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Article

Seasonal variation of water quality parameters, clinical and histological observation of some small endangered open water fishes from Mymensingh and Sylhet region

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Abstract: Seasonal variation of water quality parameters and health condition of some small endangered open water fishes are Baila (Glossogobius giuris), Gutum (Lepidocephalichthys guntea) and Tara baim (Macrognathus aculeatus) was carried out through clinical and histological observation from Kailla beel of Ishargonj upazila, Mymensingh and Surma river, Gobindogonj upazila, Sunamgonj district, Sylhet region for a period of eight months from September 2014 to April 2015. Water quality parameters like water temperature, dissolved oxygen, pH, ammonia, alkalinity, hardness, and nitrate were recorded. Water temperature, pH, alkalinity and hardness were found at unfavorable level for fish in mid November and January. Clinically it was observed that, fish was affected with fin and tail rot, gill rot, parasitic infestation, nutritional deformities, numerous red spots and patches in lateral and ventral regions, large deep whitish ulcers reached up to deep ulcers especially in December and January in both region. Samples of skin, muscle, gill, liver and kidney were collected and processed for histological observations. Major pathology in the skin and muscle were epidermis separated from dermis, presence of fungal granuloma, vacuums, hemorrhage and necrosis. Loss of primary and secondary gill lamellae, hypertrophy and primary gill lamellae separated, necrosis and hemorrhage were found in the gill. Large vacuums, necrosis and hemorrhage were observed in liver and kidney. Among the affected fish organs skin and muscle, gills were more affected than the internal organs like liver and kidney. Overall, clinical and histological observations of fishes were found to be more affected in December and January. Whereas, in the months of mid February to April, the pathological condition of fish gradually healed up to normal except few vacuums and hemorrhage. Under histopathological observations, fishes of Kailla beel were more affected than the fishes of surma river. In clinical and histopathological observation open water fishes were more susceptible due to EUS.

Keywords: seasonal variation; small endangered species; clinical and histopathological observations; water quality parameters

1. Introduction
Bangladesh is very rich in inland freshwater resources. The inland water bodies include ponds, rivers, canals, reservoirs, lakes, Oxbow lakes, floodplains and natural depressions. The closed inland water resources are estimated 7.74 hectare of which 3.71 million hectare ponds, 0.055 million hectare oxbow lakes, 2.75 million hectare shrimp farm, 1.22 million hectare seasonal water bodies; 27.11 million hectare floodplains (DoF, 2013). These water bodies are good and ideal habitat for small indigenous fish (SIS) as well as many
commercially important indigenous small fishes of Bangladesh such as catfish, cyprinids, perch, eel and snake head etc. This species of fish always remain available for daily consumption of all classes of people especially low-income groups, due to their low price, food taste and good flavor and high nutritional quality. The small indigenous species (SIS) of Bangladesh are generally considered to be those fishes which grow to a length of about 25 cm or inches (Felts et al., 1996; Hossain and Afroze, 1991). SIS has high nutritional value in terms of protein, vitamin, and minerals and these micronutrients are not commonly available in other foods (Thilsted et al., 1997). These groups of fish contain large amount of calcium and also iron and zinc (Tripathi, 1997). Moreover this small indigenous fish species are maintaining a stable and static condition of our aquatic food chain thus the biodiversity of our open water ecology have in smooth form. But natural populations of this small fish is rapidly decreasing due to over exploitation, lack of scientific management, draining out of beels, natural disaster (draught and siltation) and poor environmental conditions. Because the water quality of beels, baors, rivers and canals are decreasing day by day. As a result, fish species that breed and reared in natural waters are reducing quickly. Thus fishes of open water bodies are facing continuous stress, which leads to infection and disease. Health condition and disease has become a major problem in fish production both in culture system and wild condition in Bangladesh (Rahman and Chowdhury 1996). Common diseases of open water fishes of Bangladesh are tail and fin rot, bacterial gill rot, dropsy, various types of fungal disease, protozoan disease, parasitic disease, nutritional disease, tumors (Chowdhury, 1993). Although the existing fishes are habituated in floodplain but snakehead, catfish etc., are recorded as rare species from flood plains and beels due to the outbreak of various diseases (Hossain and Mazid, 1995). With the outbreak of epizootic ulcerative syndrome (EUS) in 1988, *Channa* spp., *Puntius* spp., *Anabas* spp. and other indigenous species of fish are seriously affected (Barua et al., 1989). The water quality parameters such as temperature, dissolved oxygen, pH, total alkalinity, total hardness and ammonia could be associated with outbreaks of disease (Subasinghe, 1995). It indicated the possible relationship between the occurrence of disease and the environmental parameters. Histopathological technique is one of the most important procedures for disease diagnosis in fish. It has been successfully used throughout the world. It was thus necessary to investigate health condition of fish through some suitable techniques. Considering the above facts the present study has been undertaken with the following objectives are to investigate water quality parameters of Kailla beel and Surma river in relation to different months and also observe the health status of selected fish species from the two different areas with the seasonal variation.

2. Materials and Methods
In the present investigation two areas were selected; they were Kailla beel of Mymensingh region and Surma river of Sylhet region. The study was conducted for a period of eight months from September 2014 to April 2015 to find out the water quality parameters and health status of a small indigenous species (Baila, Gutum and Tara baim). Water samples were collected from the Kailla beel and Surma river once in a month between 08.00 to 08.30 am and water quality parameters test on spot. Water quality parameters like temperature, pH, dissolved oxygen, ammonia, total hardness, alkalinity and nitrite were measured by using respected test kits. Fish samples from both region was collected from nearby local fisherman. A total of 15 live fishes (5 fishes from each species) were collected during each sampling from each area. The sampled fishes were clinically examined by naked eye and magnifying glass to record any external signs, injury and other abnormalities. Organs like skin and muscle, gill, liver and kidney were collected with the help of a sharp scalpel and forceps and fixed in 10% neutral buffered formalin for histopathological study. After 8 hours of fixation, the samples were trimmed in order to obtain a standard size of 1 cm³ (maximum) and placed in automatic tissue processor for dehydration, clearing and infiltration. The samples were then embedded, sectioned (5μm thickness) and stained with Haematoxylin and Eosin. Then the sections were mounted with canad a balsam and covered by cover slips and examined under a compound microscope. Photomicrograph of the stained sections was obtained by using a photomicroscope. Record of structural variations and pathologies were done from the slides and photomicrographs (Ahmed et al. 2009).

3. Results
3.1. Water quality parameters
Water quality of the Kailla beel and Surma river was studied from September 2014 to April 2015. Data regarding water temperature, pH, dissolved oxygen (DO), and ammonia analyzed and presented in Table 1.
Table 1. Monthly variations of water quality parameters of the kailla beel and Surma river.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Water body</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>Kailla beel</td>
<td>31±1.27</td>
<td>29±1.77</td>
<td>26±1.89</td>
<td>19±2.12</td>
<td>17±2.54</td>
<td>25±1.44</td>
<td>28±1.26</td>
<td>31±1.52</td>
</tr>
<tr>
<td></td>
<td>Surma river</td>
<td>32±1.33</td>
<td>30±1.54</td>
<td>27±1.76</td>
<td>21±3.25</td>
<td>19±2.88</td>
<td>27±1.33</td>
<td>28±1.22</td>
<td>33±1.01</td>
</tr>
<tr>
<td>pH</td>
<td>Kailla beel</td>
<td>6.9±0.25</td>
<td>7.0±0.54</td>
<td>6.8±0.75</td>
<td>6.6±0.24</td>
<td>6.8±0.33</td>
<td>7.0±0.14</td>
<td>7.1±0.43</td>
<td>7.0±0.32</td>
</tr>
<tr>
<td></td>
<td>Surma river</td>
<td>7.1±0.13</td>
<td>6.9±0.11</td>
<td>6.8±0.23</td>
<td>6.7±0.55</td>
<td>6.7±0.57</td>
<td>6.9±0.37</td>
<td>7.0±0.26</td>
<td>7.3±0.10</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/l)</td>
<td>Kailla beel</td>
<td>4.5±1.20</td>
<td>4.0±1.03</td>
<td>3.5±1.25</td>
<td>3.0±1.75</td>
<td>3.5±1.88</td>
<td>4.0±1.01</td>
<td>4.0±0.45</td>
<td>4.5±0.67</td>
</tr>
<tr>
<td></td>
<td>Surma river</td>
<td>4.5±1.13</td>
<td>4.5±1.20</td>
<td>4.0±1.03</td>
<td>3.5±1.75</td>
<td>3.0±1.83</td>
<td>4.0±1.22</td>
<td>4.5±0.24</td>
<td>5.0±0.35</td>
</tr>
<tr>
<td>Ammonia (mg/l)</td>
<td>Kailla beel</td>
<td>0.0±0.12</td>
<td>0.0±0.01</td>
<td>0.1±0.03</td>
<td>0.3±0.05</td>
<td>0.3±0.08</td>
<td>0.1±0.04</td>
<td>0.2±0.01</td>
<td>0.0±0.01</td>
</tr>
<tr>
<td></td>
<td>Surma river</td>
<td>0.0±0.01</td>
<td>0.1±0.02</td>
<td>0.1±0.04</td>
<td>0.2±0.06</td>
<td>0.3±0.09</td>
<td>0.1±0.05</td>
<td>0.0±0.02</td>
<td>0.0±0.00</td>
</tr>
</tbody>
</table>

3.2. Clinical observations
Clinical symptoms of investigated species in different months of the Kailla beel and Surma river were studied and recorded (Table 2).

3.2.1. Fish in Kailla beel
During the month of September and October it was observed that, all the three species were almost normal and healthy in appearance. In *G. giuris* rough skin was observed in November, scale loss at some places in, hemorrhage in gill, Fin loss and scale loss from ventral region in December and January (Fig. 2). However, in December rough skin was observed in *L. guntea* and *M. aculeatus*. Rough skin, ectoparasitic infestation in gill, and deep ulcer of ventral region were recorded in *L. guntea* in December and January (Fig. 4). Same appearance was observed in *M. aculeatus* in December (Fig. 6). In *M. aculeatus* fin loss and discoloration of gill filament were recorded in November, mild dermal lesion were observed in *L. guntea* at the same period.

3.2.2. Fish in Surma river
Healthy appearance and almost normal condition were observed during September, October and mid November in all the three species. During December and January rough skin was observed in *G. giuris* same appearance were observed in in *L. guntea* and *M. aculeatus* Weak body in last November, hemorrhage in dorsal and dorsoventral region during December and January were observed in *P. ticto*. Discolouration of gill filament during November, mild dermal lesion in December and January were recorded in *L. guntea*. In *M. aculeatus* severe necrotic lesion in head region was observed in December and loss of caudal fin in January.

**Figure 1.** *G. giuris* obtain from surma river in the month of September-october. Almost normal appearance.

**Figure 2.** *G. giuris* obtain from surma river in the month of January. Redness of eyes, hemorrhage in gill, total caudal fin loss and scale loss from dorsal region were seen.
Figure 3. *L. guntea* obtain from Kailla beel in the month of September-october. Almost healthy appearance.

Figure 4. *L. guntea* obtain from Kailla beel in the month of December. Hemorrhage in gill, Fin and total tail loss and Red spot and mass lesion in ventral region were seen.

Figure 5. *M. aculeatus* obtain from Kailla beel in the month of March- April. Almost healthy appearance.

Figure 6. *M. aculeatus* obtain from Kailla beel in the month of January. Gill rot, loss of caudal fin and whitish lesion to deep ulcer and dermal lesion in lateral region were seen.

Table 2. Clinical observation of investigated fish of the two beels in different months.

<table>
<thead>
<tr>
<th>Month</th>
<th>Beels</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>April</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kailla beel</td>
<td>Healthy appearance</td>
<td>Weak body</td>
<td>Fin erosion, mild affected</td>
<td>Whitish deep ulcer, weak body, Lesion, FL.</td>
<td>Large ulcer, weak body, DB, RP, RS.</td>
<td>WB</td>
<td>HA</td>
<td>HA</td>
</tr>
<tr>
<td></td>
<td>Surma river</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
<td>Numerous ‘R_s’, ‘WB’</td>
<td>‘RS’, weak body, EPG</td>
<td>AN</td>
<td>AN</td>
<td>AN</td>
</tr>
</tbody>
</table>

AN=Almost normal, WB=Weak body, R_s=Red spot, HA= Healthy appearance. RS= Rough skin, DB= Deformetis body, EPG= Ectoparasitic infestation in gill, FL= Fin loss.

3.3. Histopathological Observation

3.3.1. Fishes of Kailla beel

In September and October epidermis were normally arranged in all three fishes. However in December and January, a monogenean trematode was present in dermis of *G. giuris* (Fig. 9), splitted dermis, vaccum, necrosis, haemorrhage and protozoan cyst were observed in *G. giuris*, at that time same changes were found in *L. guntea* and *M. aculeatus* fishes in kailla beel. Marked hypertrophy was observed in gill lamellae, gill lamellae were splitted in some places of *L. guntea*, both the gill lamellae were ruptured with blood cells of *L. guntea*. At this time monogenetic trematode, protozoan cyst, haemoerragne were present in the gill of *L. guntea* and *M.*
aculeatus during December and January. In mid October to November, secondary gill lamellae were swollen and primary gill lamellae were almost normal in all the three species. During this period in M. aculeatus, both the gill lamellae were hypertrophied and missing in some portion having monogenetic trematode. In December and January protozoan cyst, clubbing were present in secondary gill lamellae and heavy fungal granuloma and fungal hyphae were apparent in the skin and muscle of M. aculeatus. In the month of last February to April affected fishes were gradually healed with the changes of environment.

3.3.2. Fishes of Surma river
In case of G. giuris during the month September to November muscle more or less normal. In However, in December and January, dermis splitted, necrotic muscles were scattered in empty space and dense melanocytes were observed in dermis. In M. aculeatus epidermis partly lost and separated from dermis, dermis irregularly arranged and dense melanocytes, fungal granuloma were seen in December and January in Surma river. In case of G. giuris, in September to November all fishes more or less infection free and glossy appearance. In December and January, both the gill lamellae were hypertrophied, and many monogenetic trematode, protozoan cyst, telangiectasis were present in secondary gill lamellae, hemorrhage in both gill lamellae and secondary gill lamellae were missing in some places.

4. Discussion
Several research works had been conducted in Bangladesh on disease investigation of small indigenous fish with clinical and histopathological methods as disease diagnosis tools. In the present study, small indigenous fishes like are Baila (Glossogobius giuris), Gutum (Lepidocephalichthys guntea) and Tara baim (Macragnostus aculeatus) was considered to monitor health and disease in the kailla beel from Mymensingh and Surma river from Sylhet region of Bangladesh. The results of this study are discussed below and compared with the results of other works in the relevant field.

Water quality parameters are important considerations of fish health management. In the present study, the highest water temperature 31 °C in Kailla beel and 32 °C in Surma river was recorded in September (2014). On the other hand, the lowest value of temperature was recorded 17 °C in kailla beel and 22 °C in Surma river in January. Mondal (2012) recorded water temperature were ranged from 17 to 31°C in BAU fish farm. According to Akter et al. (2009) there was a decreased value of temperature during winter season in Kailla beel of Mymensingh. In the present experiment, the pH values were ranged from 6.6 to 7.1 in Kailla beel and 6.7 to 7.3 in Surma river. Mondal (2012) recorded that pH values were ranged from 7.0 to 7.7 in BAU fish farm and Swopon fish farm throughout the experimental period. Most natural water has pH values of 6.5 to 9 (Alim, 2005). According to Swingle (1967) pH value of 6.5 to 9 is suitable for fish culture. In the present experiment, the values of dissolved oxygen were ranged from 3.0 to 4.5 mg/l in Kailla beel and 5.0 to 3.0 mg/l in Surma river. Ahmed et al (2009) reported that dissolved oxygen ranged from 5.5 to 3.0 in four beels of Mymensingh. Hossain (2000) and Kohinoor (2000) recorded dissolved oxygen values of fish ponds ranging from 3.8 to 6.9 mg/l and 2.04 to 7.5 mg/l respectively at Mymensingh region. In the present study, the values of ammonia were very much nearer that ranged from 0.0 to 0.3 mg/l in both the water bodies. Wahab et al. (1995) reported that ammonia content of 0.19 to 0.28 mg/l in their experimental areas. Akter et al. (2009) observed increased level of ammonia during winter period.

Clinically, G. giuris, L. guntea and M. aculeatus had almost normal except some fin erosions were recorded in November from Kailla beel. In January, large deep whitish ulcer reached up to deep ulcers, deformitex body, Ectoparasitic infestation in gill and Fin loss was observed from both water bodies. From research findings of Haque et al. (1999) it was observed that, large deep and whitish ulcer in the lateral and region, part of fins, scales and muscle were clinical sings of EUS affected fishes. Parveen (2001) observed that, three small indigenous fishes like P. ticto, N. nandus and C. punctatus from four beels of Mymensingh district, and observed that fishes were severely affected during the months of December and January. Ahmed et al. (2012) reported that the Tilapia was more affected from December and January and different clinical symptoms like rough skin, scale loss, red spots and dermal lesions were noticed. Ahmed et al. (2007) also observed that scale loss, ill body and rough skin, minor ulcer and small red spots in December and January. From the result of the present experiment it was observed that severities of clinical signs were increased in December and January in both region. In present study, selected species was more affected followed by Kailla beel than Surma river.
Figure 7. Cross section of affected skin and muscle of *G. giuris* in January from surma river splitted dermis, monogenean trematode, vaccum, haemorrhage, necrosis and protogoan cyst (H&E x 130).

Figure 8. Section severely affected of gill of *G. giuris* in January from surma river having monogenean trematode, haemorrhage, protozoan cyst, hypertrophy and hyperplasia (H&E x130).

Figure 9. Photomicrograph of affected skin and muscle of *L. guntea* in December from Kailla beel dermis spilled, malenomacrophage centre, vaccum, necrosis and protogoan cyst (H&E x130).

Figure 10. Cross section of severely affected of gill of *L. guntea* in December from kailla beel having monogenean trematode, haemorrhage, protogoan cyst, gill lamellae were splitted (H&E x 130).

Figure 11. Photomicrograph of affected skin and muscle of *M. aculaetus* in December from Kailla beel fungal hypae, fungal granoloma, vaccum, necrosis, and monogenean trematode (H&E x 130).

Figure 12. Cross section of severely affected of gill of *M. aculaetus* in December from kailla beel having monogenean trematode, haemorrhage, protogoan cyst, gill lamellae were splitted, clubbing, vaccum, hypertrophy (H&E x 130).
Histopathologically, it was observed in present study that all organs of fishes like gill, skin and muscle, liver and kidney was almost normal in October, mild affected in November and severely affected in December and January and infected organs were healed up to almost normal structure in March and April from both water bodies. In January, primary gill lamellae were separated; vaccum, Haemorrhage, protozoan cyst, monogenean trematode, clubbing, necrotic and hypertrophied in fishes of kailla beel. Whereas, fish organs of Surma river hemorrhage, necrosis, hypertrophied and hyperplastic in December. Konika (2011) observed less pathological changes such as hypertrophy, clubbing and few lamellar missing in Cirrhinus cirrhosus during November. According to Ahmed et al. (2012) O. niloticus gill had hypertrophy, hyperplasia, clubbing, hemorrhage in primary gill lamellae and secondary gill lamellae were lost during December and January. According to Ahmed et al. (2009) gills were healed up to almost normal structure in February and March. Muscle had fungal granuloma, fungal hyphe, Monogenean trematode, vaccums and necrosis in muscle of M. aculaetus from Kailla beel in December. However, in Surma river, muscle had splitted dermis, protozoan cyst, vacuums, necrosis and hemorrhage in January. Ahmed et al. (2010) also observed that epidermis and dermis were partly lost, necrosis, hemorrhage, fungal hypheae were seen in A.testudineus during the months of December and January. Hossoon et al. (2009) reported that severe necrosis of hepatocytes, pyknosis, vacuoles, fat droplets and hemorrhage were observed in small indigenous species during December and January. Ahmed et al. (2012) observed that in February and March, organs pathology was recovered to almost normal structure except vacuums. In the present study, internal organs such as liver and kidney were less affected than external organs like skin, muscles and gill.

The water quality parameters were in suitable range throughout the study period except temperature, and ammonia which were reduced in December and January. In December and January when water temperature was 17°C to 19°C, diseases like EUS, red spot, parasitic infestation and numerous abnormalities occurred with marked pathological changes in various organs. Fish samples were collected at monthly intervals. Under clinical observations, any injury, infection and other abnormal conditions of fish body were recorded. In present study, sample fish was almost normal during the month of September and October. The fish had numerous clinical sings seen in lateral and ventral regions during December and January from Surma river. Whereas, large deep whitish ulcers reached up to deep ulcers, red spot, rough skin, fin lose were found during December and January in fish of kailla beel. In this experiment it is observed that the fishes of surma river comparatively less affected than the fishes of kailla beel. During histopathological study, normal structure was found in September from both water bodies. Mild pathological changes were seen in the Kailla beel during November. However, pathological changes were increased in the fish in December and January. Under Pathological observation, fish was affected by epizootic ulcerative syndrome (EUS), red spot, tail and fins rots, gill rot, parasitic infestation and nutritional deformities etc. The gill and skin and muscle in fish was more affected then internal organs likes liver and kidney due to those diseases.

Conflict of interest
None to declare.

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