3.2.6 Proliferative Gill Disease

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A. Name of Disease and Etiological Agent

Proliferative gill disease (PGD) is caused by *Henneguya ictaluri* (Myxozoa: Myxosporea). Molecular data has confirmed that the actinospore stage of this parasite is *Aurantiactinomyxon* spp. and the final myxospore stage is *Henneguya ictaluri* (Pote et al. 2000).

B. Known Geographic Range and Host Species of the Disease

1. Geographical Range

This disease has been reported in commercial catfish in the Southern United States and California.

2. Host Species

Proliferative gill disease has been reported in channel catfish *Ictalurus punctatu*s and Blue catfish *Ictalurus furcatus* in the United States.

C. Epizootiology

Proliferative gill disease has been attributed to major fish losses in commercial channel catfish of all ages and sizes. Major outbreaks of this parasite occur primarily in spring (April-May) and with smaller outbreaks occurring in fall (September-October) in the Southeastern United States.

Research has confirmed that this disease is associated with the actinospore *Aurantiactinomyxon* spp. (Burtle et al. 1991; Styer et al. 1991; Bellerud et al. 1995), tentatively identified as *A. ictaluri* (Bellerud et al. 1995). It has been confirmed that the oligochaete *Dero digitata*, routinely found in catfish ponds (Bellerud et al. 1995), are infected by several myxozoans, including *A. ictalur*i. In

experimental infections where catfish were exposed to worms infected with *Aurantiactinomyxon* spp. (Styer et al. 1991) or pure *A. ictaluri* (Belem 1994), PGD-like myxozoan stages were found in the gills 5 to 6 days after exposure. Based on recent molecular data, Pote et al. (2000), further demonstrated that *A. ictaluri* actinospores, the PGD myxozoan stages present in the gills, and a subsequent myxospore gill stage, *Henneguya ictaluri* n. sp. had identical rRNA gene sequences, thus confirming that the actinospore, *A. ictalur*i, is a life stage of the myxospore, H. *ictaluri* n. sp. The proposed life cycle for this parasite is: infected *D. digitata* release the actinospore stage (Figure 1) into the aquatic environment and, upon contact with the actinospore either orally or through the skin or gills (Bellem and Pote 2002), the fish becomes infected. Development to the final *H. ictaluri* myxospore occurs in the gills (Figure 2 and Figure 3), and these spores are released into water subsequently infecting *D. digitata* (Figure 4).

D. Disease Signs

Early clinical signs of affected fish include inappetence and listlessness. As a result of the respiratory insult suffered by these fish, they are often seen in schools behind supplemental aeration devices or in the shallows along the pond bank in the early morning as they are unable to breathe in spite of sufficient dissolved oxygen levels. Mortality is often severe in the early stages of the disease and this usually dissipates after two weeks. Fingerlings are usually more susceptible to PGD although larger fish are also susceptible. However, lesions (gross and microscopic) in food fish may not reflect the clinical severity the disease (i.e. there are only a few minor lesions in the gills and yet mortality is high).

Gross lesions are limited to the gills. This disease presents acutely with gills that are often mottled red and white (Figure 5). These gills are swollen and fragile and bleed easily. The disease progresses with fractures of the cartilage in the primary gill filaments often resulting in blunted and missing filaments (Figures 6,7, 8, and 9). Chronic or healed lesions may present as misshapen gill filaments.

Histologically, PGD is characterized as a granulomatous branchitis. In acute infections, there is congestion and hemorrhage, and a mixed inflammatory infiltrate composed of mononuclear inflammatory cells, together with hypertrophy and hyperplasia of the branchial epithelium. Consequently, the lamellar troughs become occluded and are obliterated. Lysis and fractures occur in the cartilage and defects in the cartilage become apparent. Parasitic trophozoites that are most often stained intensely basophilic (in hematoxylin and eosin preparations) are sometimes evident in the inflammatory milieu. Dyschondroplasia ensues with callus type formation bridging the cartilaginous defects. The gill filaments regenerate and often a misshapen focus (kink) in the cartilage is the only residual sign of infection. The parasitic trophozoites can be seen in other non-branchial tissues (spleen, liver, brain, anterior and posterior kidneys), but there is usually no associated inflammation.

Figure 1. Actinospore stage of *H. ictaluri* in water.

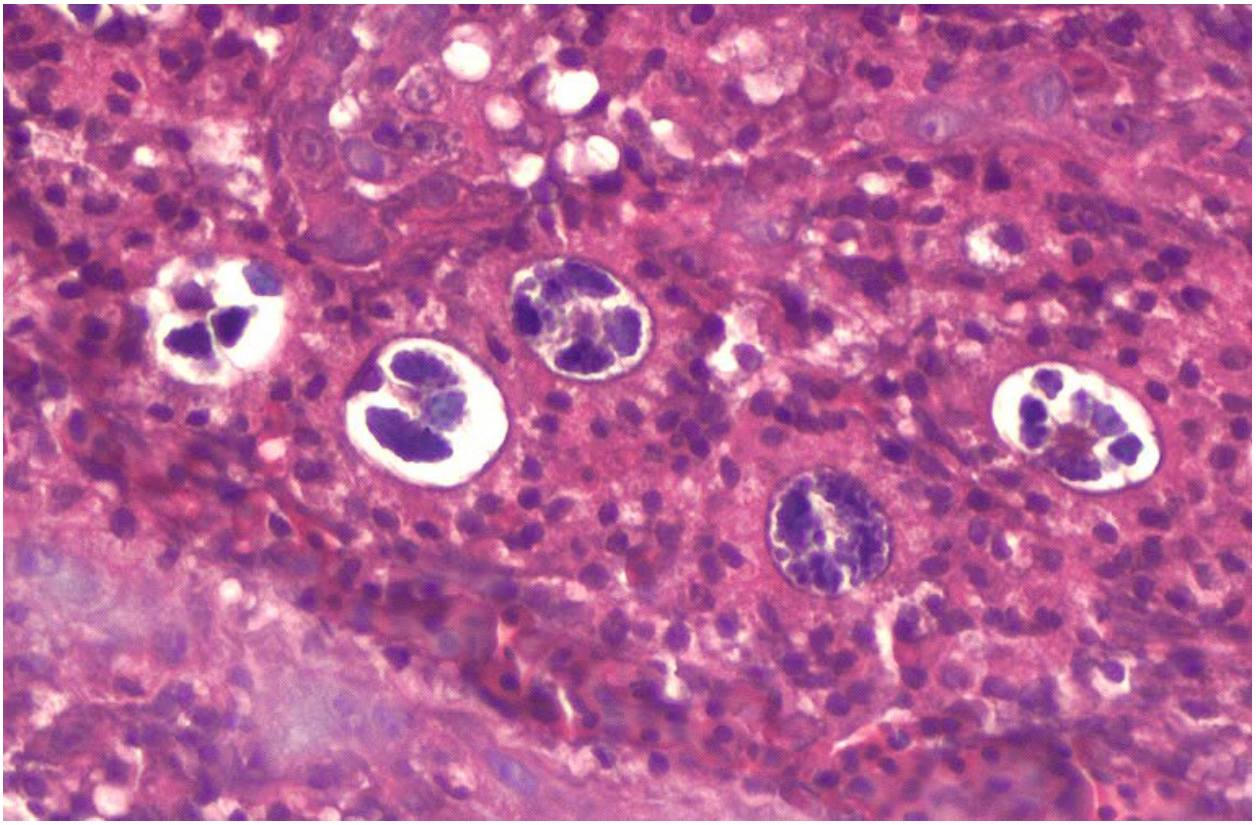

Figure 2. Histological section of early trophozoite stage of *H. ictaluri* in gills (courtesy of Andy Goodwin).

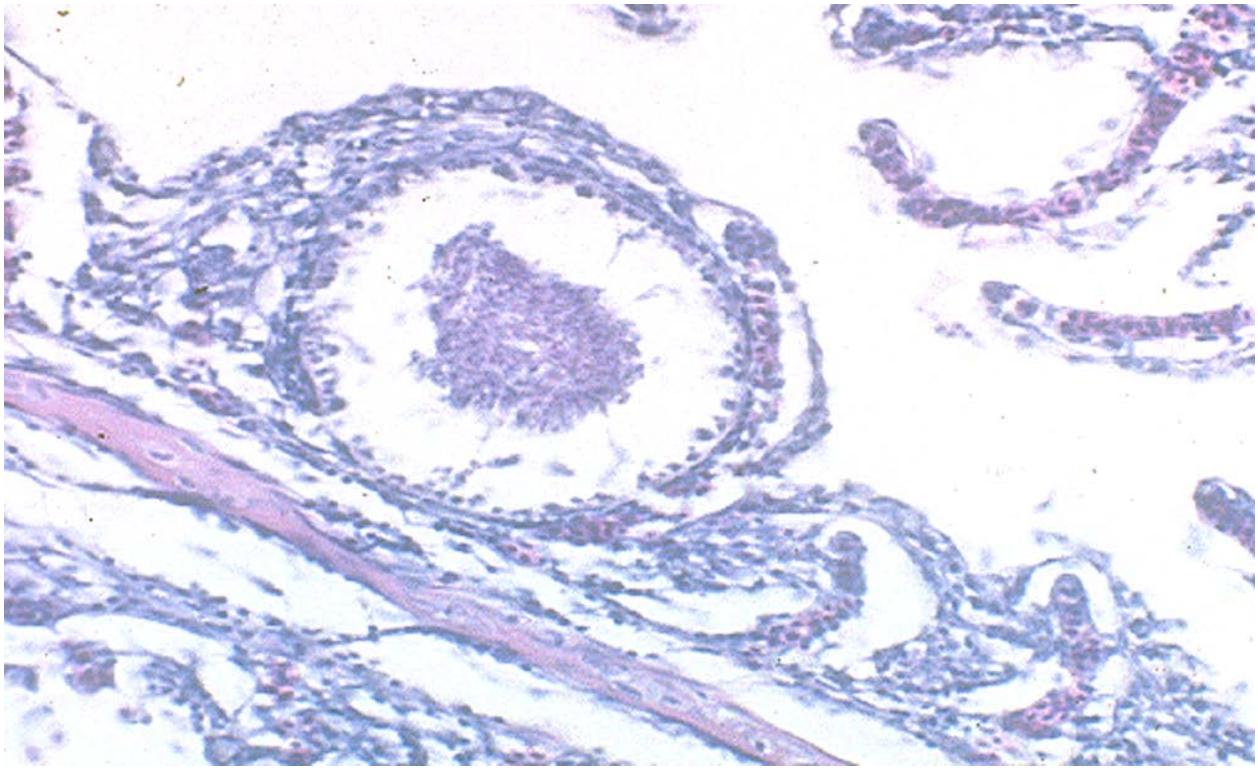

Figure 3. *Henneguya ictaluri* cyst in gills.

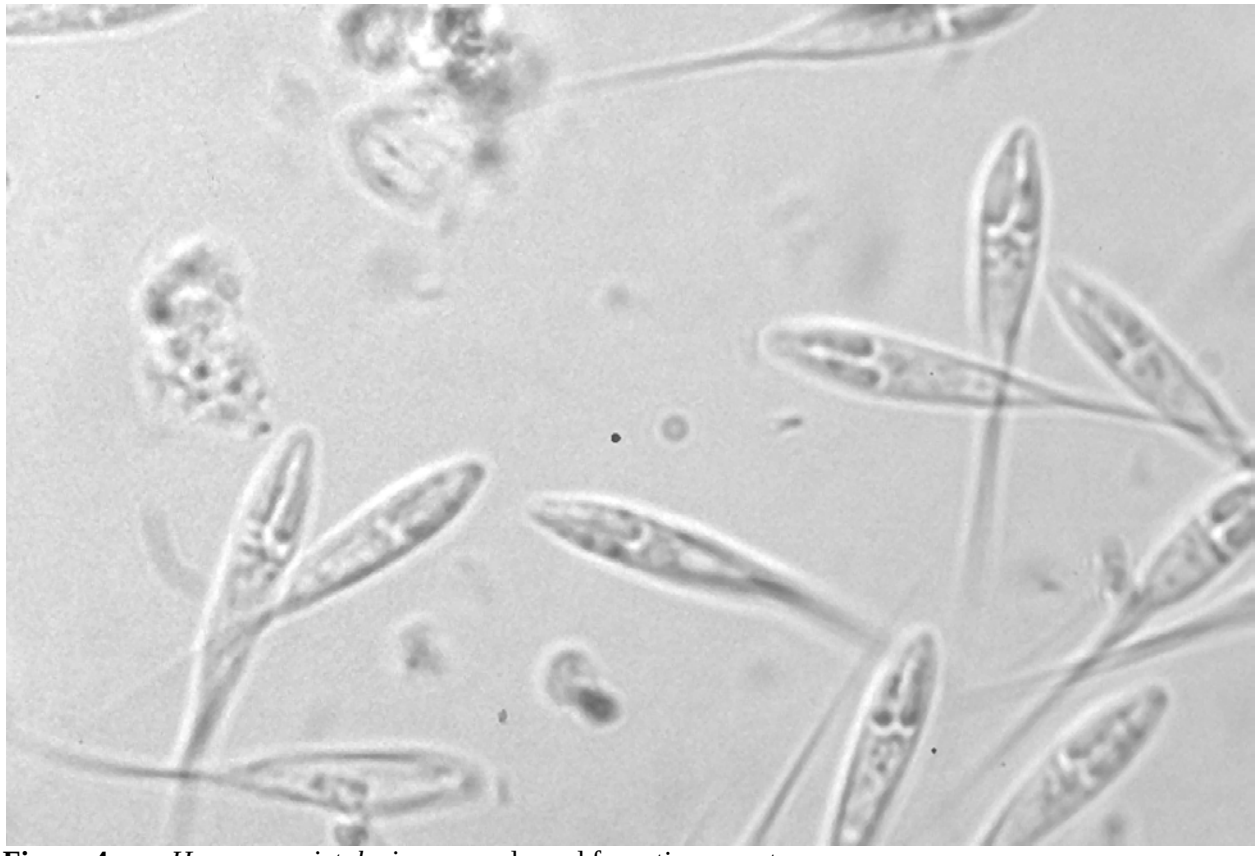

Figure 4. *Henneguya ictaluri* spores released from tissue cyst.

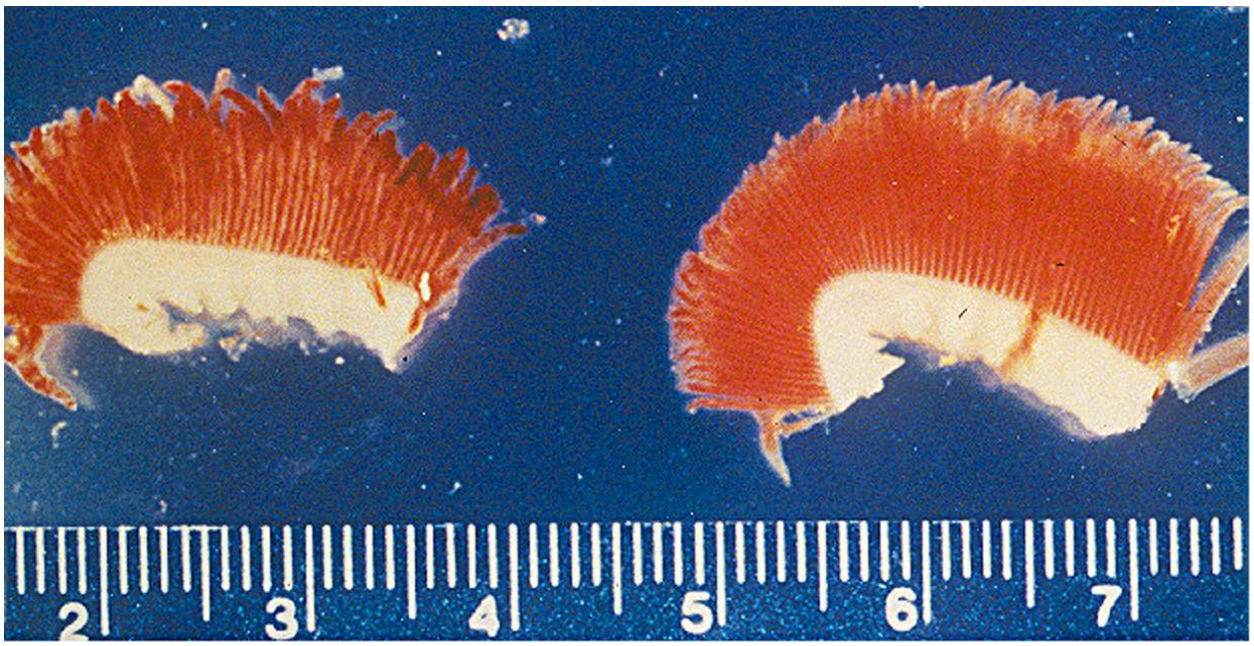

Figure 5. PGD infected gill (left) and normal uninfected catfish gill (courtesy of Larry Hanson).

Figure 6. PGD infected channel catfish demonstrating cartilage damage (courtesy of Andy Goodwin).

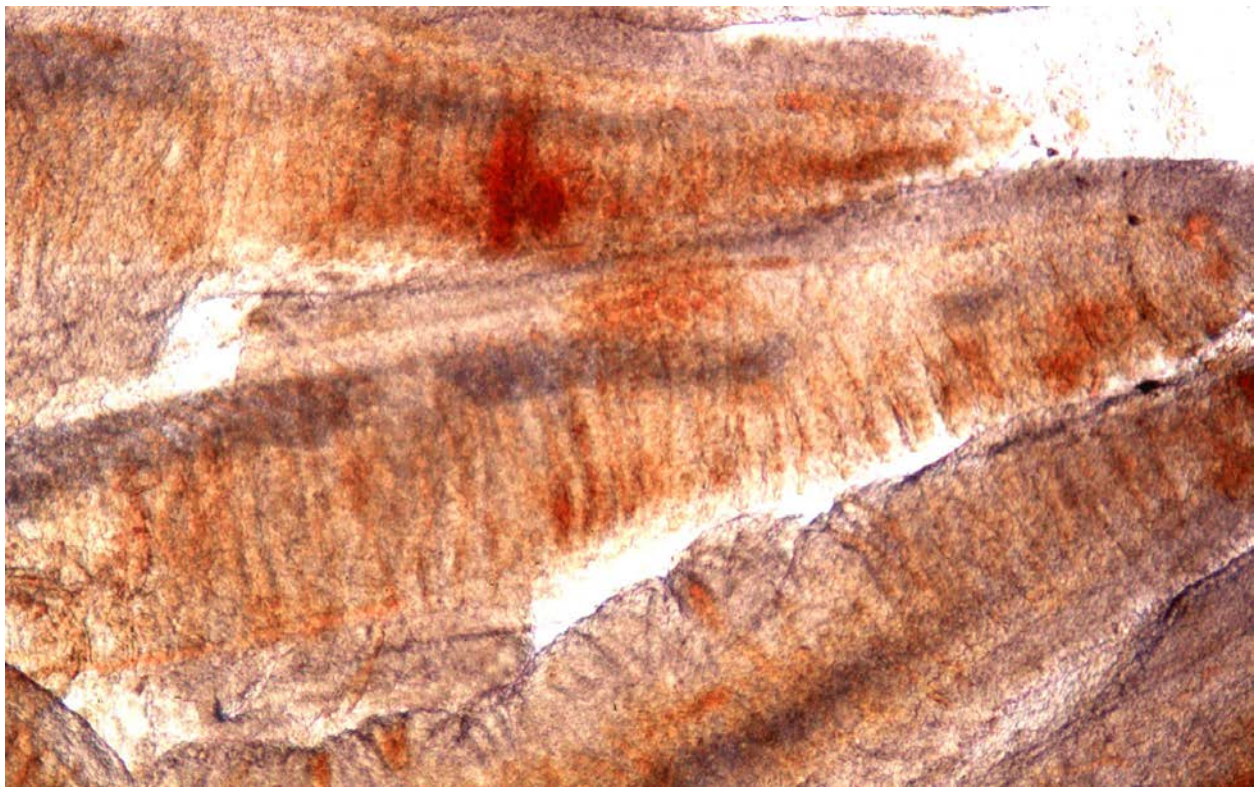

Figure 7. Wet mount of PGD infected channel catfish demonstrating cartilage damage (courtesy of Andy Goodwin).

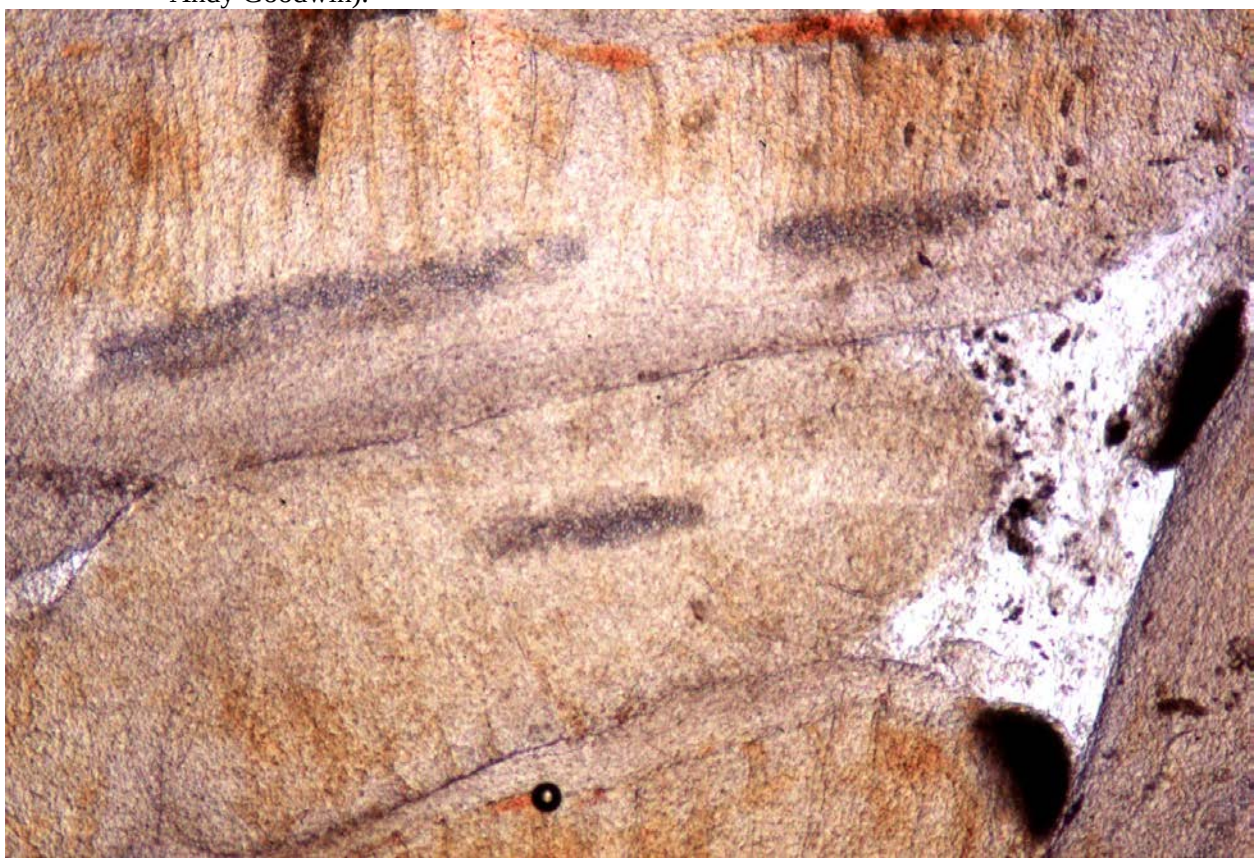

Figure 8. Wet mount of PGD infected channel catfish demonstrating cartilage damage (courtesy of Andy Goodwin).

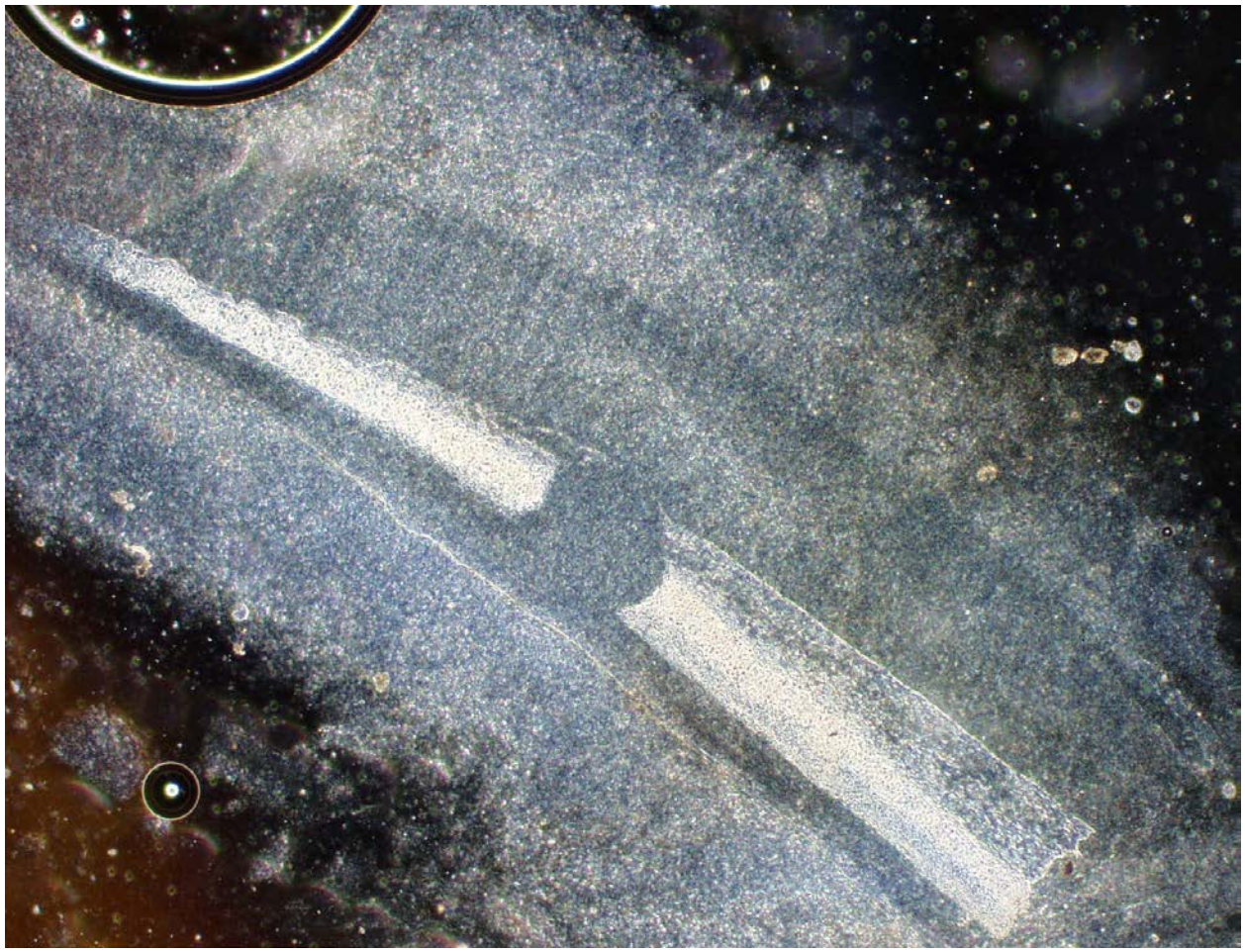

Figure 9. Phase contrast photomicrograph of a PGD infected catfish gill filament demonstrating cartilage damage and epithelial hyperplasia (courtesy of Larry Dorman).

E. Disease Diagnostic Procedures

1. Presumptive Diagnosis

Presumptive diagnosis is based on the clinical signs and the gross lesions (swollen, clubbed gill filaments together with congestion and hemorrhage). Defects in the cartilage of the branchial filaments can be detected on microscopic examination of gill wet mount preparations. These defects can also be present in subclinical cases especially during the cooler months and this may be due to a function of delayed healing.

2. Definitive Diagnosis

Presence of the parasitic trophozoites on histological preparations are required for definitive diagnosis (Figure 2). These are sometimes not readily evident and several sections may have to be examined. Only rarely are these early stages evident on gill wet mount preparations. Molecular confirmation of early gill stages of *H. ictaluri* and the final myxospore stage can be accomplished by using *H. ictaluri* specific polymerase chain reaction assay (Hanson et al. 2001; Whitaker et. al 2002; Section 1, 3.2.6.1 PGD Appendix 1).

F. Procedures for Detecting Subclinical Infections

Often the only indication of a subclinical infections are the cartilaginous defects (breaks) that are evident on microscopic examination of wet mount or histological preparations of gills. The presence of *H. ictaluri* spores can not be detected in the gills until several months after initial infection. There are numerous *Henneguya* species in channel catfish with similar spore morphology, thus identification *Henneguya ictaluri* requires molecular confirmation (Section 1, 3.2.6.1 PGD Appendix 1).

G. Procedures for Determining Prior Exposure to the Etiological Agent

No procedures have been reported.

H. Procedures for Transportation and Storage of Samples to Ensure Maximum Viability and Survival of the Etiological Agent

In order to detect the stages of this organism in the gills by visual examination, live or freshly dead fish should be kept at low temperatures (preferably on ice) prior to submission. Gill samples may also be placed in 10% buffered formalin for routine histology, however, early stages of this parasite (prior to day three post-infection) may not be detectable. Samples for molecular analysis can be frozen or placed in 10% buffered formalin or in 70% ethanol.

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