Variation in Susceptibility to Henneguya ictaluri Infection by Two Species of Catfish and Their Hybrid Cross

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Abstract.—Proliferative gill disease (PGD) in channel catfish Ictalurus punctatus is caused by the myxozoan parasite Henneguya ictaluri. There is no effective treatment for PGD, and mortalities can exceed 50% in severe outbreaks. One approach to controlling losses would be to utilize a less susceptible ictalurid species in pond culture; alternatively, one could identify the traits that convey resistance and exploit them in a selective breeding program. Challenge studies have found less severe inflammatory responses in the gill tissue of blue catfish I. furcatus and fewer mortalities than in channel catfish. However, it remains unclear whether infection and subsequent plasmodial development progress the same way in the two species. To investigate this, we compared the dynamics of H. ictaluri infection in blue catfish, channel catfish, and channel catfish × blue catfish hybrids in continuous long-term (5–7-d) and short-term (24-h) pond challenges. After long-term challenge, 66.2% of the channel catfish and 63.6% of the hybrid catfish developed characteristic PGD lesions, compared with 3.7% of the blue catfish. Quantitative polymerase chain reaction analysis detected H. ictaluri in larger percentages of channel and hybrid catfish than blue catfish (98.7% and 95.7% versus 45.9%), with significantly greater parasite DNA equivalents in channel and hybrid catfish than blue catfish. Similar findings were obtained in the short-term exposures. Histologically, channel and hybrid catfish developed severe PGD accompanied by large numbers of developing plasmodia. While mild PGD was observed in some blue catfish, the progression of lesions lagged behind that in channel and hybrid catfish. Most importantly, developing plasmodia were not observed in blue catfish, and parasite DNA was not detected 14 d after removal from the source of infection. Our findings indicate that the resistance of blue catfish to H. ictaluri infection can be overcome by large numbers of infective actinospores but that infection appears to be eliminated before plasmodial development occurs.

Since its discovery in 1981, proliferative gill disease (PGD), which is caused by the myxozoan parasite Henneguya ictaluri (Pote et al. 2000), has been one of the most devastating diseases affecting the commercial culture of channel catfish Ictalurus punctatus. The complex life cycle involves a myxospore stage in the fish and an actinospore stage shed by the benthic oligochaete Dero digitata, which is ubiquitous in commercial catfish ponds (Styer et al. 1991; Pote et al. 2000). At present, there are no effective prophylactic or therapeutic treatments for PGD and mortalities within affected populations can exceed 50% in severe outbreaks.
outbreaks (Bowser and Conroy 1985; Wise et al. 2004).

Bosworth et al. (2003) examined the genetic variation between channel catfish and blue catfish *Ictalurus furcatus* with respect to PGD resistance and determined that blue catfish are more resistant to PGD. There is also evidence that blue catfish are resistant to other important diseases of channel catfish, including those caused by *Edwardsiella ictaluri* and channel catfish herpes virus (Graham 1999). However, the culture of blue catfish is limited owing to some unfavorable production characteristics (Dunham et al. 1993).

Studies conducted by Bosworth et al. (2003) indicate that blue catfish and, to a lesser extent, channel catfish × blue catfish F1 hybrids exhibit less gill damage from PGD than channel catfish. It remains unclear whether *H. ictaluri* infection and subsequent plasmodial development progress the same way in blue and channel catfish. Specific unanswered questions are development progress the same way in blue and *H. ictaluri* PGD than channel catfish. It remains unclear whether compare the dynamics of inflammatory response seen in channel catfish, and occurs in the gills but fails to elicit the severe site of development in blue catfish, whether infection and subsequent plasmodial development progress the same way in blue and channel catfish. Specific unanswered questions are the ability of *H. ictaluri* to penetrate and reach its final host defenses before significant damage can occur in the target tissue. The purpose of this study was to confirm findings by Bosworth et al. (2003) and to compare the dynamics of *H. ictaluri* infection in blue catfish, channel catfish, and channel catfish × blue catfish hybrids using microscopic observations and molecular analysis on these respective groups during placement in ponds experiencing PGD-related mortalities.

**Methods**

*Experimental design.*—All pond exposures took place at commercial catfish operations during spring of the year, when water temperatures were within the optimal range for PGD outbreaks (16–25°C). Two separate trials were conducted. In the first trial, the development of PGD in channel, blue, and hybrid catfish was evaluated by continuously exposing all three groups to *H. ictaluri* in one of three commercial channel catfish ponds with ongoing outbreaks of PGD. Specific-pathogen-free (SPF) fingerlings (20.0–60.0 g) representing the three groups were obtained from the U.S. Department of Agriculture’s Catfish Genetics Research Unit in Stoneville, Mississippi. Before challenge, fish were held in 2,000-L fiberglass holding tanks containing 1,000 L of well water at a flow rate of 1 L/min. For each group, three net-pens constructed of 5-mm nylon mesh (to allow the free exchange of water within the confined area) were stocked with 20 fish and placed in the pond for a period of 5 or 7 d (complications with the net-pens resulted in fish only being exposed for 5 d in pond 1; see Results). To ensure that the fish were negative for PGD before challenge, three fish from each group were sampled and processed for wet-mount examination and molecular analysis before stocking. Except for pond 1, five fish were sampled from each net-pen every other day for the duration of the trial. Upon removal from the pond, fish were transported live to Thad Cochran National Warmwater Aquaculture Center in an aerated holding tank and held until sampled. Fish were euthanatized by an overdose (1,000 mg/L) of the anesthetic tricaine methanesulfonate (MS-222; Argent Laboratories, Redmond, Washington) and processed for wet-mount examination of gill clips, molecular analysis, and histopathology within 1 h of collection.

In the second experimental trial, SPF catfish fingerlings from all three groups were continuously exposed to *H. ictaluri* in commercial ponds for a period of 24 h. The fish were then relocated to aquaria and held in a controlled, parasite-free environment to determine whether *H. ictaluri* can be cleared from the host in the absence of further parasite challenge. Before challenge, three fish from each group were sampled and confirmed to be PGD negative. For each of the three groups, a net-pen was placed in each pond and stocked with 20 fingerlings. After a 24-h exposure, fish were removed from the pens, transported back to the laboratory, and held in glass aquaria containing 100 L of well water under flow-through conditions (1 L/min). Five fish from each aquarium were euthanized and processed for wet-mount examination and molecular analysis 1, 7, and 14 d postexposure. Three fish from each group that were not exposed to pond water were held in aquaria under the same conditions and sampled after 14 d as negative controls.

*Blood collection.*—Approximately 100 μL of whole blood was collected from the caudal vein of fish from trial 1 to determine whether the blood-borne stages of the *H. ictaluri* life cycle could be detected (Belem and Pote 2001). Samples were collected at 5 d postexposure from pond 1 and 7 d postexposure from ponds 2 and 3. Blood was immediately transferred to a 1.5-mL microcentrifuge tube and stored at −80°C until further processing. No blood was collected during trial 2.

*DNA extraction from blood.*—Genomic DNA for molecular analysis of the whole blood collected from trial 1 was isolated with a Gentra Puregene blood DNA isolation kit (Qiagen, Valencia, California) following the manufacturer’s suggested protocol. The purified DNA was then suspended in 200 μL of TE buffer (10 mM tris, 1 mM EDTA; pH 7.0–8.0), quantified, and stored at −80°C.
**Wet-mount examination.**—To provide an estimation of disease severity and indirectly assess the variability in challenge dose between ponds, the presence and severity of PGD lesions from both experimental trials were determined by microscopic examination of gill clip wet mounts. Approximately 40–80 filaments were taken from a left gill arch and examined microscopically. The presence of PGD was confirmed by lytic lesions in the cartilage of the gill filaments (Figure 1), and lesion scores (defined as the percentage of gill filaments exhibiting chondrolytic lesions) were determined (Wise et al. 2004, 2008; Griffin et al. 2008). An additional sample of approximately 40–80 filaments was removed from a second left gill arch and processed for molecular analysis.

**Histopathology.**—The variability in parasite development and its associated pathology between channel, blue, and channel × blue catfish exposed to *H. ictaluri* (trial 1) was assessed by histopathology. Individual gill arches were removed from the right side of each fish and placed in 10% neutral buffered formalin for a minimum of 24 h. The tissues were then processed by dehydration in a graded series of ethanol solutions of increasing strength, cleared in a series of xylenes, embedded in paraffin, and sectioned at 5 μm. The prepared slides were stained with hematoxylin and eosin. Samples were designated as positive or negative for PGD based on the presence of histological changes indicative of the disease, namely, foci of osteochondrolysis, epithelial hyperplasia, granulomatous inflammation, and multinucleated trophozoites (Figures 2, 3; MacMillan et al. 1989; Pote et al. 2000, 2003; Griffin et al. 2008; Wise et al. 2008). Histopathology was not performed on the fish from trial 2.

**Generation of quantitative PCR standards.**—To obtain pure *H. ictaluri* genomic DNA for the development of quantitative polymerase chain reaction (QPCR) standards, pond sediment was collected from a commercial channel catfish pond with an active PGD outbreak in the resident fish population. *Dero digitata* were isolated from the sediment and observed for 72 h to release *H. ictaluri* actinospores (Bellerud 1993; Pote et al. 1994; Bellerud et al. 1995). Organisms were identified morphologically according to descriptions of actinospores commonly found in commercial channel catfish ponds (Bellerud 1993).

The genomic DNA from *H. ictaluri* actinospores and SPF channel catfish was isolated with the Gentra Puregene DNA isolation kit for fresh or frozen animal tissue (Qiagen). Proteinase *K* (20 mg/mL) was added before overnight incubation at 55°C, and the remainder of the isolation was carried out according to the manufacturer’s suggested protocol.

Target regions of the 18S small subunit (SSU) ribosomal DNA (rDNA) genes were amplified from the genomic DNA of *H. ictaluri* actinospores and SPF catfish using *H. ictaluri*-specific PCR primers and generic eukaryotic 18S SSU rDNA primers, respectively (Table 1). The 25-μL PCR reaction mixtures contained 2.5 μL of 10× reaction buffer (10 mM tris, 50 mM KCl [pH 9.0], and 4.0 mM MgCl₂), 5 nM of each deoxynucleotide triphosphate, 5 pM of each primer, 0.5 units of Hot Start *Taq* DNA polymerase (Takara Bio USA, Madison, Wisconsin), and 2 μL of DNA template. The PCR was carried out on a PTC-100 thermal cycler (GMI, Ramsey, Minnesota) programmed for 1 cycle of 95°C for 10 min, 50°C for 2 min, and 72°C for 4 min followed by 35 cycles of 92°C for 1 min, 50°C for 1 min, and 72°C for 1 min, with a final extension cycle of 72°C for 5 min.

The PCR amplification products were analyzed by electrophoresis on a 1.5% agarose gel and stained (Gelstar nucleic acid stain, Cambrex, East Rutherford, New Jersey) to confirm the presence of a single product. The products were purified using the QIAquick PCR purification kit (Qiagen) and inserted into a cloning vector using the PCR4-TOPO cloning kit (Invitrogen, Carlsbad, California). The resulting plasmid clones with 18S SSU rDNA gene inserts from *H. ictaluri* and channel catfish were each grown in culture overnight, and plasmid purification was performed with the Qiagen plasmid miniprep kit. Plasmid standards were quantified with a Nanodrop spectrophotometer and the accompanying software (version 3.2.1; Nanodrop Technologies, Wilmington, Delaware) and their concentrations adjusted to 1.0 ng/μL. Plasmid DNA was then serially diluted with TE buffer and utilized for the generation of standard curves.
Quantitative PCR.—Parasite DNA equivalents in gill tissues were determined by QPCR analysis. Samples designated for molecular analysis were placed in 600 μL of cell lysis solution from a Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minnesota) and DNA isolation and quantification were carried out as described previously. At least 1 ng of total genomic DNA was added to each reaction, and all samples were analyzed in triplicate according to the QPCR protocol described by Griffin et al. (2008).

The 12.5-μL PCR reactions contained BioRad IQ supermix (BioRad, Hercules, California), 20 pM of each primer, 0.25 pM of TaqMan probe, 3 μL of template DNA, and nuclease-free water to volume. Amplifications were performed on a BioRad iCycler version 3.1 real-time PCR system programmed for 1 cycle of 95°C for 3 min and 30 s followed by 40 cycles of 95°C for 30 s, 56°C for 1 min, and 72°C for 30 s. Data collection was carried out after the 72°C elongation step at the end of each cycle. To correct for initial template variations between samples, 18S SSU rDNA plasmid standard equivalents were determined, along with H. ictaluri DNA plasmid standard equivalents, using the 18S SSU rDNA and probe

Figure 2.—Histological sections showing the progression of pathological changes in the gills of channel, blue, and channel × blue catfish held in a pond that was positive for proliferative gill disease for 7 d. Hematoxylin–eosin staining was used to enhance the images.
combinations described by Elibol-Fleming et al. (2009). Sample cycle threshold \( (C_T) \) values were compared with those of a standard curve based on serially diluted plasmid standards of the amplicons generated by \( H. \ ictaluri \) (Whitaker et al. 2001, 2005; Pote et al. 2003) or generic eukaryotic 18S SSU rDNA primers (Elibol-Fleming et al. 2009), respectively (Table 1). Reaction efficiencies \( (E) \) were calculated from the formula

\[
E = 10^{\left(1/|M|\right)} - 1,
\]

where \( M \) is the slope of the standard curve. Data were considered valid if the slope of the standard curve was between \(-3.1\) and \(-3.6\), which represent reaction efficiencies between 90% and 110% (Wong and Medrano 2005). The results were normalized against the initial concentration of 18S SSU rDNA to account for the variations in starting template quantity. The ratio of parasite standard equivalents (PSE) to host standard equivalents (HSE) was used for the purpose of comparisons.

Statistical analysis.—All statistical analyses were performed with SAS software version 9.1 (SAS Institute, Cary, North Carolina). A split plot with randomized complete block design was utilized, in which the main unit was species, the block was pond, the subsamples were sampling days, and individual fish served as sub-subsamples. Parasite standard equivalents determined by QPCR were normalized to the host 18S rDNA, and the ratio of PSE to HSE was log10 transformed before statistical analysis. All samples in which \( H. \ ictaluri \) DNA was not amplified were assigned a log value of \(-9.00\). Multiple comparisons of mean percent gill damage were analyzed by one-way analysis of variance using least-squares means. Analysis of the PSE–HSE ratios were performed separately for the two experimental trials using the MIXED procedure in SAS. Multiple comparisons were made by comparing the ratios of the two measured quantities (PSE and HSE) to the least-squares ratios obtained from the MIXED procedure. Ratios of parasite to host DNA greater than the least-squares ratio were consid-

Table 1.—Primer and probe sequences used to amplify the genomic DNA of \( H. \ ictaluri \) actinospores and blue, channel, and blue \( \times \) channel catfish.

<table>
<thead>
<tr>
<th>Name</th>
<th>Direction</th>
<th>Sequence(^a)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1–1</td>
<td>Forward</td>
<td>5’-CAAATAATCTTGCTATCATTG-3’</td>
<td>Whitaker et al. (2001)</td>
</tr>
<tr>
<td>A1–2</td>
<td>Reverse</td>
<td>5’-GCGCCACAGATTACCTCA-3’</td>
<td>Whitaker et al. (2001)</td>
</tr>
<tr>
<td>18SCCF</td>
<td>Forward</td>
<td>5’-CGGAGAGGGGACCTTAGAGA-3’</td>
<td>Elibol-Fleming et al. (2009)</td>
</tr>
<tr>
<td>18SCCR</td>
<td>Reverse</td>
<td>5’-CGCTGGGAATGGTAGTATTTG-3’</td>
<td>Elibol-Fleming et al. (2009)</td>
</tr>
<tr>
<td>HITMP</td>
<td>Probe</td>
<td>5’-[FAM]-TCACCTTGATTTGACCTCA-[BHQ1]-3’</td>
<td>Griffin et al. (2008)</td>
</tr>
<tr>
<td>18STMP</td>
<td>Probe</td>
<td>5’-[HEX]-ACCACATCAGAAGGACAGCAGG-[BHQ1]-3’</td>
<td>Elibol-Fleming et al. 2009</td>
</tr>
</tbody>
</table>

\(^a\) FAM = 6-carboxyfluorescein, HEX = hexachloro-6-carboxy-fluorescein, and BHQ1 = Black Hole Quencher-1.
Within rows, values with different letters are significantly different ($P \leq 0.05$).

### Mortalities and Fish Loss

Complications with net-pens resulting in fish loss from predation or escape occurred only in trial 1. In pond 1, fish escaped from one cage containing channel catfish and one cage containing blue catfish owing to structural failure. In addition, one channel catfish from a second cage died between days 3 and 5 postexposure. At this point the trial was terminated for pond 1. In pond 2, one blue catfish died between 5 and 7 d postexposure. No fish died during trial 2.

### Wet-Mount Examination

Based on microscopic examination of gill clip wet mounts, channel catfish and hybrid catfish had significantly greater gill damage than blue catfish in both experimental trials (Tables 2, 3). In trial 1, 3.7% of blue catfish developed chondrolytic lesions, compared with 66.2% of channel catfish and 63.6% of hybrid catfish. In trial 2, the percentage of gill damage was 0.0% for blue catfish, 9.0% for channel catfish, and 13.3% for hybrid catfish.
hybrid catfish (Table 2). All channel and hybrid catfish sampled on the last day of sampling for each pond had lesions typical of PGD. In trial 2, 6.7% of blue catfish developed clinical manifestations of the disease, compared with 33.3% of channel catfish and 24.4% of hybrid catfish (Table 3).

Not only did a greater percentage of channel and hybrid catfish have typical PGD lesions, the severity of the disease was significantly greater in those groups in both trials (Tables 2, 3). In trial 1, both channel and hybrid catfish had significantly greater lesion scores than blue catfish by the third day of the trial (Figure 4). This trend continued throughout the remainder of the challenge. The average lesion score was 0.4% for blue catfish, compared with 12.7% and 13.4% for channel and hybrid catfish, respectively (Table 2). Similarly, in trial 2, the average lesion score for blue catfish was 0.3%, significantly lower than the 2.7% and 2.9% for channel and hybrid catfish (Table 3). No PGD-related lesions were observed in blue catfish on the first or last day of trial 2 (Table 3; Figure 5).

Quantitative PCR

For all trials, blue catfish had significantly lower *H. ictaluri* DNA equivalents in gill tissue than channel and hybrid catfish, with the highest DNA equivalents consistently being observed in channel catfish. In both experimental trials, the ratio of *H. ictaluri* DNA to host DNA in blue catfish averaged nearly 3 orders of magnitude less than in channel and hybrid catfish (Tables 2, 3; Figures 6, 7). Hybrid catfish gill tissue consistently had less *H. ictaluri* DNA equivalents than channel catfish, averaging nearly one-half a magnitude lower throughout both trials. By the third day postexposure in trial 1 and the seventh day postexposure in trial 2, 100% of channel and hybrid catfish gill tissues were positive for *H. ictaluri*, a result that held over the remaining sampling days. There were no sampling events in either trial in which 100% of the blue catfish sampled were positive for *H. ictaluri*. Parasite DNA was not detected in the gills of any blue catfish 14 d after they were removed from the source of infection, and the ratio of *H. ictaluri* DNA to host DNA equivalents in blue catfish did not increase significantly over time in either experimental trial (Figures 6, 7).

Molecular analysis of fish blood from the last day of sampling in trial 1 showed the presence of *H. ictaluri* DNA in only one blue catfish. Three of the last-day channel catfish blood samples from pond 3 were lost during processing, resulting in the analysis of only 12 such samples for that day. *H. ictaluri* DNA was detected in the blood of both channel and hybrid catfish, but in a much greater percentage of the channel catfish (88.9%) than the hybrid catfish (31.1%) (Table 2).

Histopathology

Only gill tissues from trial 1 were examined histologically. On day 1 postexposure, the changes in channel catfish were predominated by congestion, hemorrhaging, and edema. There was widespread lifting of the lamellar epithelium from capillary endothelia that created clear spaces occasionally traversed by strands of fibrillar pale eosinophilic material. There were multifocal, widely scattered aneurysmal dilations of lamellar capillaries, which often ruptured and hemorrhaged into the subepithelial spaces described above and occasionally obliterated the normal architecture of the lamellar troughs. Widely present were more extensive areas of hemorrhaging that traversed the filamental bone and cartilage, spanning up to 15–20 lamellae and expanding the overall width of the affected filaments. Within these areas of hemorrhaging small collections of mononuclear inflammatory cells and the foci of lysis involving the adjacent bone and cartilage were found on rare occasions (Figures 2, 3); the organisms were not visualized, however. Epithelial changes were limited to minimal to mild hypertrophy and hyperplasia, typically in association with the foci of hemorrhaging and the bases of lamellar troughs. Moderately sized round leukocytes (interpreted as neutrophils) with moderate amounts of finely granular pale eosinophilic cytoplasm and slightly oval, eccentric nuclei were common within the filament vasculature. A band of identical cells of varying thickness infiltrated between the epithelial basement membrane and bone of large numbers of filaments. The initial changes in hybrids were comparable in all respects to those seen in channel catfish but were encountered slightly less frequently. No significant changes were observed in blue catfish tissues 1 d postexposure.

As the trial progressed to 3 and 5 d postexposure, hemorrhaging remained a significant finding in both channel and hybrid catfish gill tissues but was less severe (Figure 2). Concomitant with this was an increase in the extent of epithelial hyperplasia, the severity of inflammatory infiltrates, and the size and number of lytic bone and cartilage lesions. Epithelial hypertrophy and hyperplasia were widespread and partially to completely filled focally extensive areas of consecutive lamellar troughs. Thrombosed lamellar capillaries were occasionally present within the areas of hyperplasia. Inflammatory infiltrates shifted from predominantly granulocytic to epithelioid-like macrophages with indistinct cell borders, finely vacuolated pale eosinophilic cytoplasm, and central nuclei with vesicular to margined chromatin. Epithelial hyperplasia and macrophage infiltrates were most prominent...
FIGURE 4.—Development of proliferative gill disease in blue, channel, and blue × channel catfish fingerlings exposed for 7 d in a pond with an ongoing outbreak of the disease (trial 1). The data presented are the mean ± SE percentages of gill filaments exhibiting at least one chondrolytic lesion 1, 3, 5, and 7 d postexposure. No chondrolytic lesions were observed from blue catfish in ponds 2 and 3.
FIGURE 5.—Development of proliferative gill disease in blue, channel, and blue × channel catfish fingerlings exposed for 24 h in a pond with an ongoing outbreak of the disease and then held in an *H. ictaluri*-free environment for 14 d (trial 2). The data presented are the mean ± SE percentages of gill filaments exhibiting at least one chondrolytic lesion 1, 7, and 14 d postexposure.
FIGURE 6.—Development of proliferative gill disease in blue, channel, and blue × channel catfish fingerlings in trial 1 (see Figure 4). The data presented are the mean ± SE log_{10} transformed numbers of parasite DNA equivalents (PSE) per host DNA equivalent (HSE) in gill tissue as determined by quantitative PCR analysis.
Figure 7.—Development of proliferative gill disease in blue, channel, and blue × channel catfish fingerlings in trial 2 (see Figure 5). The data presented are the mean ± SE log e transformed numbers of parasite DNA equivalents (PSE) per host DNA equivalent (HSE) in gill tissue as determined by quantitative PCR analysis.
adjacent to the foci of osteochondrolysis. Mantles of macrophages were immediately apposed to lytic gill filaments and were separated from epithelial cells by a basement membrane. Macrophages were further distinguished from epithelial cells, which also had vesicular nuclei but typically possessed a prominent nucleolus and a finely granular amphophilic cytoplasm. Lymphocytic infiltrates were increased and moderate in number, but limited primarily to filament and lamellar epithelial surfaces.

As the trial progressed, histological examination of blue catfish revealed widely scattered, small foci of minimal hemorrhaging limited predominantly to lamellar capillaries. Larger hemorrhages associated with gill filaments, as described for channel catfish, were extremely rare. There were also scattered, multifocal, mild foci of epithelial hyperplasia that typically filled lamellar troughs, resulting in the fusion of 2–3 lamellae. Thrombosed capillaries were occasionally present within these foci. Rarely, focal lysis of filament bone and cartilage was present, but it was not associated with severe epithelial hyperplasia or inflammatory infiltrates, as was seen in the channel and hybrid catfish.

By the final days of the trial (5 or 7 d postexposure), hemorrhaging continued to diminish in both channel and hybrid catfish, while hyperplasia and macrophage infiltrates continued to increase (Figure 2). Small numbers of elongated plump fibroblasts were visible in areas of osteochondrolysis and bordered rare irregular foci of proliferating dysplastic cartilage. In both channel and hybrid catfish, large spherical developing plasmodia surrounded by mantles of macrophages were located adjacent to the foci of osteochondrolysis (and less frequently within lamellar troughs). Plasmodia possessed numerous small centrally located, hyperbasophilic nuclei with an outer rim of clear to pale eosinophilic cytoplasm (Figure 3). Conversely, examination of blue catfish tissues 5 and 7 d postexposure revealed changes that closely resembled those seen in channel and hybrid catfish on days 1–3 of the trial, including the scattered presence of osteochondrolysis. However, developing plasmodia were not observed in any blue catfish tissue sections.

**Discussion**

This study provides further confirmation that although some species of Myxozoa are not host specific there is significant variation in the susceptibility of and associated pathology in the hosts they do infect. Yokoyama et al. (1995) demonstrated that sporoplasm release by the actinospore stage of *Myxobolus cultus* could be induced in the presence of mucin from a wide variety of fish species. This suggests that host susceptibility to *M. cultus* is more a product of immunological responses after penetration of the sporoplasm than recognition of a specific host by the actinospore. *Enteromyxum leei* and *Kudoa thrysites* both infect a wide range of host species across many different families and genera. Currently, the host record of *Enteromyxum leei* contains over 40 species of both freshwater and marine fish. Given its wide host range and ability to infect both freshwater and marine fishes, *E. leei* is of major concern for both aquaculture and native fisheries in the Mediterranean area, as any number of native fishes could serve as reservoir hosts for the parasite (Diamant et al. 2006). Similarly, *K. thrysites*, which can cause postharvest myoliquefaction, is considered a significant threat to pen-reared Atlantic salmon *Salmo salar* in Canada, the United States, and Ireland. As of 2006, this parasite had been reported from 37 species of marine teleosts, representing 18 different families of fish from the coastal waters of North America, South America, Europe, Asia, Africa, and Australia (Whipps and Kent 2006).

Alternatively, only members of the family Salmoideae are known to develop ceratomyxosis (which is caused by the myxozoan parasite *Ceratomyxa shasta*), although susceptibility varies between species and genera (Bartholomew 1998). In naturally occurring outbreaks, rainbow trout *Oncorhynchus mykiss* developed severe infections, whereas the severity of the disease was significantly lower in brook trout *Salvelinus fontinalis* and brown trout *Salmo trutta* from the same water supply (Schafer 1968). In controlled studies, rainbow trout, cutthroat trout *Oncorhynchus clarkii*, chum salmon *Oncorhynchus keta*, and fall Chinook salmon *Oncorhynchus tsawytscha* all demonstrated high susceptibility to *C. shasta*, whereas brown trout, sockeye salmon *Oncorhynchus nerka*, and spring Chinook salmon were relatively resistant (Zinn et al. 1977).

Similarly, *Myxobolus cerebralis*, the causative agent of salmonid whirling disease, is able to infect numerous species of salmonids; however, not all infected fish present clinical signs of the disease (skeletal deformities, blackened caudal regions, and whirling behavior), and the pathology associated with infection is variable between species. Rainbow trout are highly susceptible to the disease, as are sockeye salmon and cutthroat trout. Conversely, coho salmon *Oncorhynchus kisutch* demonstrate relatively low susceptibility (O’Grodnick 1979; Wagner et al. 2002a; Gilbert and Granath 2003). Brown trout are normally asymptomatic; however, they can exhibit darkened caudal regions when exposed to high numbers of actinospores. Although whirling behavior is absent, myxospores have been observed in digests of
cartilaginous tissues from the head and spine, but at much lower numbers than in rainbow trout exposed to the same dose (Hedrick et al. 1999a). Bull trout *Salvelinus confluentus* are susceptible to infection, although this species rarely demonstrates clinical signs of the disease and under experimental conditions parasite numbers and associated lesions resolved over time (Hedrick et al. 1999b). Lake trout *Salvelinus namaycush* appear to be refractory to infection by *M. cerebralis*. They did not demonstrate clinical signs when naturally exposed in a contaminated stream, and myxospore development was not observed (O’Grodnick 1979). Likewise, in controlled laboratory challenges lake trout challenged with low (200) and high (2,000) numbers of actinospores per fish were parasite negative by spore enumeration 22 weeks postexposure (Blazer et al. 2004). In another controlled study, 100% of the lake trout exposed to 1,000 *M. cerebralis* actinospores per fish were positive by PCR 5 weeks postexposure but less than 15% were positive by spore enumeration 20 weeks postexposure, suggesting that the parasite can invade and initially infect the host but that lake trout have the ability to clear the infection (Wagner et al. 2002b; Blazer et al. 2004).

This study suggests that *H. ictaluri* is unable to complete its life cycle in blue catfish, a phenomenon similar to that of *M. cerebralis* in lake trout. Infection and plasmodial development did not progress within this species, and when the fish were removed from the source of actinospores the infection appeared to resolve. These data also support previous work showing that blue catfish develop lesions characteristic of PGD during severe outbreaks but that they are significantly more resistant to it than both channel and hybrid catfish (Bosworth et al. 2003). Only 6 of the 159 blue catfish examined from trial 1 and 3 of the 45 blue catfish examined from trial 2 demonstrated gross lesions indicative of PGD. All blue catfish with chondrolytic lesions were from ponds in which channel and hybrid catfish exhibited the ability to clear the infection, suggesting there was a larger number of infective actinospores in the pond environment.

Although our QPCR analysis detected *H. ictaluri* DNA in the gill tissues of many blue catfish, developing plasmodia were not visualized in histological sections. Furthermore, the parasite levels detected by QPCR did not significantly increase over the long-term challenge, and 14 d after fish were removed from the source of infection parasite DNA decreased to undetectable levels. By comparison, parasite DNA levels in the gills of channel and hybrid catfish increased even after fish were removed from the source of infection, suggesting replication and proliferation of the parasite within the host.

One limitation of QPCR analysis is that the assay can only determine whether or not target DNA is present in a given sample; it is not indicative of the viability of the target organism and does not distinguish between an active infection and a DNA artifact. Assuming that the actinospore stage of the organism was present in pond water at the time of sampling, and considering the sensitivity of the QPCR assay, the small amounts of *H. ictaluri* DNA detected in blue catfish gills could represent an infection that had not undergone further development or surface-associated DNA contaminants trapped in gill tissues. The absence of detectable *H. ictaluri* DNA in blue catfish by day 14 of trial 2 suggests that although the organism is initially able to penetrate blue catfish tissues, the infection is short-lived. Conversely, in both trials the gills of channel and hybrid catfish consistently had significantly greater parasite DNA equivalents than those of blue catfish. This finding, which was corroborated by the presence of developing plasmodia in histologic sections, indicates there was an active infection and the proliferation and uptake of this parasite occurred to a much greater extent in channel and hybrid catfish.

The QPCR analysis of the blood collected on the last day of each trial indicated that even among heavily infected channel and hybrid catfish a greater percentage of the channel catfish had *H. ictaluri* DNA in their blood. The presence of *H. ictaluri* in the blood supports the findings of Belem and Pote (2001), who suggested that after penetrating host tissues, sporozoites of *H. ictaluri* are transported by blood vessels to different organs, such as the spleen, kidneys, and liver, before residing in the gills. This also suggests that the course of infection and degree of tissue migration of *H. ictaluri* are not identical in channel and hybrid catfish, even though the gross presentation of the disease is similar. Although the biological significance of this finding is unclear, it suggests that *H. ictaluri* does not complete its development as efficiently in hybrid catfish as it does in channel catfish. The presence of *H. ictaluri* DNA in the blood of one blue catfish from the most heavily infested pond (pond 1 in trial 1) supports previous reports of a high degree of natural resistance in blue catfish (Bosworth et al. 2003). However, the findings from this study indicate that the parasite can still overwhelm the host’s defenses and gain limited entry when actinospores are present in large numbers (although in a more resistant host the infection may be eliminated before myxospore development is complete).

Histologically, the progression of lesion development among the three ponds and their respective groups of catfish was qualitatively similar throughout trial 1, although the time course and severity of lesions
varied among the individual ponds. This is attributable to differences in infective actinospore levels among the ponds, which resulted in different challenge doses among the three treatment units. Despite this, lesions typical of severe PGD and plasmodial development occurred in both channel and hybrid catfish in all ponds over time. In contrast, blue catfish were significantly less affected in all ponds. Although PGD lesions were present in some fish, they were encountered far less frequently and their overall impact was minimal to mild. Most significantly, developing *H. ictaluri* plasmodia were not visualized in blue catfish from any trial despite their being numerous in the gills of channel and hybrid catfish from the same exposure group.

Although blue catfish do not appear to be entirely resistant to *H. ictaluri* infection, the findings of this study support previous work indicating the impacts of the disease are significantly less in that species than in channel catfish and channel × blue catfish hybrids. Furthermore, the observed lack of developing organisms in blue catfish tissue sections, minor gill damage, and apparent clearance of the organism 14 d after removal from the source of infection suggest that the later life stages observed in channel and hybrid catfish do not occur in blue catfish or do so only rarely. In summary, *H. ictaluri* appears to possess the ability to gain entry into blue catfish and reach its target gill tissue when there are a large number of infective actinospores, but this is followed by rapid elimination of the organism before the development of plasmodia.

The resistance of blue catfish to a number of important pathogens that affect channel catfish has led to a reexamination of its potential for aquaculture purposes. Proliferative gill disease is the most significant parasitic infection affecting channel catfish culture and represents a major impediment to the profitability of commercial pond operations. This study expands our understanding of a favorable characteristic of blue catfish, namely, its lower susceptibility to *H. ictaluri* infection. Further research is needed to identify the specific mechanisms that limit the susceptibility of the blue catfish, as this information could prove invaluable in a selective breeding program directed at increasing resistance to PGD. The inability of *H. ictaluri* to progress and complete its development in blue catfish also suggests that this species could be used to interrupt the life cycle of the parasite through crop rotations, effectively preventing the accumulation of *H. ictaluri* in channel catfish pond operations.

**Acknowledgments**

The authors thank Shelley Meador, Dax Hinton, Cynthia Doffitt, Marlena Yost, Angela Brandon, and Esteban Soto (Mississippi State University, College of Veterinary Medicine) for technical assistance in sample collection and preparation and Debbie Boykin (U.S. Department of Agriculture, Agricultural Research Service) for assistance with the statistical analysis. This research was supported by the Mississippi State University College of Veterinary Medicine and the Mississippi Agricultural and Forestry Experiment Station (MAFES). This is MAFES publication PS-11540.

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