Investigation of health status of juvenile exotic carps from various farming conditions of Bangladesh

Gosh, K.*, A.C. Barman¹, M.A. Alam¹, and A.H.A. Rashid¹
Department of Aquaculture, Bangladesh Agricultural University, Mymensingh 2202
¹Bangladesh Fisheries Research Institute, Mymensingh
*Correspondence author

Abstract
Health status of juvenile silver carp, Hypophthalmichthys molitrix and silver barb, Barbodes gonionotus were investigated in three fish farms following different farming conditions through clinical and histopathological examinations for a period of nine months. Here the fishes and water quality parameters were sampled on monthly basis. Among the water quality parameters, water temperature has a distinct effect on fish health observed during the winter season. Different clinical signs like scale loss, dermal lesion, fin erosion were observed, while histopathologically necrosis, pyknosis, inflammation, haemorrhage, hypertrophy, vacuoles, missing of gill lamellae and clubbing were evidenced in the investigated fishes. The study showed that pathological symptoms were mainly increased during the winter season and H. molitrix exhibited severe pathological symptoms in compare to B. gonionotus during the investigation. It was also found that fishes of BAU farm was comparatively in the best condition, while, the fishes of other farms were severely affected during the experimental observations. In addition, disease like Epizootic Ulcerative Syndrome (EUS), protozoan disease and suspected bacterial colonies were clearly evidenced in the fishes of Government and NGO fish farms.

Key words: Fish disease, Hypophthalmichthys molitrix, Barbodes gonionotus, Histopathology

Introduction
Aquaculture development in the country has intensified recently through increased stocking densities, artificial feeding and fertilization (Mazid and Banu 2002). In this development, introduction of exotic fishes with the Indian major carps has brought a new dimension in augmenting the fish production ((Ullah and Khan 2003). Though intensification has magnified the fish production, it may also lead to water quality deterioration and susceptibility to infection (Mazid and Banu 2002). Thus, the carp fishes were affected by different range of diseases and parasites (Ahmed and Hoque 1999). Common diseases of freshwater fishes of Bangladesh are tail and fin rot, bacterial gill rot, dropsy, fungal disease, protozoan disease, parasitic disease, nutritional diseases and tumors (Chowdhury 1993). In composite carp culture system, fishes are often
stressed by various living and non-living factors resulting in the outbreak of diseases (Das et al. 2000). Basically, fish remains in such an environment, which is loaded with innumerable agents like chemical pollutants, bacteria, virus, parasites and fungus etc. that are either individually or in combination can inflict the body tissue or system producing disease of several kinds (Post 1987). Moreover, their internal and external biology is also altered by other physical, chemical and biological factors of the environment. These factors determine whether an etiological agent can or will cause disease among fishes or not (Post 1987). In addition, incidence of fish diseases was also depended on season, which tends to fluctuate with temperature. It was reported that up to 31% of extensive carp farms and 24% of semi-intensive carp farms were affected with diseases (Chowdhury 1997). In these view, it is obvious to investigate the occurrence of diseases by using suitable diagnostic techniques. However, clinical investigation provides information on the nature of diseases whereas; histopathological technique gives a way to unearth the fish health related condition. But in Bangladesh, these techniques have been using for diagnosing of fish diseases to a very limited extent due to poor technical know how and laboratory facilities (Moniruzzaman 2000). Thus, the present work was undertaken to identify the pathological changes and disease occurrence in Barbodes gonionotus and Hypophthalmichthyes molitrix, collected from different fish farming systems of Mymensingh district of Bangladesh for the assessment of their health conditions.

Materials and methods

The experiment was conducted for a period of nine months from April to December 2005 in three different fish farms i.e., Bangladesh Agricultural University (BAU) fish farm, Government and NGO fish farm. The BAU fish farm is located at the southern side of the Faculty of Fisheries, BAU, Mymensingh, whereas the other fish farms were at the Fulpur upazilla under the Mymensingh district. Silver barb (Barbodes gonionotus) and Silver carp (Hypophthalmichthyes molitrix) were sampled from the three experimental ponds (one pond from each fish farm) considering as the experimental fish. Each pond was prepared following the standard procedure of semi-intensive carp polyculture system practiced in Bangladesh (Table 1). Stocking ratio were maintained at the rate of 35-40 per decimal with Labeo rohita (rui), Catla catla (catla), Cirrhinus cirrhosus (mrigal), Hypophthalmichthyes molitrix (silver carp), Ctenopharyngodon idella (grass carp), Barbodes gonionotus (silver barb), Cyprinus carpio (carpio) in BAU fish farm, whereas 60-65 numbers in NGO farm and 70-80 in Government fish farm maintaining the same species. Poultry droppings was applied in both the Government and NGO fish farms at a rate of 1.5 kg/ decimal and 1 kg/decimal respectively during October to December instead of cowdung for pond fertilization (Table 1).
Table 1. Data on Fish Farming Practices in different Farms included in the Experiment

<table>
<thead>
<tr>
<th>Fish farm</th>
<th>Area (Acre)</th>
<th>Depth (m)</th>
<th>Stocking Density (no/dec.)</th>
<th>Fertilization (Kg/dec.)</th>
<th>Supplementary feeding</th>
<th>Fish spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAU</td>
<td>0.02</td>
<td>1</td>
<td>35-40</td>
<td>Urea 0.2, TSP 0.1, Compost 2, Cowdung 1</td>
<td>Rice bran: Oilcake Feeding rate (of body wt)</td>
<td>3:1, Rui, Catla, Mrigal, Silver carp, Grass carp, Carpio</td>
</tr>
<tr>
<td>Govt.</td>
<td>0.023</td>
<td>2</td>
<td>70-80</td>
<td>Urea 0.2, TSP 0.1, Cowdung 1, Poultry dropping 1</td>
<td>Maize bran: Oilcake Feeding rate (of body wt)</td>
<td>6:1, 3%</td>
</tr>
<tr>
<td>NGO</td>
<td>0.025</td>
<td>1.8</td>
<td>60-65</td>
<td>Urea 0.1, TSP 0.1, Cowdung 1, Poultry dropping 2</td>
<td>Rice bran: Oilcake Feeding rate (of body wt)</td>
<td>3:1, 4%</td>
</tr>
</tbody>
</table>

Sampling was carried out at monthly intervals. During each sampling, 6 fishes from each farm were collected with the help of seine net. Plastic bags were used for transporting the fish samples in the 'Fish Disease Laboratory' of the 'Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Water quality parameters i.e., pH, dissolve oxygen, temperature, total hardness, conductivity and total dissolve solids were estimated at monthly intervals between 0800 to 0900 hours by using a sterilized disposable bottle. Standard procedures and methods were followed by using HACH'S kit (Model FF-1A).

The sampled fishes were examined thoroughly by naked eye and then placed it under a low powered dissecting microscope to observe any kind of injury, infection or other abnormalities. Samples for histopathological study were collected from five organs i.e., skin, muscle, gills, liver and kidney through dissection. Then the samples were fixed in 10% neutral buffered formalin immediately after collection. After 12 hours of fixation, the samples were trimmed into a standard size (2 cm³) and then processed in an automatic tissue processor for dehydration, clearing and infiltration (SHANDON, CITADEL 1000). The samples were sectioned at 5 µm by using microtome machine, stained with routine Haematoxylin and Eosin (H & E) and mounted with Canada balsam. The stained slides were then examined under a compound microscope (OLYMPUS) and photographs were taken by using a photo micrographic camera (OLYMPUS, Model CHS, Japan).
Results

Water quality parameters from different fish farms were studied for about nine months and the recorded values of the investigated fish farms were almost nearer during the experimental period (Table 2).

Table 2. Water quality parameters (Mean± SE) sampled at monthly intervals from fish farms

<table>
<thead>
<tr>
<th>Water Quality Parameters</th>
<th>BAU Fish Farm</th>
<th>Govt. Fish Farm</th>
<th>NGO Fish Farm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>27.44 ± 0.40</td>
<td>27.16 ± 0.62</td>
<td>27.65 ± 0.28</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/L)</td>
<td>87.62 ± 0.49</td>
<td>91.93 ± 0.24</td>
<td>92.00 ± 0.19</td>
</tr>
<tr>
<td>Conductivity (μS)</td>
<td>210.36 ± 0.97</td>
<td>216.22 ± 0.19</td>
<td>220.33 ± 0.27</td>
</tr>
<tr>
<td>Total dissolved solids (ppm)</td>
<td>176.00 ± 0.024</td>
<td>180.66 ± 0.85</td>
<td>171.11 ± 0.32</td>
</tr>
</tbody>
</table>

Clinically both silver carp (*H. molitrix*) and silver barb (*B. gonionotus*) were observed bright, healthy and normal during the period of April to June whereas, scale loss with minor infectious symptoms in the skin were evidenced during July to October in all the investigated fish farms. These scenarios were found quite different during November and December. In the BAU fish farm, different symptoms like fin erosion, slight skin losses and haemorrhagic lesions were observed around the dorsoventral regions in *H. molitrix*, followed by *B. gonionotus*. Here, *H. molitrix* of Government fish farm had shown mild pathological symptoms during this period (November and December) having only rough skin and mild epidermal loss, while, serious scale losses was appeared in the lateral region of *B. gonionotus*. On the other hand, investigated fishes of NGO fish farm had several scale losses and rough skin symptoms observed in November. In addition, some other significant symptoms were appeared in the experimental species during December. *H. molitrix* had various scale losses along with bright reddish spot at dorsoventral region, while *B. gonionotus* had weak body and rough skin in caudal and ventral region.

Histopathologically skin and muscle of both the experimental species of BAU fish farm were severely affected during November and December other than April to June. Parts of epidermis along with dermis were lost and separated with sloughing off condition. In addition, necrosis, ruptured myotomes as well as missing were seen in many places, thus vacuolation was noticed. But mild pathology was observed during July to October. Similar symptoms were also observed in both the fishes of NGO and Government farms (Figs. 1 & 6). However, several distinct and well developed fungal granuloma and its traces were noticed in the affected muscle of *H. molitrix* in the above two fish farms during the colder season.
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Fig. 1. Cross section of skin and muscle of sharptunti from NGO fish farm during July to October. Epidermal loss (v), vacuoles (v), haemorrhage (h) were noted. H & E x 125.

Fig. 2. Section of gill in sharptunti from Govt. fish farm during July to October. Secondary gill lamellae (SGL) were greatly hypertrophied (hp) and necrotized (n). H & E x 420.

Fig. 3. Section of liver in sharptunti from NGO fish farm during November & December. Hepatocytes were highly haemorrhaged (h), had pyknotic cells (pc), fat droplets (fd) and vacuoles (v). H & E x 420.

Fig. 4. Cross section of kidney in sharptunti from Govt. fish farm during July to October. Kidney tubules had pyknosis (pc). H & E x 420.

Fig. 5. Section of gill in silver carp from NGO fish farm during November & December. Secondary gill lamellae were greatly hypertrophied (hp) & clubbed (cb). H & E x 420.

Fig. 6. Section of skin and muscle of silver carp from Govt. fish farm during November & December. Epidermal loss (v), Vacuoles (v) was noticed. H & E x 125.

Fig. 7. Cross section of kidney in silver carp from Govt. fish farm during July to October. Necrosis (n), haemorrhage (h), vacuoles (v), pyknotic cell (pc), suspected bacterial colonies (BC) having tubular degeneration (td). H & E x 420.

Fig. 8. Section of kidney in silver carp from Govt. fish farm during November & December. Necrosis (n), hemorrhage (h), vacuoles (v), melanin (m) pigments and protozoan cysts (PC). H & E x 420.
Among the organs, gills of the investigated fishes were also found affected in the BAU fish farm followed by NGO fish farm. It includes marked hypertrophy, clubbing, haemorrhage, missing of gill lamellae observed mainly in November and December (Fig. 5). But in case of Government fish farm, the intensity of gill pathology in *H. molitrix* was remarkably higher than the *B. gonionotus* causing severe hypertrophy, necrosis, clubbing, haemorrhage and pillar cells disruption in and between the primary and secondary gill lamellae. Both the gill lamellae were found to have swollen at its tips and many inflammatory cells were accumulated at the base (Fig. 2.).

Internal organs such as liver were severely affected in both the experimental fishes of NGO and Government fish farms having massive necrosis, pyknosis and inflammation (Fig. 3). But in the BAU fish farm, liver of the investigated fishes had shown less pathology compared to the other fish farms having some kinds of minor necrosis.

However, kidney of *B. gonionotus* of both the Government and NGO fish farms had shown different pathological changes like haemorrhage, pyknosis, and necrosis along with suspected bacterial colonies in the surroundings of renal cells during November and December (Fig. 4.). All the above mentioned symptoms were also observed in *H. molitrix* but more intensely (Figs. 7 & 8). Not only these but also, some protozoan cysts were evidenced in the kidney tubules of *H. molitrix*, which indicated it's susceptibility to disease (Fig. 8).

**Discussion**

Clinically both the investigated fishes were observed infected during the winter season. In *Hypophthalmichthys molitrix* rough skin, scales loss and weak body was observed in November whereas, haemorrhages with reddish bright spots were evidenced at dorsoventral region of skin during December. In contrast, scale loss and rough skin was observed in *Barbodes gonionotus* during November, but serious haemorrhages in dorsoventral and lateral region had found during December. However, both the fishes were recorded to have minor abrasions during July to October and had almost normal condition during April, May and June. 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 by Hoque (1998), Islam (1999), Moniruzzaman (2000) and Roy *et al.* (2006).

Nevertheless, histopathologically *H. molitrix* of both NGO and Government fish farms had several fungal granulomas and traces in its muscle. Noga and Dykstra (1986) were of the opinion that marked granuloma, inflammatory response were shown by fish infected with *Aphanomyces* sp. Hoque *et al.* (1999) also reported that large deep and whitish ulcers were recorded in the lateral region (near dorsal fin) and caudal region, where part of fins, scales and muscles were lost in most of the EUS affected fish. The result was also agreed with the observation of Moniruzzaman (2000), Roy *et al.* (2006) and Akter *et al.* (2006), Ahmed and Hoque (1999). Thus, it could be mentioned that *H.*
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*H. molitrix* of NGO and Government fish farms were infected with Epizootic Ulcerative Syndrome (EUS).

Moreover, gills of investigated fishes were also observed infected in the investigated fish farms having hypertrophy, clubbing, haemorrhage, missing of gill lamellae occurred mainly in November and December. Islam *et al.* (1999) found that gills of local and exotic carp species were severely affected during colder months i.e., December and January when compared with other months. Almost similar gill pathology was also found by Ahmed and Haque (1998), Roy *et al.* (2006) and Akter *et al.* (2006). Thus, it could be reported that, infection of gills were also depended on seasonal variations.

On the other hand, histopathologically liver were severely affected observed in both the NGO and Government fish farms exhibiting necrosis, pyknosis and inflammation, while BAU fish farm had minor infections. Ram and Singh (1988) made similar opinion that liver exhibited varying degree of pathological changes. Afifi (1996) also observed that vascular changes in the liver due to the endothelial lining of the blood vessels and prevascular cuffing.

In kidney, investigated fishes of both the Government and NGO fish farms had different pathological changes and suspected bacterial colonies. Some protozoan cysts were also found in *H. molitrix* around its renal surroundings, which indicated it's susceptibility to disease. Similar opinion was also given by some authors, such as silver carp is susceptible to many diseases caused by parasitic protozoan (Ribelin and Migaki 1975). In addition, Bejerano *et al.* (1979) reported that bacterial infections can lead to death in silver carp. The scientist also observed that bacteria were introduced from poultry feces used to fertilize carp ponds. Mi *et al.* (1993) described septicemia, a common symptom of bacterial infection on silver carp, as a process of acute hemorrhagic inflammation accompanied with functional disorder in the heart, kidney and brain. He *et al.* (1992) isolated more than 10 strains of pathogenic bacteria from silver carp in Shashi District, China. From the result, it could be mentioned that *H. molitrix* of Government fish farm was severely affected and diseases like EUS, suspected bacterial colonies and protozoan infection occurred during colder months.

In contrast, when we consider the fish farms, BAU fish farm had comparatively less pathological symptoms in the investigated species among the other fish farms. Low stocking density of BAU fish farm could be one of the reasons having of less infection. On the other hand, clinically and histopathologically, internal organs like liver and kidney of the investigated fishes found affected in colder months. Ahmed and Hoque (1999) reported that histopathologically the internal organs like kidney and liver were more affected in compare with other organs. Similar pathological symptoms in kidney of major carp were observed by several authors like Islam (1999), Roy *et al.* (2006), and Akter *et al.* (2006). Thus, it could be mentioned that the health condition of BAU fish farm had in improved conditions, whereas Government fish farm was the most severely affected. Disease like Epizootic Ulcerative Syndrome (EUS) was clearly evidenced in the fishes of Government and NGO fish farms.

Clinically and histopathologically, different distinct effects of water temperature and season were also observed in the experimental fishes. Both the exotic carps had
almost normal condition during April to June whereas, minor pathological changes in organs were observed in July to October. But marked and distinguishing pathological changes in organs were evidenced in November and December. Similar results was also reported by different authors like Ahmed et al. (2004), Roy et al. (2006) and Akter et al. (2006), who examined through using clinical, parasitological and histopathological techniques on three small indigenous fishes and found that all fishes were severely affected during the month of November, December and January. It was also agreed by Hossain and Paul (1993), who observed that the outbreak of disease was highest when water temperature was at the lowest. So, it could be assumed that low water temperature might play a role in the health condition changes in exotic fishes.

Again when we consider individual fish species, H. molitrix was more susceptible to infectious diseases e.g., EUS, suspected bacterial infection and protozoan diseases than B. gonionotus. Furthermore, fishes of Government farm had shown different pathologies and infectious symptoms over the other fish farms. This is may be due to poor management practices i.e., high stocking density, poor quality of fingerling, low quality in feed management and less attention on the health condition of fishes. The correlation between disease and stocking density was observed by Duijn (1973) and stated that high stocking density often cause fish disease in cultural system. Thus, it could be suggested that more preventive and cautionary measures would necessary to be carried out in this fish farm to prevent diseases in order to have healthy and disease free fish. According to Srivastava (1975), success of the implementation of various fisheries development program depends, to the extend, on the intensification of fish disease research, as the improvement of fish yield can be achieved from healthy fish stock. In this situation, either facilities for disease diagnosis should be introduced or affected fish should be sent to the diagnostic laboratory immediately for diagnosis of diseases in order to have a healthy and disease free fish production.

References


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