. Notify the laboratory you are coming with the sample. Keep live fish in a bucket with a battery-operated aerator or a plastic bag with well-oxygenated water. For dead fish, wrap them in wet paper towels in a plastic bag, then place the bag on ice in a cooler.

The Diagnostic Laboratory

Once the case history is recorded, a general evaluation by the diagnostic laboratory is initiated. First, the diagnostician will test water chemistry parameters, such as total and unionized ammonia, nitrite, pH, total hardness, total alkalinity, and chloride. (Water chemistry measurements for marine systems include ammonia, nitrite, nitrate, salinity or specific gravity, and pH). Measurements of chlorine, dissolved oxygen, hydrogen sulfide, iron, and copper are sometimes warranted.

Fish are measured and weighed and abnormal changes to the external body appearance are recorded. A complete fish necropsy will include biopsies of external tissues (skin, gill, and fin). These tissues are examined under the microscope, primarily looking for parasites, although some bacteria (e.g., columnaris-causing bacteria), fungi, and effects of water quality and nutrition can sometimes be assessed. The fish are opened to examine the internal organs for lesions and growths. The kidney is sampled for bacterial culture, and other organs (e.g., brain, liver, spleen, swim bladder) may also be cultured as deemed necessary. Tissues may be taken for histological processing and virology (see Table 1 below). Squash preparations of internal organs are examined under the microscope, primarily for observation of parasite infection. Bacterial, viral, and fungal agents and nutritional imbalances can often be suspected microscopically but must be confirmed by other methods.

In general, the laboratory can make an initial diagnosis of water quality problems or parasitic infection at the time of examination, and appropriate treatment can be recommended. However, in many cases, more time will be needed to determine the cause of disease. Growth of bacteria on culture media usually requires 24 to 48 hours, and sensitivity testing to determine the proper antibiotic to use takes an additional 24 to 48 hours. If tissues are submitted for histology or virology, one to two weeks turnaround time is common.

The Value of the Sample: What Can It Tell Us?

Fish diseases are often caused by a combination of factors, such as water quality, nutrition, and/or infection by pathogens (bacteria, fungi, parasites, or viruses). Therefore, starting any treatments before accurately diagnosing the problem will make it more difficult to pinpoint the primary cause(s). Furthermore, inappropriate treatment can compromise the fish's immune system, leading to secondary infections and masking the original problem. By knowing the history and keeping good records of your system, you may be able to anticipate and control common problems, such as poor water quality, that often lead to immune system suppression and infection by secondary disease agents.

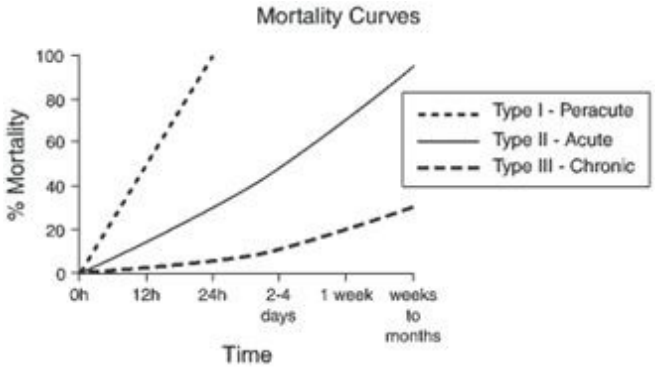

Figure 1. By graphing the percent mortality (number of dead fish/total number of fish in the population) over time, one can determine if affected fish are suffering from a peracute, acute, or chronic type pathogen.

One way to help determine a cause of death is to map a mortality curve (Figure 1). A mortality curve is a graph that indicates how many fish died at different points in time, from the beginning of the disease outbreak until the present time, or the end of the outbreak. If most of the fish in a system demonstrate abnormal behavior and die within 24 hours, it follows a "Type I mortality curve." Agents that create this peracute reaction are usually environmental, namely a change in water quality. For example, low dissolved oxygen in ponds can result in peracute mortality (see IFAS fact sheet FA27). Toxic chemicals or overdosed medications can also cause a peracute response. A "Type II mortality curve" occurs when fish appear debilitated over

a few days to a week, with progressively higher mortalities. Causes of acute mortality are usually infectious agents, such as bacteria or viruses. Finally, fish that act lethargic, decrease feeding, or die over a period of up to several weeks or months indicate a chronic pattern of disease. A variety of causes, including parasites, bacteria, viruses, toxins (e.g., heavy metals) and poor nutrition, can cause this "Type III mortality curve."

Because a wide variety of agents can cause a range of disease signs in fish, the sooner you submit water and fish to the laboratory, the more accurate the diagnosis will be. With the help of a diagnostic laboratory, proper treatment can be initiated more rapidly and losses can be minimized greatly. Additionally, how a sample is handled from the onset will determine how complete and accurate the diagnosis will be. This is called diagnostic usefulness (Table 1). As mentioned above, live moribund fish are the best sample for identifying pathogens (i.e., parasites, bacteria, fungus, and viruses). Fish that won't survive transport can be useful specimens as well, if placed on ice immediately and examined within 24 hours. In general, fish that have been dead for greater than six hours are useless for most external parasites, bacterial isolation, or histology. This is because parasites fall off after death, and decomposition causes bacteria to overgrow the tissues. Parasite examination and histology are particularly difficult to perform on fish once they've been frozen; however, frozen specimens can be useful for identifying bacterial or viral pathogens. Fish fixed in 10% buffered formalin are best utilized for histological evaluations; however, tissue sections must be appropriately sized to fix properly. Other diagnostic tests, such as bacterial and viral isolation, are no longer possible once tissues have been fixed in formalin.

Summary

When a fish becomes sick or dies, it is important to respond correctly to determine why the fish's health is compromised. Know your system by keeping good records of water chemistry parameters, water changes, and recent additions of fish. This will help your fish health professional to determine an accurate diagnosis. Separate water and representative fish samples are usually required by the diagnostic laboratory. How the samples are collected and shipped will determine their usefulness in the examination, and consequently, the accuracy and effectiveness of the final assessment and recommendation.

Reference

Noga, E.J. 2010. Fish Disease: Diagnosis and Treatment, Second Edition. Wiley-Blackwell, Ames, IA.

Appendix

There are fees associated with any diagnostic procedure performed by state agencies or by private consultants. Typically, state agencies are open Monday through Friday from 8:00 am to 5:00 pm. Some private consultants may have after-hours services at an additional cost. All laboratories must be notified prior to sample submission.

State Specialists:

UF/IFAS Extension Aquaculture Specialists:

UF College of Veterinary Medicine
Aquatic Animal Health Program
2015 SW 16th Ave
Gainesville, FL 32608
(352) 294-4198
Vm-aquaticAHP@ad.ufl.edu

UF/IFAS School of Forest, Fisheries and Geomatics Sciences
Tropical Aquaculture Laboratory (commercial aquaculture/industry clients only)
1408 24th Street SE
Ruskin, FL 33570
(813) 671-5230

Florida Department of Agriculture and Consumer Services, Division of Animal Industry:

Bronson Animal Disease Diagnostic Laboratory
2700 N John Young Pkwy
Kissimmee, FL 34741
(321) 697-1400

Table 1. Diagnostic usefulness of fish specimens for identifying pathogens. How the sample is handled will determine how complete and accurate a diagnosis will be. Specimens labeled "dead" are those that died within 12 hours of examination. Specimens labeled "iced" or "frozen" are those animals that were moribund and were placed on ice or immediately frozen after death for transport. Fixed tissues are from fish immediately placed in 10% buffered formalin after death. This table is modified from Noga, 2010.

	External Parasites	Internal Parasites	Bacterial Isolation	Viral Isolation	Cellular Changes
Live	Best	Best	Best	Best	Best
Dead	Poor	Good	Poor	Fair	Poor
Iced	Fair/Good	Best	Fair	Good	Fair
Frozen	Fair	Good	Good	Good	Poor
Fixed	Fair/Good	Fair/Good	Poor	Poor	Best

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