SRAC
Southern Regional Aquaculture Center

SRAC Publication No. 475
September 2015
Revision

Proliferative Gill Disease *(Hamburger Gill Disease)*

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Proliferative gill disease (PGD) is a significant parasite disease of farm-raised channel catfish. It causes severely swollen gills with broken gill cartilage. The Mississippi State University College of Veterinary Medicine Aquatic Research and Diagnostic Laboratory in Stoneville reports PGD as the third leading cause of mortalities in the Mississippi Delta region, behind only columnaris (caused by the bacteria *Flavobacterium columnare*) and enteric septicemia of catfish (ESC; caused by the bacteria *Edwardsiella ictaluri*). PGD was first reported in 1981 and is now considered the leading parasitic disease in the channel catfish industry. It accounts for about 20 percent of the cases submitted to the Mississippi Delta diagnostic lab. About 80 percent of these PGD cases occur between March and May. The prime temperature range for the disease is 61 to 77 °F (16 to 25 °C). Mortalities can exceed 50 percent and one case caused 100 percent mortality in 3 days. High mortality is not unlikely because the severe gill swelling makes it difficult for the fish to extract adequate dissolved oxygen from the water, which leads to suffocation. The swelling, lack of distinct gill structure ("mashed" appearance), and red hemorrhagic areas (aneurisms) next to white necrotic (dead) gill tissue cause the gills to look like raw hamburger meat (Fig. 1).

Figure 1. Lack of distinct gill structure in these "mashed" gills affected by proliferative gill disease (PGD; hamburger gill disease) in this channel catfish.

Clinical Signs and Diagnosis

PGD occurs most often in the spring, but it can occur in the fall. Exceptional cases have been reported in the winter (at a water temperature of 43 °F; 6 °C) as well as during the summer (92 °F; 33 °C). Common early signs of a PGD outbreak are reduced feeding activity and fish listlessly congregating in shallow areas, especially near incoming water or behind an aerator, in an attempt to get more oxygen. The skin of channel catfish affected with PGD usually looks normal and healthy; the gills, however, are severely affected. (Other organs such as the liver, kidney, spleen, brain, heart, and stomach also harbor the parasite but the pathology is usually not severe.) In advanced stages, the gill filaments do not lie flat and filaments on one gill arch are not distinct from filaments on other arches. The gills often look mashed (like raw hamburger) and may bleed when touched or when the fish are simply lifted from the water.

Microscopic examination of the gills at low magnification (40x provides a good view over a large area of the gills) reveals extreme swelling caused by an abnormally large number of cells (hyperplasia) that can form abrupt

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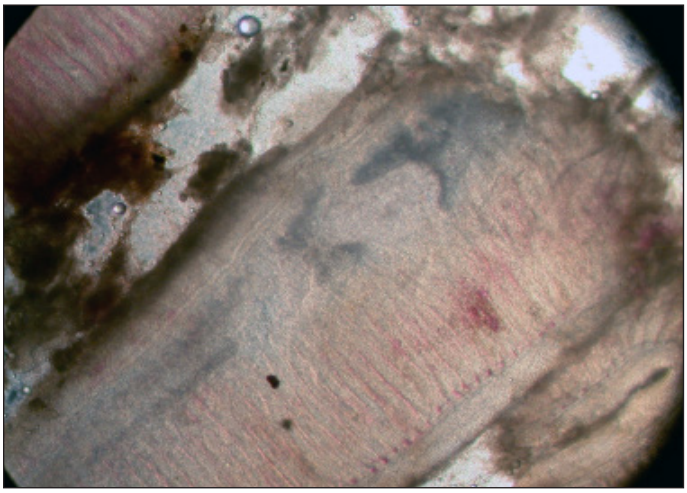

Figure 2. Gaps in this channel catfish's broken gill cartilage (called chondrolysis) cause a loss in gill structure and give the gills the "mashed," unstructured, raw hamburger appearance.

and grotesque protrusions from the gill surface. Also at this low magnification, numerous breaks and notches in the gill cartilage are apparent in several filaments, a condition termed "chondrolysis," a destruction of cartilage (Fig. 2). The gill cartilage appears as a dark gray band (supporting "rod") along the edge of the gill filament. When this supporting cartilage has gaps and holes in it, it cannot adequately support the gills; this leads to the collapsed/mashed "hamburger gill" appearance. Parasite cysts are seen only occasionally in wet mount slides under the microscope and appear as small, round, pale areas. When these areas are cut into microscopically thin slices during histology and stained with hemotoxylin (blue) and eosin (red) (H&E stain), they appear as basophilic (blue) cell clusters (which are generative cells or early stages of the developing myxospore) inside the cyst's cytoplasm (Fig. 3). Sometimes mature *Henneguya ictaluri* spores are found in the gill cysts.

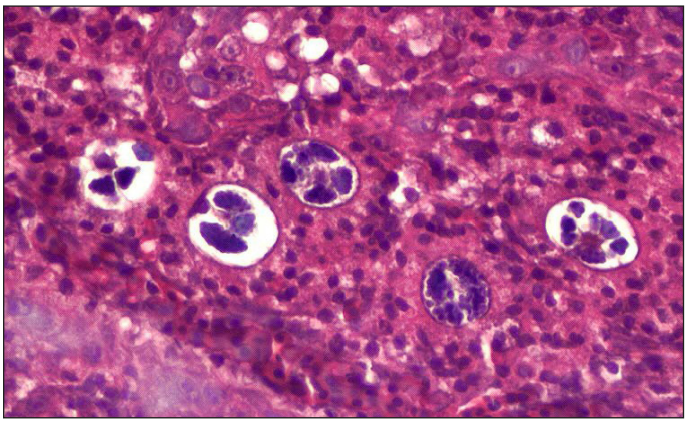

Figure 3. H&E-stained histology of multi-nucleated trophozoite stage of *Henneguya ictaluri* in channel catfish gills. (Photo courtesy of Dr. Andy Goodwin, from American Fisheries Society Fish Health Section Blue Book 2014)

These are the end result of the parasite's life cycle and often appear 2 to 3 months after initial infection.

In 2001, Julia Whitaker and four other researchers at Mississippi State University developed a polymerase chain reaction (PCR) assay to detect *Henneguya ictaluri* and *Aurantiactinomyxon ictaluri,* the PGD-causing organisms, and in 2005, Whitaker, Pote, and Hanson used the test to identify the infective parasite stage from pond water samples. This technology makes it possible to screen ponds as potential sites of infection, which helps catfish producers determine where and when they can safely stock channel catfish fingerlings. Griffin and others (2008) improved on this surveillance method by developing a real-time PCR test (often referred to as qPCR, **quantitative** polymerase chain reaction), which is more conveniently and quickly performed than conventional PCR. Real-time PCR is also advantageous in that it can numerically approximate actinospore concentrations in the pond water, whereas conventional PCR assays show only the presence or absence of the parasites.

Cause and Disease Course

The parasite causing proliferative gill disease is a myxosporean, a group of spore-forming parasites that alternate between two hosts, an annelid worm that hosts the actinospore stage of the parasite and a vertebrate (fish, amphibians, birds, reptiles, or mammals) that hosts the myxospore stage. The oligochaete (annelid) *Dero digitata* (and sometimes *Dero furcata*) hosts the actinospore, *Aurantiactinomyxon ictaluri.* This actinospore leaves the *Dero digitata* host and invades a channel catfish host where it develops into the myxospore stage, *Henneguya ictaluri* (Pote, Hanson, and Shivaji, 2000). This species of *Henneguya* is different from the commonly found *Henneguya exilis* that often forms cysts and spores in the gills of freshwater fish. These authors demonstrated that there is a distinct genetic difference and, during the myxospore stage, morphological difference between the two. In 2014, Rosser and five other researchers showed that blue catfish (*Ictalurus furcatus*) are refractive to PGD and blue × channel catfish hybrids are partially refractive and may not allow the PGD parasite to complete its life cycle. So, basically, channel catfish (and sometimes their hybrids with blue catfish) are the only species susceptible to PGD caused by *Henneguya ictaluri* and channel catfish are most likely the only host in which *Henneguya ictaluri* can complete its life cycle.

The PGD life cycle can be summarized as

- *Aurantiactinomyxon ictaluri,* the actinospore stage, develops in a *Dero digitata* oligochaete/annelid worm living in the pond mud.

- The *Dero digitata* worm releases the actinospore stage in its feces into the pond water. This *Aurantiactinomyxon ictaluri* actinospore floats in the water with its three wing-like caudal projections (or caudal processes) that act as buoyancy floats (Fig. 4).

Figure 4. This *Aurantiactinomyxon ictaluri* actinospore floats in the water with its three wing-like caudal projections (or caudal processes) that act as buoyancy floats.

- The *Aurantiactinomyxon ictaluri* actinospore attaches to the fish's surface and releases its plasmodial sporoplasm, containing infectious cells, into the fish (most notably into the channel catfish's gills). This causes a severe inflammatory response by the fish (including the lysis or degradation of the chondrocytes or cartilage cells), leading to respiratory distress. This PGD condition also involves fusion of the gill lamellae (the feathery projections on the gill filament) due to swelling (hypertrophy) and a large increase in the number of gill cells (hyperplasia). It is seen most often in younger fish, especially those stocked into new ponds, but older fish and established ponds are affected at times. Larger concentrations of the infective actinospores are required to infect older fish.
- Multicellular spores form within the plasmodia inside the fish (mostly in the gills of the channel catfish). Some of the spores mature into *Henneguya ictaluri* spores, are released into the pond environment, and are ingested by *Dero digitata* worms, thus completing the life cycle.

Figure 5 illustrates this life cycle. It should be noted that many fish parasitologists prefer not to use the *Aurantiactinomyxon ictaluri* nomenclature because this organism is genomically identical to *Henneguya ictaluri.* Before this was known, the two stages of *Henneguya ictaluri* were regarded (and named) as two distinct organisms. The authors have chosen to retain the use of both names in this publication to allow readers to make the connection to earlier literature on PGD.

Prevention and Treatment

Although most proposed preventive measures or treatments for PGD have not been demonstrated to work conclusively, certain methods have been used with some degree of anecdotal success. Using the observation that PGD occurs primarily in new ponds has led to the recommendation that new or renovated ponds be filled at least partially with water from adjacent, established ponds. Also, noting that the actinosporean stage of PGD is carried in worms dwelling in pond bottom mud, one may conclude that stocking animals that consume these worms could reduce the number of available hosts for PGD. Smallmouth buffalo fish (*Ictiobus bubalus*) and freshwater shrimp (giant Malaysian prawns, *Macrobranchium rosenbergii*) have been proposed as possible predators of these *Dero* spp. worms. In some cases, excellent prevention of PGD has been achieved by applying hydrated lime to dry ponds prior to filling. The caustic nature of hydrated lime and the associated pH change in the pond soil can significantly reduce the amount of *Dero* spp. worms present prior to filling.

One scientifically proven preventive measure is performing qPCR (described in the Diagnosis section) on water samples from a pond, which quantifies the concentration

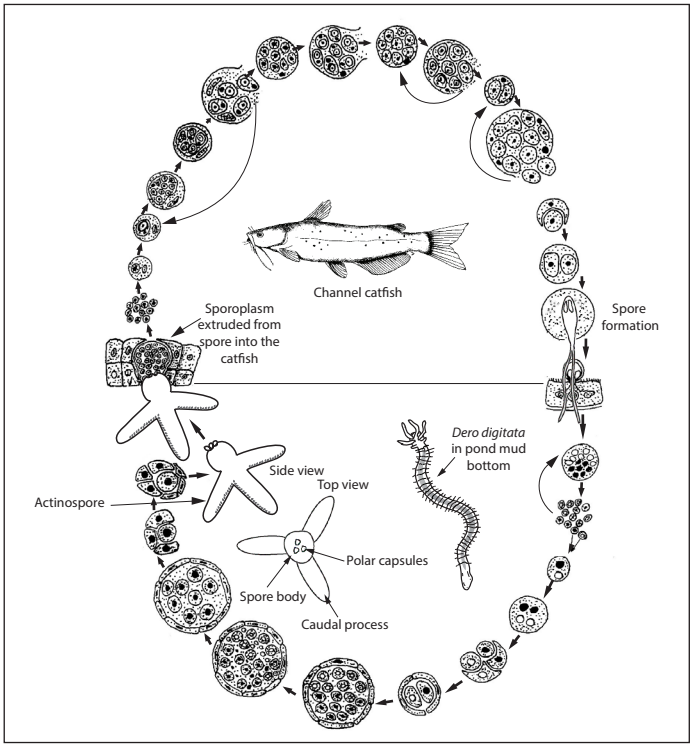

Figure 5. Life cycle of *Henneguya ictaluri,* causative organism of proliferative gill disease, showing the development of its myxospore stage in channel catfish and actinospore stage in *Dero digitata.* (Photo courtesy of Wyvette Williams, Kentucky State University)

of the PGD infective actinosporean stage so that aquaculturists can assess the risk of stocking or re-stocking that particular pond. The alternative approach to measuring this risk is to stock healthy sentinel fish in a cage and note whether they become infected with PGD. Compared to qPCR, this method is inconvenient, time consuming (1 to 2 weeks), labor intensive, and may be inconclusive.

The question has been posed as to whether stocking channel catfish fingerlings in the fall to expose them to PGD pathogens during cold weather (when their immune system is suppressed) would reduce the incidence of PGD the following spring. This theory is based on observations that catfish exposed to PGD when they are immunosuppressed tend to develop resistance to the disease.

As for treatment, catfish farmers have often pumped water across the levee from an older, healthy pond into a pond infected with PGD with some degree of success in reducing mortalities. It might also be noted that when PGD-infected catfish are removed from the infected pond, they recover quickly.

Although no good, proven treatments exist, it is important to note that once a PGD outbreak is over, there does not tend to be a recurrence of the disease in that pond for the rest of that season.

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This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2010-38500-21142. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

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The work reported in this publication was supported in part by the Southern Regional Aquaculture Center through Grant No. 2010-38500-21142 from the United States Department of Agriculture, National Institute of Food and Agriculture.