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IFAS Extension

FA55

Submission of Fish for Diagnostic Evaluation[1]

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Introduction

Determining the cause of a fish's illness and death can be difficult and frustrating for anyone who works with fish. Disease problems are commonly misdiagnosed and fish are often incorrectly treated with over-the-counter medications. A vicious cycle arises when the first treatment doesn't work and another one is tried, then another, and so on. Not only is this method a waste of time and money, but it usually does more harm than good to the fish in question. In addition, over-treatment, and the time lag from problem onset to submission could allow secondary infections (caused by agents that invade fish tissue after the damage by the initial agent has occurred) to take hold. Therefore, it is important to contact a diagnostic laboratory as soon as a disease problem is noticed 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 their demise is determined, the faster a response can be taken to rectify the situation. Whether it is a pond, home aquarium, production farm, or a fish kill observed in the wild, it is imperative to respond quickly and correctly. The following is the proper procedure to submit fish and water samples to a diagnostic facility. Following these steps should result in 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 system and animals that live there. Keeping records of water chemistry parameters, water changes, species in the system, and recent additions can accelerate 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 system involved?
- How old is the system?
- What are the species and numbers of each species in that system?
- What are their sizes and ages?
- Which species are in trouble? Which are not?
- Have there been recent additions? Which species and when?
- When was abnormal behavior or death first noticed?
- Number of sick fish per day? Number of mortalities per day?
- What weather changes were observed (important in pond cases)?
- Have there been problems in this system before?
- Have there been problems with this/these species before?

1. This document is FA55, one of a series of the Fisheries and Aquatic Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Original publication date November 2001. Revised February 2010. Reviewed June 2013. Visit the EDIS website at http://edis.ifas.ufl.edu.

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Behavioral Changes:

- What are the fish doing (e.g., are they flashing, is their breathing rate increased, are they lethargic)?
- What are the positions of the fish in the water column (at surface, vertical, lying on the bottom, near the aerator or pond edge)?
- Are the fish eating? 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 popped out?
- 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 and how often is fed per day?
- Has there been any change in feeding or system maintenance recently?
- When was the last water change? How much was changed?

Previous Treatments:

- When was the last treatment?
- What was the treatment(s) and dosage(s)?

Submission of a Water Sample

Fish spend their entire life in water, which means when a fish gets sick, that environment must be tested. **No matter how clean you think your water is, no matter what your water source is, and even if your system is flow-through, you must have recent records of your water quality parameters (e.g. temperature, pH, ammonia, nitrite, dissolved oxygen) and submit a water sample for analysis.** A good diagnostic laboratory will first test the water for any deviation in quality.

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 remarkably during transport. For example, while the fish is being transported to the diagnostic laboratory, its metabolic activities (i.e., respiration, 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 in cold storage 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 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) or showing signs of distress. Dead fish are rarely acceptable for diagnostic tests. However, if the fish are in good condition, that is, their eyes are clear and the gills are red, they may have some 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. Usually three to five fish will be sufficient. This ensures an accurate diagnosis of the population as a whole.

If the fish are alive and appear to be able to make 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 regulate temperature. This can then be placed in a cardboard shipping carton and shipped overnight. If the fish are dead or will not make the transport, the fish should be kept moist with wet paper towels in a heavy ply plastic bag. Keep the sample cold packed with ice 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).

There are commercial overnight carriers that will take live and dead fish, if they are properly packed as mentioned above. 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. 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, and moribund/dead fish wrapped in wet paper towels in a plastic 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 surface (see physical changes above) 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 (i.e. *Flexibacter columnaris* or *Flavobacterium columnare*), fungus, and effects of water quality and nutrition can sometimes be assessed. The fish are opened to expose the internal organs and examined for lesions and growths. The kidney is sampled for bacterial culture (the brain, liver, spleen, swimbladder, and other organs 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 infestation. Bacterial 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 and parasitic infection within 24 hours of receipt of a sample. Most cases can be adequately assessed at that time and treatment can be recommended. However, in some cases, more time will be needed to determine the causative agents. Growth of bacteria on specialized media usually requires 24 to 48 hours, and sensitivity testing to determine the proper antibiotic to use takes an additional 24 hours. If tissues must be submitted for histology or virology, one to two weeks turn around 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, parasites, or viruses). Therefore, starting any treatments before accurately diagnosing the problem will make it more difficult to pinpoint the primary cause. Furthermore, the fish's immune system becomes compromised, 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 eliminate common problems that are the underlying cause or that allow infection by secondary disease agents.

One way to aid the fish health specialist in determining 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 fish demonstrate abnormal behavior or the majority of fish in your system die within 24 hours, it follows a "Type I mortality curve." Agents that create this **peracute** reaction are environmental, namely a change in water quality. For example, low dissolved oxygen in ponds can result in peracute mortality (see IFAS fact sheet FA-27). Toxins such as chemicals and drug over-treatment 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 this **acute** mortality are generally due to infectious agents, such as bacteria or viruses. Fish that act lethargic, decrease feeding, or die over a period of a week to a few weeks undergo a

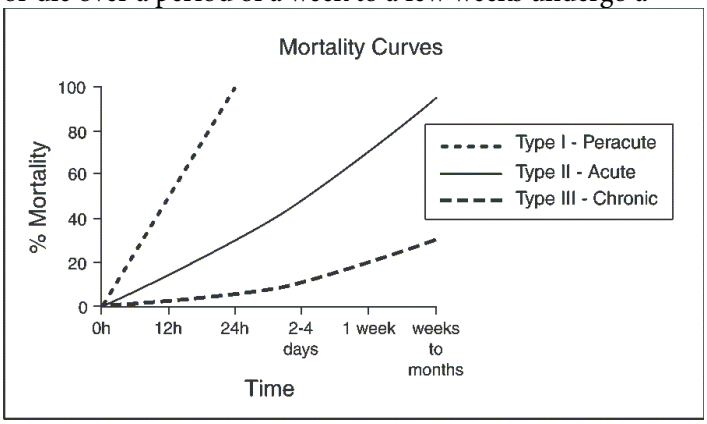

Figure 1. By graphing the percent mortality (number of dead fish/total number of fish in the population) over time, one can determine if affected fish are suffering from a peracute, acute, or chronic type pathogen.

chronic reaction. A number of factors, including parasites, bacteria, viruses, toxins (e.g. heavy metals) or poor nutrition can cause this "Type III mortality curve."

Because a wide variety of agents can create a range of symptoms in fish, the sooner you submit water and fish to the laboratory, the more accurate the diagnosis will be. Proper treatment can be initiated more rapidly and losses can be minimized greatly. Additionally, how that 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 specimens are the best when identifying pathogens (i.e., parasites, bacteria, fungus, and viruses). Live fish placed on ice immediately and examined less than 24 hours later are useful specimens as well. However, in general, fish that have been dead for greater than six hours are useless for most external parasites, bacterial isolation or histology. Parasites fall off and bacteria involved in decomposition overgrow tissue as soon as the fish dies. Fish that have been frozen immediately generally lose any external parasites and histology of tissues is impossible. However, frozen specimens are useful in determining 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.

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 changes or additions of fish. This will help when speaking with a fish health professional and will facilitate the process of making an accurate diagnosis. Separate water and representative fish samples are usually required by the diagnostic facility. 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.

References

Noga, E.J. 1996. Fish Disease. Diagnosis and Treatment. Mosby-Yearbook, Inc., St. Louis, MO.

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.

Assistance from State Agencies:

Department of Fisheries and Aquatic Sciences
7922 NW 71st Street
Gainesville, FL 32653
(352) 392-9617, ext. 230
pettyd@mail.vetmed.ufl.edu
hollowayj@mail.vetmed.ufl.edu

Tropical Aquaculture Laboratory
1408 24th Street SE
Ruskin, FL 33570
(813) 671-5230
rpy@ifas.ufl.edu
dbpouder@ifas.ufl.edu

Zoological Medicine Service
College of Veterinary Medicine
P.O. Box 100125
Gainesville FL 32610-0136
(352) 392-4700
pettyd@mail.vetmed.ufl.edu

(Note: DACS facilities require referral by a licensed veterinarian)
Kissimmee Diagnostic Laboratory
2700 N. Bermuda Avenue
Kissimmee, FL 34741
(407) 846-5200

Live Oak Diagnostic Laboratory
P.O. Drawer O
Live Oak, FL 32060
(904) 362-1216

Table 1. Diagnostic usefulness of 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. Specimens labeled "iced" or "frozen" are those animals that were moribund and placed on ice or immediately frozen for transport. Fixed tissues are from live fish immediately placed in 10% buffered formalin. This table is modified from Noga, 1996.

	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