Corneal Hypertrophy in Farmed Chocolate Mahseer, *Neolissochilus hexagonolepis*, from (Uttarakhand), India

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**Abstract:** Corneal hypertrophy was observed in fingerlings of chocolate mahseer, *Neolissochilus hexagonolepis* (McClelland, 1839) from Uttarakhand, India. The advanced stage of infection was marked with damage of cornea and shrunken eye. Skeletal deformity was also recorded in some of the infected fishes. Samples of eye and other organ were collected for histopathological, parasite and microbial investigation. Histological examination of the eye indicated ocular collapse, entirely damaged cornea, disruption of vitreous, aphakic globe, oedema of choroid and optic nerve and, deformed retina. Microscopic observations of wet mounts and histopathology ruled out infectious agents like microsporidia, myxosporidea, cystercooidiasis, atypical mycobacteria, digeneans and fungi. PCR for viral aetiology did not yield any significant amplicons. Further detail study related to physic-chemical parameter need to be carried out to ascertain the exact cause of this disease.

**Keywords:** *Neolissochilus hexagonolepis*, Pathology, Eye, Corneal hypertrophy, Chocolate mahseer.

**Introduction**

Occurrence of disorder of the eye of the fishes has been widely reported by various researchers throughout the world (Boylan *et al.*, 2011; Shahi and Mallik, 2013). Majority of the fish eye lesions involved swelling of the orbit or discoloration of the cornea, which is attributed to biotic (Olufemi and Roberts, 1986) and abiotic stress (Hughes *et al.*, 1981). Eye disorder can occur in epidemic proportions, and in several cases, an infectious microbial aetiology has also been suggested (Roberts, 2001; Evely *et al.*, 2011).

There are sporadic published reports of pop eye disease and eye disorder from India (Shome *et al.*, 1999; Shahi and Mallik, 2013). However, in coldwater aquaculture practices of India, eye disorder is one of the significant factor affecting the production of rainbow trout, *Oncorhynchus mykiss* (Walbaum) and golden mahseer, *Tor putitora* (Hamilton, 1822) (Shahi and Mallik, 2013). Recently this incidence is also observed in fingerlings of chocolate mahseer, *Neolissochilus hexagonolepis* (McClelland, 1839), a freshwater fish, inhabiting the hill streams and rivers of India, Nepal, Bangladesh, Myanmar, China and Indonesia (Froese and Pauly, 2011). This species is a popular food and game fish in north and northeast region of India. Over the few years, chocolate mahseer is considered as candidate species for Indian aquaculture production, due to its high market demand and standardization of breeding technology. However, due to overexploitation, habitat destruction and other anthropogenic activities, this fish has been declared near threatened (NT) by the IUCN red list (Arunachalam, 2010). The present study reports corneal hypertrophy in chocolate mahseer fingerlings from mid Himalayan region (altitude of 1370 meter above sea level) of India.

**Materials and Methods**

For species diversification, the fry of chocolate mahseer was brought to mahseer hatchery of Directorate of Coldwater Fisheries Research,
After physical examination, the fish were killed by overdose of tricaine methanesulfonate (MS 222). Eye, liver, kidney, brain, spleen, kidney and gill tissues were fixed with 10% neutral buffered formalin for histopathological analysis. The fixed samples were routinely processed to 5 µm thicknesses, stained with haematoxylin and eosin (H and E) and observed with a light microscope. Wet mount preparations of gill, skin scrapping, eye and other organs were also observed and there was no evidence of any parasites in the samples. To find out a possible viral aetiology, a degenerate primer was designed to target the DNA polymerase of aquatic herpes viruses, iridoviruses, poxviruses and adenoviruses (Hanson et al., 2006).

Results and Discussion

The water quality parameters of rearing pond from which samples of chocolate mahseer was analyzed as per the standard methods and is shown in Table 1.

Histological examination of the eye indicated ocular collapse, entirely damaged cornea, disruption

Table 1 Physico-chemical parameters of water from chocolate mahseer pond.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Average and standard error</th>
</tr>
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<tbody>
<tr>
<td>Temperature (°C)</td>
<td>25.03 ± 3.20</td>
</tr>
<tr>
<td>pH</td>
<td>8.40 ± 0.32</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>4.26 ± 0.93</td>
</tr>
<tr>
<td>Conductivity (µm/s)</td>
<td>136.00 ± 9.50</td>
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<tr>
<td>TDS (ppm)</td>
<td>68.00 ± 11.64</td>
</tr>
<tr>
<td>NH₃-N (mg/L)</td>
<td>0.36 ± 0.04</td>
</tr>
<tr>
<td>NO₂-N (mg/L)</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>NO₃-N (mg/L)</td>
<td>4.18 ± 0.94</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>9.45 ± 2.80</td>
</tr>
<tr>
<td>PO₄³⁻-P (mg/L)</td>
<td>0.83 ± 0.08</td>
</tr>
<tr>
<td>Alkalinity (mg/L)</td>
<td>108.00 ± 9.61</td>
</tr>
<tr>
<td>Hardness (mg/L)</td>
<td>96.00 ± 4.82</td>
</tr>
<tr>
<td>SO₄²⁻ (mg/L)</td>
<td>132.60 ± 11.23</td>
</tr>
<tr>
<td>Potassium (mg/L)</td>
<td>12.58 ± 0.59</td>
</tr>
</tbody>
</table>

Bhimtal (29.35°N and 79.56°E), Uttarakhand from Mehao Lake (28.14 °N and 95.94 °E) of Arunachal Pradesh, India in the year 2008. The fish were bred in captivity during august 2012. While carrying out a routine survey in the month of summer (June 2013), the malformation in eye (Fig. 1A, prevalence 32 %) of fingerlings (weight 3.26 ± 1.9g; length 6.46 ± 3.9cm) was recorded. Adults were found to be free from any visible eye disorder. In fingerlings, cornea of the eye was hypertrophic (Fig. 1B and C), and the advanced stage of infection was marked with damage of cornea and shrunken eye (Fig. 1D). Some of fishes were having only eye ball. In majority of the fish, the abnormality was unilateral. Affected fish also exhibited spinal and operculum deformity (Fig. 2). The deformity consisted of curvature in the dorsal ventral plain, and extended from the middle of the dorsal fin to the caudal fin. The fish were lethargic and swimming abnormally by tilting to one side.
of vitreous, aphakic globe, oedema of choroid and optic nerve and, deformed retina (Fig. 3A). The lens capsular epithelium of eye was also disrupted (Fig. 3B). Oedema and disruption of optic nerve was massive (Fig. 3C). The other internal organs did not reveal any pathological or inflammatory changes on histological examination.

Microscopic observations of wet mounts and histopathology ruled out infectious agents like microsporidia, myxosporidea, cystercoeldiasis, atypical mycobacteria, digeneans and fungi. PCR for viral aetiology did not yield any significant amplicons.

Cornea is an important structure that is rarely mentioned in the fish disease literature due to the rare occurrence of pathology, and is the most vulnerable part of eye to any traumatic damage. Though the exact cause of the corneal hypertrophy in this study could not be established, but trauma such as plankton bloom, high temperature and change in osmotic pressure during summer month could be the cause of disorder. At the onset of southwest monsoon the prevalence of this disease was reduced and we could not find newly affected fingerlings of chocolate mahseer. This strongly indicates that the physio-chemical parameter of the water may induce the hyperplasia, as feed and other management practice was similar during the entire culture period. The planktonic bloom was prominent during the summer due to low water exchange rate and stagnant nature of water body in shallow rearing condition (earthen pond). The cornea is also likely to suffer secondary infection from virus. Papillomavirus and lymphocystis virus, have been linked to several piscine hyperplasia (Ottesen et al., 2007; Noga, 2010). The primer set employed in this study is capable of amplifying every alloherpesvirus and iridovirus tested to date including agents that induce proliferative cutaneous lesions (Hanson et al., 2006). But viral amplicon was not present in PCR amplification. However, if at all virus is the cause, it might belong to a family not readily amplified by the employed primers or there is low titer of the virus.

Bacteria such as Streptococcus spp. are known to cause the eye disorder in cultured fish (Nakatsugawa, 1983). However, in most of the cases, the bacterial eye disease shows the symptoms of corneal opacity, haemorrhage in the eye and exophthalmia. The similar symptom was not observed in the eye of chocolate mahseer.

To the author’s knowledge, this is the first report documenting the occurrence of corneal hypertrophy in pond reared fingerlings of chocolate mahseer. Further study is needed to ascertain the exact cause of this disorder.

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References


