Investigation on changing pattern of health status of juvenile *Hypophthalmichthys molitrix* from farming systems in Bangladesh

Kamal Gosh¹ • Gias Uddin Ahmed¹ • Mst Nahid Akter²

¹ Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
² Department of Aquaculture, Faculty of Fisheries, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh

Abstract
An experiment was carried out to investigate the health status of *Hypophthalmichthys molitrix* among three different fish farms at Mymensingh district of Bangladesh. Fish sampling and water quality parameters were collected on a monthly basis. Samples of skin, muscle, gills, liver and kidney were examined through histological technique. Results from water quality parameters suggested that water temperature had a distinct effect on fish health of *H. molitrix*, which was mostly evident during the colder months. Infectious symptoms were usually evident in July to October and the severity of pathology was increased in November and December. These were evident from their clinical signs that include scale loss, haemorrhagic lesion, and reddish spots or from their histopathological symptoms such as necrosis, pyknosis, inflammation, haemorrhage, hypertrophy, missing of gill lamellae, vacuums, tubular and pillar cells degeneration, inflammation, clubbing, and suspected bacterial colonies. Results suggest that the health condition of the fishes from BAU fish farm was the best over the other two farms, whereas Government fish farm followed by NGO fish farm were severely affected with EUS, protozoan and bacterial infection. Moreover, internal organs like liver and kidney were more affected than skin, muscle and gills.

Keywords: Health status; *Hypophthalmichthys molitrix*; fish farming; disease

INTRODUCTION
Inland water resources of Bangladesh are considered to be one of the richest in the world and potential for fisheries development (Islam 1989). At present, not only the major carps but also exotic fishes are commonly cultured in our inland aquaculture. Introduction of such exotic fishes, and their culture with the indigenous species have often produced significant socio-economic benefits to fish farming communities (Arthur and Subasinghe 2002). However, the production trend of these exotic fishes has, to some extent, declined due to several reasons, where disease is playing, probably the major role (Duijn 1973). Review study suggests that disease is a major problem in the aquaculture and fisheries sector of Bangladesh (Rahman and Chowdhury 1996). This may include Epizootic Ulcerative Syndrome (EUS), tail and fin rot, fungal, parasitic and bacterial infections (Chowdhury et al. 1999). Several species like *Myxobolus pavlovskii* (Lucky 1978; El-Matbouli and Hoffmann 1991); and *Trichodinia* sp. (Bauer et al. 1973) are also prevalent, primarily in high-density culture situations of *H. molitrix*. Hemorrhages, septicemia, lesions, gill damage are the common symptoms of such
fish disease outbreak (Chowdhury 1993, 1998). The causative agent of these diseases may include several factors that are parasites/pathogens, malnutrition, physical and chemical changes of water quality, rate of water flow in hatchery, pollution, pesticide, dirty living condition, weakness or damage caused by other diseases (Duijn 1973). In general, fish lives in such an environment, which is loaded with innumerable agents like chemical pollutants, bacteria, virus, parasites and fungus etc., which are either individually or in combination can inflict the body tissue or system that can produce disease (Post 1987). This can incur huge economic loss to the fish farmer, who adopts *H. molitrix* in their polyculture system. Hence, it is important to investigate the disease scenarios of *H. molitrix* so that the probable fish loss can be prevented ahead of time. Based on that ground, this research was conducted in three different farming scenarios in Bangladesh by using the clinical and histopathological techniques. It is expected that this research will explore the pathology and occurrence of fish diseases in *H. molitrix* in relation to season and environment, which may likely to use further to prevent the probable disease problem in Bangladesh.

**METHODOLOGY**

The present experiment was conducted on *H. molitrix* cultured in three different fish farming scenarios (randomly selected) in Mymensingh district of Bangladesh for a period of 9 months (April 2005 to December 2005). These three fish farms were Bangladesh Agricultural University (BAU) fish farm, located at southern side of the Faculty of Fisheries, BAU, Mymensingh (24°38′3″N and 90°16′4″E); the Government fish farm; and NGO fish farm. Last two fish farms were located at Phulpur upazilla (24.9500°N and 90.3500°E) in Mymensingh district. Three replicate ponds from each of the aforementioned farm were selected for the present experiment. Each pond was prepared by following the standard procedure of semi-intensive carp polyculture system of Bangladesh. Stocking density was maintained at the following rate in these replicated fish ponds (Table 1).

Sampling was carried out on a monthly basis by collecting 6 individuals of *H. molitrix* per farm with using seine net. Fish samples were then transported to the Fish Disease Laboratory of the Faculty of Fisheries with plastic bags. Water quality parameters like pH, dissolved oxygen, temperature, total hardness, conductivity and total dissolve solids were estimated during each sampling period between 0800 to 0900 hours. Standard procedures and methods were followed by using HACH’s kit (Model FF-1A).

The sampled fishes were examined just after taking out from the container to observe external symptoms with recording any injury and infectious conditions of fish body. The following clinical signs were usually evident in investigated fish that include; AN = almost normal (i.e., no evidence of external injury or infection); AH = apparently healthy (i.e., very few evidence of external injury or infection), B = bright (i.e., shiny eye and body color); DV= dorsoventral; H = haemorrhage (i.e., an escape of blood from a ruptured blood vessel, especially when profuse); sl= skin loss; RS = rough skin; SL = scale loss; V = ventral; WB = weak body (i.e., evidence of external injury or infection or apparently looks thin); L = lateral; Rsp = reddish spot (i.e., reddish spot were evidenced due to hemorrhage or other infectious agents or so).

**Table 1: Stocking density of three different farming scenarios used in BAU, government and NGO fish farms in Bangladesh**

<table>
<thead>
<tr>
<th>Fish</th>
<th>Stocking density (No./decimal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BAU</td>
</tr>
<tr>
<td><em>Labeo rohita</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Catla catla</em></td>
<td>4</td>
</tr>
<tr>
<td><em>Cirrhinus cirrhosus</em></td>
<td>6</td>
</tr>
<tr>
<td><em>Hypophthalmichthys molitrix</em></td>
<td>12</td>
</tr>
<tr>
<td><em>Ctenopharyngodon idella</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Puntius gonionotus</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Cyprinus carpio</em></td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>40</strong></td>
</tr>
</tbody>
</table>

For histological analysis, various organs such as skin and muscle, gills, liver and kidney were collected by a sharp scalpel and forceps, which were preserved in 10% neutral buffered formalin. Then the samples were placed in an automatic tissue processor for dehydration, clearing and infiltration (SHANDON, CITADEL 1000). The samples were then embedded, sectioned (5 µm) and stained with Haematoxylin and Eosin. Then the sections were mounted with Canada balsam and covered by a cover slip and examined under a compound microscope (OLYMPUS). Photomicrographs were then taken by using a photomicroscope (OLYMPUS, Model CHS, Japan).

**RESULTS**

The range of the recorded water temperature in all the investigated ponds of the three fish farms was in the range of 27.16 to 27.65 °C. This data was matched with previous study of water temperature (24-31 °C) on *H. molitrix* that was reported for maximum growth by Mahboob and Sheri (1997). Results from the clinical observations of fishes (in all three fish farms) suggested that *H. molitrix* was appeared comparatively bright, healthy and normal during April to June.
Conductivity (µs) 210.36±71.97
pH 7.21±0.20 7.14 ± 0.22 7.05±0.32

fishes of BAU fish farm, which was followed by NGO fish
Among the organs, gills were also found affected in the
during November and December (Figure 2b). Islam et al.
haemorrhage, and missing of gill lamellae (Figures 2a and
2c). But the intensity of gill pathology was remarkably
higher in Government fish farm that caused hypertrophy,
necrosis, clubbing, haemorrhage and pillar cells disruption
around the primary and secondary gill lamellae, mainly
during November and December (Figure 2b). Islam et al.
(1999) found that gills of local and exotic carp species
were severely affected during colder months (i.e.,
December and January) in compare to other months.
Almost similar gill pathology was also found by Ahmed

Table 2: Monthly variations of water quality parameters
collected from the replicated ponds (N=81)

<table>
<thead>
<tr>
<th>Water quality parameters</th>
<th>Mean±SE BAU</th>
<th>Govt.</th>
<th>NGO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>27.44±2.40</td>
<td>27.16±1.62</td>
<td>27.65±2.28</td>
</tr>
<tr>
<td>Dissolved oxygen (ppm)</td>
<td>5.36±0.23</td>
<td>5.02±0.27</td>
<td>5.17±0.38</td>
</tr>
<tr>
<td>pH</td>
<td>7.21±0.20</td>
<td>7.14 ± 0.22</td>
<td>7.05±0.32</td>
</tr>
<tr>
<td>Hardness (mg/l²)</td>
<td>87.62±9.40</td>
<td>91.93±13.24</td>
<td>92.00±8.19</td>
</tr>
<tr>
<td>Conductivity (µs)</td>
<td>210.36±71.97</td>
<td>216.22±42.19</td>
<td>220.33±87.27</td>
</tr>
<tr>
<td>Total dissolved solids (ppm)</td>
<td>176.00±32.24</td>
<td>185.66±27.85</td>
<td>171.11±19.32</td>
</tr>
</tbody>
</table>

But these scenarios were slightly changed in July to
October by showing minor pathological symptoms that
included rough skin, scale loss, ill health and reddish
spots. In November, fishes from BAU fish farm showed
weak body, scale loss and fin erosion, while slight scale
losses with haemorrhagic lesions were observed around
the dorsoventral regions in December. Almost similar
kinds of symptoms were observed in the fishes of NGO
fish farm. But fishes from Government fish farm showed
different minor pathological symptoms such as weak
body, scale and skin losses with reddish spot in lateral
region and rough skin (Table 3). Ahmed and Hoque (1999)
mentioned that clinical symptoms like grey white necrotic
areas were increased in December, January and February
in various carps of Bangladesh. Similar symptoms have
also been reported previously (Hoque 1998; Islam 1999;
Moniruzzaman 2000; Akter et al. 2006; Roy et al. 2006).

From the histopathological point of view, skin and muscle
(in all three fish farms) showed zero meaningful changes
during April to October. But significant pathological
symptoms were evidenced during November and
December by showing epidermal loss with a sloughed off
condition. Moreover, ruptured and missing myotomes
were evidenced in this period that resulted vacuolation
on the fish body (Figure 1a).

Similar symptoms were also prevailed in fishes of Govt.
and NGO fish farms in association with fungal granuloma
in the affected muscle (Figures 1b and 1c). Noga and
Dykstra (1986) were also observed marked granulomas
with inflammatory response in the investigated carps
infected with Aphanomyces spp. Hoque et al. (1999)
reported quite similar large deep and whitish ulcers in
most of the EUS affected fish. These results were also
agreed by Ahmed and Hoque (1999), Moniruzzaman
(2000), Hilaire et al. (2005) and Roy et al. (2006).

Table 3: Monthly variation of clinical signs in different fish farms in Hypophthalmichthys molitrix

<table>
<thead>
<tr>
<th>Fish Farms</th>
<th>Months</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAU</td>
<td>AN &amp; B</td>
<td>AN &amp; B</td>
<td>AN &amp; AH</td>
<td>AN &amp; AH</td>
<td>RS</td>
<td>SL &amp; ill health</td>
<td>WB, SL &amp; fin erosion</td>
<td>H in DV region &amp; SL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Govt</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
<td>RS</td>
<td>AH &amp; SL</td>
<td>AH, RS &amp; SL</td>
<td>WB &amp; RS</td>
<td>SL &amp; sl with reddish spot in L region</td>
<td></td>
</tr>
<tr>
<td>NGO</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
<td>SL &amp; sl</td>
<td>Red spot in V</td>
<td>AH &amp; Rsp in DV</td>
<td>SL &amp; RS</td>
<td>SL &amp; bright reddish spot in DV region</td>
<td></td>
</tr>
</tbody>
</table>

AN, almost normal; AH, apparently healthy; B, bright; DV, dorsoventral; H, haemorrhage; sl, skin loss; RS, rough skin; SL, scale loss; V, ventral; WB, weak body; L, lateral; Rsp, reddish spot.

Among the organs, gills were also found affected in
the experimental fishes of Government and NGO fish
farms having massive necrosis, haemorrhage and
vacuoles in hepatocytes (Figures 3b and 3c), mostly
during November and December. But the fishes from BAU
fish farm showed less pathological symptoms by
exhibiting minor vacuums (Figure 3a). Ram and Singh
(1988) also made similar opinion that liver exhibited
varying degree of pathological changes. Affii (1996) also
reported such vascular changes in the liver that was
probably due to the endothelial lining of the blood vessels
and post-vascular cuffing. Similar results were also found
by Rodgers and Drinan (1993). Thus, it could be
mentioned that liver of *H. molitrix* may likely to show
pathological changes when the disease outbreak
occurred.

Internal organ such as liver was also severely affected in
Figure 1: Cross section of skin and muscle of fish from BAU (a), Govt. (b) and NGO (c) fish farms during Nov-Dec. Arrow indicates loss of epidermal partially in (a), epidermal in (b) and epidermis in (c). r, myotomes; v, vacuoles; fg, fungal granuloma. H & E x 125.

Figure 2: Cross section of gill of fish from BAU (a), Govt. (b) and NGO (c) fish farms during Nov-Dec. In (a) secondary gill lamellae were hypertrophied (hp), necrotized (n), hemorrhaged (h), and vacuoles (v) were seen. In (b) secondary gill lamellae were greatly hypertrophied (hp), hemorrhaged (h), lost (arrow) and clubbed (cb). In (c) gill lamellae were greatly hypertrophied (hp), hemorrhaged (h), largely missed from origin. H & E x 420.

Figure 3: Cross section of liver of fish from BAU (a), Govt. (b) and NGO (c) fish farms during Nov-Dec. In (a) small vacuoles (v) had in hepatocytes. In (b) marked disruption in hepatocytes having large vacuoles (v), haemorrhage (h) and necrosis (n). In (c) hepatocytes had haemorrhage (h) and some vacuoles (v). H & E x 420.

Figure 4: Cross section of kidney of fish from BAU (a), Govt. (b) and NGO (c) fish farms during Nov-Dec. In (a) melanomacrophage (mm) and mild vacuoles (v) were seen. In (b) haemorrhage (h), vacuoles (v), pyknotic cell (pc) and bacterial colonies (bc) having tubular degeneration (td). In (c) haemorrhage (h), vacuoles (v), and pyknotic cells (pc) with suspected bacterial colonies (bc). H & E x 420.
Histopathological study of kidney tissue of *H. molitrix* from the fishes of Government and NGO fish farms showed different renal pathological changes like haemorrhage, pyknotic cells, and vacuoles with the presence of suspected bacterial colonies. These colonies occupied the surroundings of kidney tubules, especially prevalent during November and December (Figures 4b and 4c). Mi et al. (1993) reported that septicemia, a common symptom of bacterial infection on silver carp, was a process of acute hemorrhagic inflammation accompanied with functional disorder in the heart, kidney and brain. This was evident in He et al. (1992) research, where the authors reported and isolated more than 10 strains of pathogenic bacteria from the silver carp in China. Similar opinion was also reported for silver carp as it is susceptible to many diseases caused by parasitic protozoan (Ribelin and Migaki 1975). Histopathological changes of the liver, kidney, spleen and intestine for the twist disease of *H. molitrix* parasitized by *M. drjagini* was also described by Yu and Wu (1992). It was also agreed by Chowdhury (1998) and Moniruzzaman (2000). In contrast, the kidney sample from the fishes of BAU fish farm was found almost in normal structure by showing few melanomacrophage and vacuum space formation (Figure 4a).

Overall, it could be inferred that fish kidney can be affected by disease that may likely to include suspected bacterial colonies together with pyknotic cells.

In a nutshell, it can be mentioned that fishes from BAU fish farm had shown less pathological changes in compare to the fishes from the Government and NGO fish farms (Table 4). Stocking lower amount of fish in the BAU fish farm could be one of the reasons that might reduce the disease problem in the fishes. Moreover, clinically and histopathologically, internal organs like fish liver and kidney of all the fish farms found affected in colder months (Tables 3 and 4). Ahmed and Hoque (1999) reported that histopathologically, the internal organs like kidney and liver were more affected in winter. Similar pathological symptoms of kidney in major carp species were also observed by Islam (1999), Akter et al. (2006) and Roy et al. (2006). Thus, it could be mentioned that fishes from BAU fish farm had improved health conditions over the other two fish farms, whereas Government fish farm was the most severely affected. Disease like EUS was clearly evidenced in the fishes of Government and NGO fish farms, mainly during colder months. Nevertheless, internal organs like liver and kidney were more affected than skin, muscle and gills.

### Table 4: Histopathological observations of various fish organs collected from three different fish farms

<table>
<thead>
<tr>
<th>Fish farms</th>
<th>Organs</th>
<th>Months</th>
<th>Apr-Jun</th>
<th>Jul-Oct</th>
<th>Nov-Dec</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAU</td>
<td>Skin &amp; muscle</td>
<td>Almost normal</td>
<td>'e' lost partly</td>
<td>'e' partially lost, 'r' myotomes &amp; 'v' were seen.</td>
<td></td>
</tr>
<tr>
<td>Gill</td>
<td>Few 'cb' seen.</td>
<td>'sgl' had 'h'.</td>
<td>'gl' were 'hp' &amp; 'h'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>Almost normal with minor 'n'</td>
<td>mild 'v' found</td>
<td>'hpt' had minor 'v'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>Almost normal</td>
<td>Almost normal</td>
<td>Minor 'v' &amp; 'mm' had in 'kt'.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Govt.</td>
<td>Skin-muscle</td>
<td>Almost normal</td>
<td>'e' lost partly</td>
<td>'e' lost, 'r' myotomes, 'v' present &amp; 'fg' in muscle were seen</td>
<td></td>
</tr>
<tr>
<td>Gill</td>
<td>Few missed</td>
<td>'sgl' were 'hp' &amp; 'cb' and lost</td>
<td>'sgl' were 'hp', 'n', 'h', 'cb' and missed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>'hpt' had massive 'n'</td>
<td>'v' &amp; 'pc' found</td>
<td>'hpt' had marked 'n', 'h' &amp; 'v'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>Mild 'v' present, almost normal</td>
<td>Marked 'n', 'h' 'v' &amp; 'pc' had in 'kt'.</td>
<td>Marked 'h', 'v' &amp; 'bc' had in 'kt'.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGO</td>
<td>Skin-muscle</td>
<td>Almost normal</td>
<td>'e' lost partly, with 'v' seen</td>
<td>'e' lost, 'r' myotomes, marked 'v' seen in 'm' with distinct 'fg'</td>
<td></td>
</tr>
<tr>
<td>Gill</td>
<td>'sgl' were 'hp' &amp; 'cb'</td>
<td>'sgl' were 'hp' &amp; 'cb' severely</td>
<td>'sgl' were 'hp', missed, &amp; 'h'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>Minor 'v'</td>
<td>Large 'v' &amp; 'pc' found</td>
<td>Large 'v' with 'h' found</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>Minor 'pc'</td>
<td>Marked 'n', 'h' &amp; 'v' had in 'kt'</td>
<td>Marked 'h', 'pc' &amp; 'v' with a distinct 'bc' in 'kt'.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

bc, bacterial colony; cb, clubbing; d, dermis; e, epidermis; fg, fungal granuloma; fd, fat droplets; gl, gill lamellae; h, haemorrhage; hp, hypertrophied; hpt, hepatocytes; ht, hematopoietic; in, inflammatory; kd, kidney tubule; m, myotome; n, necrosis; p, pyknosis; pc, pyknotic cells; pl, primary gill lamellae; pcl, pillar cells; sgl, secondary gill lamellae.
In summary, it could be inferred that water temperature had a distinct effect on fish disease, which was, to some extent, inversely related. This was evident from the comparison of highest versus lowest pathological symptoms in warmer and colder months, mainly in November and December (Tables 3 and 4). Similar observations were also made by Palisoc (1990), Barua (1994) and Chinabut (1994) on EUS outbreaks in Philippines, Bangladesh and Thailand. They reported that the reduction of water temperature together with heavy rainfall, low alkalinity and pH fluctuations were the predisposing factor for the EUS occurrence. Ahmed et al. (2004) also examined and concluded that clinically and histopathologically, fishes were found more affected during the colder months (December and January) in Bangladesh. Hence, it could be mentioned that the investigated fish was affected more during November and December, which was likely to be related to seasonal variation and/or water temperature.

CONCLUSION

In conclusion, low water temperature had an inverse impact on fish pathological symptoms that led to increasing pathological symptoms in connection to disease outbreak, mostly to the fishes of Government and NGO fish farms. Water temperature was reached to the lower point during that period, mainly in November and December and hence, outbreak of disease like EUS, bacterial and protozoan diseases could occur. Moreover, internal organs like liver and kidney were more affected than skin, muscle and gills. Hence, it is suggested to take precautionary measurement for the fishes of Government and NGO fish farms in order to obtain healthy and disease free fish. Nevertheless, introduction of pathogens and infected fish to the water bodies from other sources should be prevented as far as possible. However, till now very few steps have been taken to prevent and control the diseases of cultured fishes. Srivastava (1975) reported that the success of implementing various fisheries development program depends on the intensification of fish disease research effort, as the improvement of fish yield can be achieved from healthy fish stock.

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CONTRIBUTION OF THE AUTHORS

Kamal Gosh
Conceived the study, designed and conducted the experiment.

Gias Uddin Ahmed
Provided intellectual advice on the research and experimental design

Mst Nahid Akter
Wrote the manuscript.