The Effects of Proliferative Gill Disease on the Blood Physiology of Channel Catfish, Blue Catfish, and Channel Catfish × Blue Catfish Hybrid Fingerlings

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Abstract.—This study was conducted to gain a better understanding of the effect of exposure to the myxozoan Henneguya ictaluri (the agent of proliferative gill disease [PGD]) on host physiology by measuring the variation in selected blood characteristics in three differently affected host taxa (channel catfish Ictalurus punctatus, blue catfish I. furcatus, and blue catfish × channel catfish hybrid fingerlings). Forty-five fish of each host taxon were exposed to PGD, and 10 fish of each host taxon were sampled at 24, 96, and 168 h. Fish were weighed, blood was collected and analyzed for a suite of physiological variables, and wet mount preparations of gill clips were examined grossly for the presence of cartilage breaks. The results of this study are consistent with the current knowledge regarding H. ictaluri infections in blue catfish, channel catfish, and blue catfish × channel catfish hybrids. Chondrocytic lysis was observed in channel catfish and hybrid catfish at 96 and 168 h but was not observed in blue catfish. There was an observed reduction in oxygen partial pressure (pO₂) and an increase in carbon dioxide partial pressure (pCO₂) at 96 h in the blood of channel catfish and hybrid catfish, but these changes were not exhibited by blue catfish. For all species, pH decreased as lactate concentrations increased. The lack of physiological changes and the absence of H. ictaluri sporozoites and DNA in gill tissue lead us to speculate that H. ictaluri is unable to establish infection in blue catfish. Current research investigating the mechanisms of infection and portals of parasite entry into blue catfish, channel catfish, and blue catfish × channel catfish hybrids is underway to better elucidate the defenses employed by blue catfish against H. ictaluri.

From 1997 to 2005, proliferative gill disease (PGD) made up nearly 20% of the diseases diagnosed by the Aquatic Diagnostic Laboratory at the Thad Cochran National Warmwater Aquaculture Center (TCNWAC), Stoneville, Mississippi. Proliferative gill disease, or “hamburger gill,” has caused major economic losses in commercial production of channel catfish Ictalurus punctatus since 1981 and is one of the most devastating parasites affecting catfish aquaculture today (Bowser et al. 1985; Camus et al. 2006).

Proliferative gill disease is the result of infection by the actinospore stage of the myxozoan parasite

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Henneguya ictaluri (Bowser et al. 1985; MacMillan et al. 1989; Styer et al. 1991; Pote and Waterstrat 1993; Pote et al. 2000; Whitaker et al. 2005). Because this disease is characterized grossly by swelling, hemorrhage, inflammation, and motting of the gill filaments, PGD can be easily recognized pondside in even moderate infections. Infection and proliferation of the parasite in gill tissue lead to chondrocytic lysis, observed as breaks in the filamental cartilage, and a strong correlation exists between the percentage of broken gill filaments and pond mortalities (Bowser et al. 1985; MacMillan et al. 1989; Mitchell et al. 1998; Wise et al. 1999, 2004; Bosworth et al. 2003). Hypertrophy and hyperplasia of the gill epithelium along with an influx of inflammatory cells associated with the developing sporoplasm significantly reduce the gill surface area, inhibiting the catfish’s ability to carry out gas exchange and osmoregulation (Bowser et al. 1985; MacMillan et al. 1989; Mitchell et al. 1998). Infected fish exhibit behavior similar to that of fish affected by oxygen stress; such fish congregate behind aerators and swim listlessly near the surface and along pond banks.

Outbreaks of PGD are highly unpredictable, and mortality rates can approach 100% in severe outbreaks. The disease can cause significant losses in all sizes of fish, even when dissolved oxygen levels are kept near saturation. Currently, there are no therapeutic or prophylactic treatments for PGD, and biosecurity methods have been unsuccessful (Mischke et al. 2001; Tucker et al. 2003).

Although myxozoans have been found in numerous species of fish, they tend to be relatively host and site specific (Lom 1987). Blue catfish I. furcatus and channel catfish × blue catfish hybrids exhibit less gill damage than do channel catfish when held in ponds experiencing an ongoing PGD outbreak (Bosworth et al. 2003; Griffin 2008). Blue catfish are also resistant to other diseases that affect channel catfish, such as enteric septicemia of catfish and channel catfish virus (Giudice 1966; Graham 1999). The objective of this study was to gain a better understanding of the effect of H. ictaluri exposure on host physiology by measuring the variation in selected blood characteristics in three differently affected host taxa.

**Methods**

As part of a larger study (Griffin 2008), specific-pathogen-free channel catfish, blue catfish, and blue catfish × channel catfish backcross hybrid fingerlings were exposed to a pond containing an ongoing subclinical infection of PGD as diagnosed by the TCNWAC Fish Diagnostic Laboratory. Exposure methods consisted of confining fish (n = 45) in three replicate cages per host taxon. Fish (n = 10) from each host taxon were sampled at 24, 96, and 168 h. Upon sampling, fish were weighed, blood was collected, and wet mount preparations of gill clips were examined for the presence of cartilage breaks. The remaining fish (n = 15 fish/host taxon) were sampled at 168 h and were analyzed molecularly and histologically for H. ictaluri infection.

Quantitative evaluation of the infection was determined by calculating the percent of primary gill lamellae containing lytic lesions in the cartilage of approximately 40–80 gill filaments from each fish (Wise et al. 2004; Griffin 2008). Gill tissues designated for histological examination were processed routinely by dehydration in a graded series of ethanol solutions of increasing strength, followed by clearing in a series of xylenes, embedding in paraffin, sectioning at 6-μm thickness, and staining with hematoxylin and eosin (Griffin 2008). Histological confirmation of PGD infection was based on the presence of parasitic cells in combination with the characteristic histopathological changes associated with the disease (Griffin 2008).

Molecular confirmation of H. ictaluri infection was carried out according to the protocol described by Whitaker et al. (2005) and modified by Griffin (2008). Gill filaments (40–80 per fish) were homogenized in 600 μL of cell lysis solution from the Puregene DNA Isolation Kit (Genta Systems, Inc., Minneapolis, Minnesota) and were incubated for 10 min at 95°C. After lysis, 3 μL of proteinase K (20 mg/mL; enzyme code 3.4.21.64; IUBMB 1992) were added to the homogenate, mixed by inversion, and incubated at 55°C until all tissue homogenate had dissolved. The remainder of the isolation was carried out according to the manufacturer’s suggested protocol. The purified genomic DNA was resuspended in 50 μL of Puregene DNA hydration solution and quantified using a NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, Delaware); the DNA concentration (host and parasite) was then adjusted to 10 ng/μL by use of Puregene DNA hydration solution (Griffin 2008).

Using established H. ictaluri-specific primers (H. ictaluri-1: 5′-CAAAAGTTTCTGCTATCATTG-3′; H. ictaluri-2: 5′-TGAGGTAATCTGTGCGCT-3′) and thermal cycling protocols, polymerase chain reaction (PCR) amplification was performed (Hanson et al. 2001; Whitaker et al. 2001, 2005; Griffin 2008). The 25-μL PCR reaction mixture contained 2.5 μL of TaKaRa Taq Hot Start Version 10× PCR buffer (TaKaRa Bio, Inc., Otsu, Shiga, Japan), 2 μL of 25-mM deoxynucleotide triphosphates, 10 pmol of each primer (H. ictaluri-1 and H. ictaluri-2), 0.625 units of TaKaRa Taq Hot Start Taq polymerase (enzyme code
2.7.7.7), 13.75 μL of nuclease-free H₂O, and 2 μL of DNA template (~20 ng). The reaction mixture was cycled on an MJ Research PTC-100 thermal cycler as follows: (1) an initial denaturation step of 94°C for 10 min; (2) 35 cycles of 92°C for 1 min, 50°C for 15 s, and 72°C for 15 s; and (3) a final extension step of 72°C for 5 min. The PCR products were electrophoresed on a 1.5% agarose gel and were stained with Gelstar nucleic acid stain (Cambrex, East Rutherford, New Jersey) to confirm the presence of DNA product.

To obtain blood, each fish was quickly removed from the pond, transported to the laboratory, and anesthetized with tricaine methanesulfonate (MS-222) at a concentration of 0.2 g/L of water. Blood was then collected via the caudal artery with a heparinized syringe. Blood samples were placed on ice and analyzed within 30 min using a Stat Profile Critical Care Xpress blood gas analyzer (Nova Biomedical). Blood samples were analyzed for hematocrit, pH, oxygen partial pressure (pO₂), carbon dioxide partial pressure (pCO₂), lactate, and calcium.

Statistical analysis.—An analysis of variance (ANOVA) was used to detect differences in the percentage of gill breaks in channel catfish and hybrid catfish at 96 and 168 h. No statistical test was conducted on the percentage of gill breaks in blue catfish because no gill breaks were present. An ANOVA was used to detect differences in blood physiological variables between the different sampling times and different host taxa. Data were analyzed with the Statistical Analysis System version 9.1 (SAS Institute, Inc., Cary, North Carolina), and significance testing was conducted at the 0.05 probability level.

Results

Mean (±SE) weights of channel catfish, blue catfish, and hybrid catfish used in this study were 54.97 ± 2.84, 18.52 ± 1.34, and 43.98 ± 3.70 g, respectively. Chondrocytic lysis was observed in channel catfish and hybrid catfish at 96 and 168 h but was not observed in blue catfish. The remaining fish (n = 15 fish/host taxon) were examined molecularly and histologically, and all hybrids and channel catfish were positive for *H. ictaluri* infection. Three channel catfish died during the 96- and 168-h sampling periods, but no mortalities occurred among the blue catfish or the hybrids. None of the blue catfish were positive for *H. ictaluri* infection when examined at the molecular, histological, and gross levels (Figure 1).

The percentage of gill breaks was significantly higher in the hybrid catfish than in channel catfish (P < 0.0001), but there were no differences between 96 h (14.87 ± 1.6% for channel catfish, 22.08 ± 3.3% for hybrid catfish) and 168 h (9.38 ± 2.5% for channel catfish, 22.61 ± 3.9% for hybrid catfish; P = 0.40) for the individual host taxa and there was no interaction between host taxon and sampling time (P = 0.34). No statistical comparisons were done on blue catfish because no gill breaks were present at any time during sampling; no gill breaks were present in any host taxon at 24 h.

The values of blood gases (pO₂; Table 1; pCO₂; Table 2) were significantly different between host taxa (pO₂; P = 0.0055; pCO₂; P = 0.0006) and sampling times (pO₂; P = 0.01; pCO₂; P < 0.0001). The host taxon × sampling time interaction was not significant for pO₂ (P = 0.13) but was significant for pCO₂ (P < 0.0001). There was an observed reduction in pO₂ at 96 h in the channel catfish and hybrid catfish, but this was not apparent in blue catfish. There was a corresponding increase in pCO₂ at 96 h. The 24- and 168-h values were similar for both pO₂ and pCO₂.

Hematocrit values were significantly different among the host taxa (P < 0.0001); results for blue catfish were significantly different from those for channel catfish and hybrid catfish. Hematocrit values were also significantly different between the sampling times (P < 0.0001), but no difference was observed between the control and 24 h. A significant host taxon × sampling time interaction was evident (P < 0.0001). Hematocrit (mean percent ± SE) remained constant for blue catfish across sampling times (24 h: 16.45 ± 0.4; 96 h: 16.57 ± 0.9; 168 h: 17.68 ± 0.6). Hematocrit values for channel catfish decreased over time (24 h: 25.87 ± 0.6; 96 h: 17.83 ± 0.1; 168 h: 17 ± 1.1). In hybrid catfish, hematocrit showed a decrease at 96 h (16.62 ± 0.8), but hematocrit at 168 h (22.82 ± 0.9) was similar to the level detected at 24 h (21.85 ± 0.9).

For all species, pH decreased as lactate concentrations increased. Hybrid catfish had the lowest pH (mean ± SE) at all sampling times (24 h: 7.18 ± 0.01; 96 h: 7.06 ± 0.01; 168 h: 7.02 ± 0.03) compared with channel catfish (24 h: 7.19 ± 0.02; 96 h: 7.21 ± 0.02; 168 h: 7.14 ± 0.01) and blue catfish (24 h: 7.26 ± 0.01; 96 h: 7.15 ± 0.01; 168 h: 7.18 ± 0.02). The pH was significantly different between the host taxa (P < 0.0001), with pH values for hybrid catfish being significantly different from those for channel catfish and blue catfish. The pH was also significantly different between the sampling times (P < 0.0001); no difference was observed between 96- and 168-h values, but a significant host taxon × sampling time interaction was observed (P < 0.0001). Lactate concentrations (mean mmol/L ± SE) increased over time in both channel catfish and hybrid catfish; concentrations for channel catfish were 4.75 ± 0.3 at 24 h, 6.42 ± 0.5 at 96 h, and 6.14 ± 0.3 at 168 h. For hybrid catfish, lactate was 4.63 ± 0.3 at 24 h, 6.29 ± 0.3 at 96 h, and 5.73 ± 0.5 at 168 h. For blue catfish,
**FIGURE 1.**—A 1.5% agarose gel of polymerase chain reaction products amplified from gills of fish held for 168 h in a pond containing an ongoing infection of proliferative gill disease. Lanes 1, 18, and 35 are negative controls; lanes 2, 19, and 36 are *Henneguya ictaluri* positive controls; lanes 3–17 are gill samples from blue catfish; lanes 20–34 are gill samples from blue catfish × channel catfish hybrids; and lanes 37–48 are gill samples from channel catfish. The marker is a 100-base-pair molecular weight standard.

**TABLE 1.**—Mean (±SE) oxygen partial pressure percentage (pO$_2$; mm Hg) in three catfish taxa (channel catfish, blue catfish, and channel catfish × blue catfish hybrids) at three different sampling times after infection with *Henneguya ictaluri*. Within a row, values followed by different letters are significantly different among taxa (ANOVA: $P < 0.05$).

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Channel catfish</th>
<th>Blue catfish</th>
<th>Hybrid catfish</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>22.54 ± 2.6 z</td>
<td>36.75 ± 3.9 y</td>
<td>35.2 ± 4.6 y</td>
</tr>
<tr>
<td>96</td>
<td>17.49 ± 1.6 z</td>
<td>34.94 ± 4.5 y</td>
<td>19.88 ± 2.3 z</td>
</tr>
<tr>
<td>168</td>
<td>30.5 ± 3.4 z</td>
<td>33.52 ± 4.1 z</td>
<td>44.59 ± 12.3 y</td>
</tr>
</tbody>
</table>

**TABLE 2.**—Mean (±SE) carbon dioxide partial pressure percentage (pCO$_2$; mm Hg) in three catfish taxa (channel catfish, blue catfish, and channel catfish × blue catfish hybrids) at three different sampling times after infection with *Henneguya ictaluri*. Within a row, values followed by different letters are significantly different among taxa (ANOVA: $P < 0.05$).

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Channel catfish</th>
<th>Blue catfish</th>
<th>Hybrid catfish</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>12.9 ± 0.4 z</td>
<td>11.44 ± 0.3 z</td>
<td>12.99 ± 0.3 z</td>
</tr>
<tr>
<td>96</td>
<td>14.13 ± 0.5 y</td>
<td>17.03 ± 0.4 z</td>
<td>18.25 ± 0.4 z</td>
</tr>
<tr>
<td>168</td>
<td>16.34 ± 0.5 z</td>
<td>12.75 ± 0.5 y</td>
<td>15.84 ± 0.9 z</td>
</tr>
</tbody>
</table>
lactate concentration increased slightly at 96 h \((4.17 \pm 0.2)\) but decreased at 168 h \((3.13 \pm 0.3)\) to a level similar to the 24-h concentration \((3.21 \pm 0.2)\). Lactate concentrations were found to be significantly different between the host taxa \((P < 0.0001)\), with concentrations for blue catfish being significantly different from those for channel catfish and hybrid catfish. Lactate concentrations were also significantly different between the sampling times \((P < 0.0001)\), but no difference was observed between 96 and 168 h; the host taxon \(\times\) sampling time interaction was not significant \((P < 0.2022)\).

Calcium concentration did not vary significantly between host taxa \((P = 0.26)\) or sampling times \((P = 0.08)\), but there was a significant host taxon \(\times\) sampling time interaction \((P = 0.0004)\). Calcium levels (mean mmol/L \(\pm\) SE) were 1.18 \(\pm\) 0.03 at 24 h, 1.13 \(\pm\) 0.03 at 96 h, 1.16 \(\pm\) 0.02 at 168 h for channel catfish; 0.99 \(\pm\) 0.03 at 24 h, 1.26 \(\pm\) 0.02 at 96 h, and 1.08 \(\pm\) 0.05 at 168 h for blue catfish; and 1.17 \(\pm\) 0.02 at 24 h, 1.15 \(\pm\) 0.04 at 96 h, and 1.15 \(\pm\) 0.05 at 168 h for hybrid catfish.

**Discussion**

In a previous study of channel catfish in the Mississippi Delta (Leard et al. 1998), hematocrit, calcium, and pH levels of healthy channel catfish sampled in May and June were comparable to the initial levels found in this study. The results of this study support the previous findings of Bosworth et al. (2003), who demonstrated that susceptible families of hybrid catfish had significantly greater gill damage than did susceptible families of channel catfish and blue catfish. The lack of physiological changes in blue catfish and the absence of *H. ictaluri* trophozoites and DNA in blue catfish gill tissue suggest that *H. ictaluri* is unable to establish infection in this species. Current research investigating the infection mechanisms and portals of parasite entry into blue catfish, channel catfish, and blue catfish \(\times\) channel catfish hybrids is underway to better elucidate the defenses employed by blue catfish against *H. ictaluri* infection.

Observed changes in the physiological variables in this study support previous findings that gill damage in response to *H. ictaluri* infection is more severe in channel catfish and blue catfish \(\times\) channel catfish hybrids than in blue catfish (Bosworth et al. 2003). The severe inflammation induced by penetration and proliferation of the organism in gill tissue severely limited the ability of the gills to carry out gas exchange. Inhibition of osmoregulation resulted in a decrease in \(pO_2\) and \(pH\) and, in turn, a subsequent increase in \(pCO_2\) and lactate formation as the catfish underwent oxygen stress. Dissolved oxygen levels were monitored throughout the study and never reached levels that would induce oxygen stress in healthy fish. Changes in hematocrit values observed in channel catfish and blue catfish \(\times\) channel catfish hybrids can be attributed to the severe hemorrhaging that occurred in the gills. This significant loss of blood prevents oxygen from circulating throughout the body of the fish, further exacerbating the problem of getting sufficient oxygen to the tissues within the fish. Hematocrit values in the blue catfish \(\times\) channel catfish hybrids seemed to recover as the catfish responded to blood loss, but there are not enough data to confirm this.

Calcium concentrations did not vary significantly across sampling times or host taxa. Previous research on different fish taxa (hybrid striped bass *Morone saxatilis*, hybrid tilapia *Oreochromis* spp., channel catfish, and rainbow trout *Oncorhynchus mykiss*) under acute stress showed that while the initial levels of calcium were not always the same among these taxa, calcium levels of fish exposed to stressors did not vary significantly among taxa (Hrubec and Smith 1999). This lack of variance leads us to believe that gas exchange is a much more significant factor than osmoregulation in PGD-infected catfish and that the actual cause of death is suffocation rather than an inability to maintain osmotic balance.

The significant damage incurred in the gill tissues of channel catfish and hybrid catfish appears to inhibit gas exchange but does not appear to affect osmoregulatory ability. This suggests the catfish are able to compensate for reduced osmoregulatory capacity, but respiratory function is still inhibited. At this time, the mechanisms employed for this compensation are unknown.

Similar effects on blood gas have been demonstrated for rainbow trout infected with *Flavobacterium branchiophilum*, the causative agent of bacterial gill disease in salmonids. Byrne et al. (1995) demonstrated that rainbow trout experimentally infected with *F. branchiophilum* and fed a normal diet demonstrated significant hypoxia in comparison with similarly infected fish given a restricted diet, suggesting that decreased blood oxygen due to reduced gas exchange can be exacerbated by increased oxygen demands brought on by feeding. This may indicate that similar conditions occur in catfish suffering severe PGD. Therefore, to alleviate potential losses attributed to PGD, dissolved oxygen should be maintained near saturation, feed should be restricted to reduce oxygen demand, and a minimum chloride level of 100 mg/L should be maintained to decrease osmoregulatory demand in oxygen-stressed fish (Wise et al. 2004).

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