Parasitology and Necropsy of Fish

**Abstract:** Parasitic diseases are common in fish. Diagnosis can be made through gill biopsy, skin cytology, fecal examination, or necropsy. Common parasites include protozoa, helminths, and crustaceans. Determining the cause of death in a fish is important for maintaining the health of other fish in the same environment. Due to rapid autolysis, fish necropsies should be performed promptly after death. Samples should be preserved in 10% neutral buffered formalin. Squash preparations, tissue imprints, microbiology, and virology are also useful in obtaining a diagnosis.

Parasites are a common problem in aquaculture and can negatively affect fish appearance and health, as well as have financial repercussions. Therefore, practitioners must be able to obtain and maintain samples from both live and deceased fish and administer basic treatment to minimize the risk to the patient and to other fish in its environment.

**Parasitology**

In a confined setting such as a home aquarium or pond, parasites can impair growth and reproduction and cause substantial morbidity and mortality. Parasites of the gills can cause irritation, leading to hyperplasia and increased mucus production, which may result in decreased respiration and ion-exchanging capabilities. On the skin, parasites can cause defects that predispose the affected fish to osmotic imbalances and serve as a portal of entry for viruses, fungi, and bacteria. In the intestine, parasites compete for nutrients and cause ulcerations, inflammation, and emaciation.

**Signs**

Clinical signs of a parasitic infection are related to parasite feeding activity. Pruritus may be exhibited by fish as flashing—quickly turning laterally in the water to display the lighter-colored ventrum. Other signs may include excess skin and gill mucus with epithelial growth, clinical hypoxia (pale gills, rapid opercular movement, piping at the surface), secondary infections, and weight loss.

**Sampling**

Because most parasites that infect fish are microscopic, it is necessary to view diagnostic samples under a light microscope. Skin scrapings and gill biopsies can detect external parasites. If possible, multiple affected fish should be examined because ectoparasites are not always distributed evenly. Although small, debilitated, or calm fish may not require sedation, it is usually practical to provide it for a thorough examination. However, some fish parasites may detach from the body under the influence of sedation.

Once anesthetized, the animal may be raised slightly out of water, supported with gloved hands, or placed on a damp or well-lubricated surface (e.g., a damp synthetic chamois cloth) for a short duration. Nonpowdered latex gloves should be worn to prevent transmission of zoonotic diseases such as fish tank granuloma (finger sores caused by atypical Mycobacterium spp) and to minimize disruption of the fish’s protective mucus layer. A plastic coverslip or dull scalpel blade is used to gently scrape the skin of the fish. Noticeable lesions, the area behind the pectoral fins, or along the base of the dorsal fins (where water flow is decreased) are good places for sampling. Other places that may yield a high number of parasites include the caudal peduncle or the ventral side of the mandible. The coverslip is then placed on a glass slide containing a drop of the fish’s environmental water and examined under light microscopy at 40x to 400x.
Fungal hyphae, bacteria, and parasites may be seen, along with tank debris, fish scales, mucus, pollen spores, and salt crystals.

Biopsies of the gills are handled in much the same manner. The operculum is gently lifted, and iris or suture removal scissors are inserted perpendicularly to remove a few gill tips from the gill arch. The gill tips are then placed on a drop of environmental water on a slide and gently covered with a coverslip, taking care to keep the filaments separate but not pressed down. Many parasites are detected by movement, and sterile saline can kill freshwater parasites, as sterile water can kill parasites from marine fish. A small amount of bleeding may be noted from beneath the operculum once the animal is returned to the water. Applying general pressure often stops this bleeding. If the hemorrhage is severe, silver nitrate may be used for hemostasis. Small biopsy samples of suspicious fin lesions (e.g., redness, fraying) may also be taken.

Fecal samples can show intestinal parasites, but they may be difficult to obtain in an aquatic habitat and are often contaminated with nonpathogenic organisms. One sampling method involves using a vent wash with a rubber catheter and sterile saline solution. The sample is then placed on a slide with a coverslip and examined by light microscopy. Fish often defecate when anesthetized, providing a fresh fecal sample for evaluation. Feces may also be obtained via syringe from the container in which the animal is transported, or a swab can be passed through the vent to obtain a fecal sample. The samples can be processed using Ziehl-Neelsen or Fite stain to detect acid-fast organisms.

Organisms
Protozoa are the most common infectious agents in fish. These organisms feed on skin and gill surfaces and have a direct life cycle. Their reproduction cycle is temperature dependent, which affects treatment. Many parasites infect both saltwater and freshwater fish, whereas others have saltwater and freshwater counterparts. Trichodina spp (freshwater, saltwater), often described as “scrubbing bubbles” or “flying saucers,” are usually indicators of poor water quality or overcrowding. They can survive off the host for 1 or 2 days and can be transported with plants and aquarium furniture. Although they are not problematic in low numbers (fewer than five per low-powered field), heavy infestations can cause epithelial damage resulting in anorexia, loss of body condition, and low-level mortality.

Ciliates such as Tetrabymena (freshwater), Uronema (saltwater), Ichthyophthirius (freshwater), Cryptocaryon (saltwater; Figure 1), Chilodonella (freshwater), and Brooklynella (saltwater) spp can cause gill and skin lesions and may give rise to more serious disease if they invade internal organs. Infections often appear as small, white patches on the skin,
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around the eye. Because these organisms can survive off the host, the environment must be cleaned and disinfected in addition to treating the fish. Fish can develop severe osmotic imbalance due to parasitic damage to the skin and gills. Dinoflagellates such as *Piscinoodinium* (freshwater) and *Amyloodinium* (saltwater) spp are commonly found on the gills but may also affect the skin, fins, and gastrointestinal (GI) tract. In severe infections, the skin may become velvety gold in appearance (*velvet disease*). Mortality is attributed to severe osmotic imbalance. *Sporonucleus* spp (freshwater, saltwater) have been isolated from head lesions of discus *Symphysodon* spp and angelfish *Pterophyllum* spp (*hole-in-the-head disease*) and from the digestive tract of both freshwater and saltwater fish. Monogenean flatworms such as *Neobenedenia*, *Dactylogyrus*, and *Gyrodactylus* spp are also fairly common. These parasites may be found on the gills and skin, but *Dactylogyrus* spp are generally found on gill tissue. The life cycle is direct; the eggs of dactylogyrids hatch into free-swimming larvae that locate a fish and begin maturation. Gyrodactylids (FIGURE 2), on the other hand, bear live young that spread to other fish through direct contact. *Neobenedenia* spp are marine parasites that can be found on the gills, skin, and eyes (FIGURE 3). Eggs from this parasite have long strands that stick to tank substrates and decoration, requiring additional vigilance during treatment. Large numbers of these organisms can be lethal to fish, as both the attachment of the parasite by hooks and/or suckers and its feeding activity cause mechanical damage to the skin and gills.

Digenean trematodes have indirect and often complex life cycles involving two or more intermediate hosts. They are generally found in the GI tract or musculature of the host. As they cannot complete their life cycle without intermediate hosts, these parasites are often an incidental finding in aquarium fish. However, they may cause severe internal damage in large numbers. Intermediate hosts (e.g., mollusks) should be removed from the environment to prevent fish-to-fish transmission. Final hosts (e.g., birds) should be deterred from outdoor ponds.

Lernaea spp (*anchor worms*), *Ergasilus* spp, fish lice, and isopods appear as tiny pill bugs or crab-like creatures. In low numbers, they may cause local inflammation and ulceration that can lead to secondary infections. Some of these organisms have also been implicated as potential vectors for viral pathogens. Most of these parasites can be seen with the naked eye and removed manually.

Other parasites that cause clinical disease in fish can be encysted in various tissues or reside in the GI tract or other organs (e.g., gallbladder). In our experience, these parasites include various species of cestodes, nematodes, acanthocephalans (FIGURE 4), and coccidia.

*Neobenedenia* parasites found in a skin scraping from a gray angelfish (*Pomacanthus arcuatus*), with an inset of *Neobenedenia* eggs from the same sample using light microscopy (200× magnification).

*Figure 4*

Acanthocephalan sp found incidentally during a postmortem examination of an Atlantic saury (*Scomberesox saurus*).
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**Treatment**

Although specific therapeutic options vary for each organism, chemical treatment is of temporary value if water quality and management are poor. Tank hygiene should be improved first. Many parasitic infections of fish are treated with medicated baths (e.g., formalin, praziquantel, metronidazole) or altered salinity. In saltwater fish, copper sulfate is very effective for treatment of infection with *Cryptocaryon* spp. Lufenuron baths have been used to control crustacean parasites.

GI parasites may be treated by incorporating medication in a gel food form, but only if the animal is eating. Gel food is available commercially or may be homemade and is useful for administering common antiparasitic drugs (e.g., fenbendazole, metronidazole, praziquantel, pyrantel pamoate). These drugs can also be given via gastric lavage using an appropriate-sized rubber catheter. Metronidazole is particularly effective in treating intestinal *Spirogyra* spp. Oral drugs are also helpful in reducing numbers of *Ichthyophthirius* spp on fish. One study has shown nutrition to be an important factor; fish fed medium-chain fatty acids had significantly fewer parasites than those that did not receive the supplement. Ivermectin should be avoided because it can cause neurologic signs and death in fish at therapeutic doses. A useful reference for therapy and drug dosages is *Exotic Animal Formulary*, 3rd edition.

**Necropsy**

Postmortem examination is one of the fish practitioner’s most valuable tools and is essential to assess the infectious disease risk to other fish in the patient’s environment. Dead fish should be kept moist and placed on ice or refrigerated. Delay is inadvisable, as autolysis is rapid. If a fish has been dead for more than a few hours (6 to 8 hours maximum), the histopathologic value of the tissues is decreased, but gross and parasitologic evaluation can still be productive. Tropical fish (freshwater or saltwater) undergo autolysis more quickly, and necropsy should be performed as soon as they are found; they should not be refrigerated for longer than 1 to 2 hours.

Before necropsy, the weight and length (FIGURE 5) of the dead fish should be measured. The condition of the fins, scales, gills, and eyes should be noted, along with any other abnormalities (FIGURE 6). Radiographic images are useful for documenting skeletal abnormalities (FIGURE 7), and skin scrapings and gill biopsies should be performed. In the absence of lesions, samples can be taken from the lateral line and the area caudal to the pectoral fins.

Following external and parasitologic examination, gross necropsy is performed using a systematic, consistent approach. Most fish are positioned in right lateral recumbency for bet-
Scissors are inserted into the ventral operculum and used to cut caudally through the pectoral girdle to create a “window” into the body cavity. The scissors are then brought up to the dorsal operculum, and an incision is made caudally along the lateral line, extending to follow the abdominal curve ventrally in the direction of the vent. To complete the window, the incision is then extended from the pectoral girdle along the ventral midline to the vent, and the body wall is removed19 (Figure 8). Bone cutters or pruning shears can be used on larger or thick-scaled fish. Stomach configurations vary depending on the type of food consumed, but the anatomy of most types of bony fish is similar.

Organ consistency and color should be noted, as well as the presence and characteristics of any free abdominal fluid. Free abdominal fluid should be evaluated grossly for color, consistency, and specific gravity, as well as microscopically for the presence of bacteria and parasites. If the organs appear to be fresh, samples may be taken for histologic analysis. Many fish are less than 4 cm in fork length (e.g., neon tetras) and may be preserved whole in 10% neutral buffered formalin fixative after the creation of a full-thickness slit through the ventrum. Fish 4 cm or greater in length require more preservation; a needle on a syringe can be used to fill the GI tract with 10% formalin fixative. Sections of organs from larger fish may also be placed in 10% formalin fixative.

Squash preparations and tissue imprints of organs can assist in the diagnostic process. Squash preparations of samples of the GI tract, which are made by “squashing” a small piece of tissue between a slide and coverslip, can be useful in identifying protozoa and other parasites. Tissue imprints of lesions and organs are made by blotting the tissue dry with a tissue and then pressing it several times on a clean slide. These fresh imprints are helpful in assessing bacterial and fungal infection before culture.

Bacterial culture should be conducted on any abnormal tissue before the organ is disturbed and preferably on a freshly dead fish, as bacterial invasion of fish skin and other organs occurs rapidly after death. The use of 70% alcohol and a butane flame to sterilize the organ surface and biopsy tools can help to reduce contaminants. Organ surfaces may also be decontaminated by searing with a hot scalpel blade. Samples may be obtained with sterile loops, blades, or swabs and should be either plated immediately or placed in a trans-
port medium. Columbia agar with 5% defibrinated sheep blood is a good general-purpose medium for culturing freshwater and saltwater bacteria. The samples should be kept cool on freezer blocks during transport and delivered rapidly to the laboratory. Delays and lack of temperature control may confound results.

Because fish kidneys are involved in phagocytosis, highly vascular, and protected from contamination by surrounding structures, kidney cultures are desirable when bacteremia is suspected. The kidneys may be approached by either peeling back the swimbladder or (in small fish) cleaning the dorsal aspect of the fish with alcohol and cutting over the midback until the kidney is exposed. The submission laboratory should be experienced in culturing fish organisms, and maintaining the cultures at 71°F to 77°F (22°C to 25°C) or at the fish’s water temperature. It is best to alert the laboratory if certain organisms are suspected. Some organisms (e.g., *Flavobacterium* spp, *Vibrio* spp) require special media and temperatures, and *Mycobacterium* spp may take several months to culture, so polymerase chain reaction testing may be of use.

If the fish is large enough or a viral entity is suspected, it is a good idea to freeze portions of the spleen, liver, and kidney. This preserves samples for further diagnostic testing if histopathology findings indicate a viral etiology.

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References

CE TEST
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1. Parasitic diseases in fish can be diagnosed using
   a. radiography.
   b. gill biopsy.
   c. Wood’s lamp.
   d. ultrasonography.

2. Parasitic infections in fish can cause all of the following except
   a. poor growth.
   b. poor reproduction.
   c. gill hyperplasia.
   d. none of the above

3. Which clinical sign is indicative of parasite feeding relative to a parasitic infection?
   a. flashing
   b. pus
   c. reddish gills
   d. rubbing against the tank wall

4. The most helpful method(s) for external parasite detection in fish is/are
   a. lice comb.
   b. skin scrapings and gill biopsies.
   c. running tape over the skin.
   d. microscopic examination of 1 mL of aquarium water.

5. The most common parasites detected in fish are
   a. nematodes.
   b. trematodes.
   c. protozoans.
   d. cestodes.

6. can be seen with the naked eye and removed manually.
   a. Lernaea spp
   b. Digenean trematodes
   c. Cryptocaryon spp
   d. Ichthyophthirius multifilis

7. The most common route for treatment of parasitic diseases in fish is
   a. oral.
   b. injectable.
   c. water bath.
   d. direct topical.

8. is one of the most valuable tools for diagnosing infectious disease in fish.
   a. Radiology
   b. Endoscopy
   c. Necropsy
   d. Hematology

9. Which components are necessary for a complete fish necropsy?
   a. skin scrapings and gill biopsies to check for parasites
   b. an overall gross external examination
   c. squashing preparations of organs
   d. all of the above

10. If bacteremia is suspected, the is the best organ for obtaining direct cultures in fish necropsy.
    a. liver
    b. kidney
    c. gill
    d. swimbladder