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Skeletal and swim bladder deformities in rohu, *Labeo rohita* (Hamilton, 1822) from grow-out pond, Tamil Nadu, India

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Abstract
The present study was conducted to investigate the skeletal deformities and swim bladder malformations in rohu (*Labeo rohita*). The fish were collected in the newly constructed cultured pond, Bhuvanagiri, Tamilnadu, India. Various patterns of skeletal deformities were observed in 13 of 497 sampled specimens (2.6 %). Deformities were diagnosed using a variety of clinical, radiographic and histopathological techniques. Witnessed deformities included kyphosis and lordosis. The swim bladder was deformed, with no connection between the two major chambers and with absence of pneumatic ducts. Histopathological investigations of ventral musculature tissue samples revealed the congestion of blood capillaries, dermal edema, inflammatory cells, as well as excess of melanophores and muscular edema. Observed deformities were confirmed using radiographic and histopathological techniques. The explanation of the witnessed deformities could not be safely determined, but possible attributions of causes are discussed.

Keywords: rohu, skeletal deformities, radiography, histopathology

1. Introduction
Rohu, *Labeo rohita*, is a cyprinid species, native inhabitant of rivers, streams, lakes and canals. It is widely cultured in Indian aquaculture (Dutta *et al*., 2013) [1]. Hora (1942) [2] and Law (1944) [3], both have described hump backed catla, these were the earlier records of deformities on freshwater carps of India. Similarly, Dutta *et al*. (2013) [1] observed deformations such as lordosis, kyphosis, scoliosis, disposition of dorsal and anal fins in reared *L. rohita*.
Various types of skeletal deformities has been reported in different fish species; these include lordosis, scoliosis, jaw deformities, spondylolisthesis, stump body, pugheadness, doublefins, semi-opened operculum, crossbite and finfusion (Alharbi, 2001; Cunningham *et al*., 2005; Issa, 2008) [4-6]. A wide spectrum of aetiology has been suggested such as genetic disorders, inbreeding depression, temperature fluctuation, low pH, parasitism, vitamin C deficiencies and environmental pollution (Orska, 1962; Koumoundouros *et al*., 1997; Eissa & Moustafa, 2008) [7-9].
This study aimed to investigate the deformities in reared rohu, *L. rohita*. Efficient methods namely, radiographic and histopathology were used to confirm the deformities.

2. Materials and Methods
2.1 Fish sampled
In March 2017, a total of 497 market size fishes were examined while harvesting from newly constructed semi-intensive cultured fish pond located at Bhuvanagiri (11.47°N 79.63°E), Tamil Nadu, India.

2.2 Clinical and morphological examination
The abnormal and normal fishes were collected and brought to the fish pathology laboratory for further investigation. Clinical and morphological examinations of fishes were performed at the fish pathology laboratory. Based on morphological examination the deformed fishes were grouped and the representative fishes from each group were examined with radiography and histopathology.

2.3 Radiography
A radiograph with a technique chart utilising 50kV, 10–20 mA and 10 cm thickness was used as described previously by Eissa *et al*. (2009) [10].

2.4 Histopathology
For histological analysis, ventral musculature tissue samples of deformed & normal fishes were fixed in 10% phosphate buffered formalin solution as per the standard procedure. Further the tissue samples were embedded in paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin (H&E) as described by Bancroft *et al*. (1996) [11].

3. Results and Discussion
3.1 Clinical and morphological examination
Out of 497 harvested rohu 13 (2.6 %) exhibited deformities. Deformed fish specimens were compared with normal (Fig. 1A-C) to ascertain deformations. No significant differences between the normal and deformed fishes weight. Morphological examination of deformed rohu indicated a large, abnormal growth at the anterior dorsal region, ventral region and caudal region (Fig. 1B). Similarly, a large lateral bulge truncated on post dorsal region or caudal region with depression was noted (Fig. 1C), from these Nine out of thirteen were grouped under deformed fish only with kyphosis

and four were clubbed as combined deformed fishes (with both kyphosis and lordosis).

3.2 Radiography

Radiographic examination was confirmed that the fish which was grouped as combined deformities has the spinal curvature in dorsal (kyphosis), ventral (lordosis) and post dorsal (kyphosis) region with exaggerated curves. Also, it was noted that the two major chambers in hydrostatic organ of swim bladder was disconnected with no pneumatic ducts (Fig. 2B). Whereas the fish with kyphosis was shows only post dorsal spinal curvature (kyphosis) with the normal architecture of swim bladder (Fig. 2C).

3.3 Histopathology

Histopathological investigations revealed that both the group of deformities were shows the congestion in blood capillaries, dermal edema and muscular edema in ventral musculature tissue. Apart from the ventral muscular architectural difference, the pathogenic cum stress response markers like inflammatory cells, excess melanophores were also observed (Fig. 3A-D).

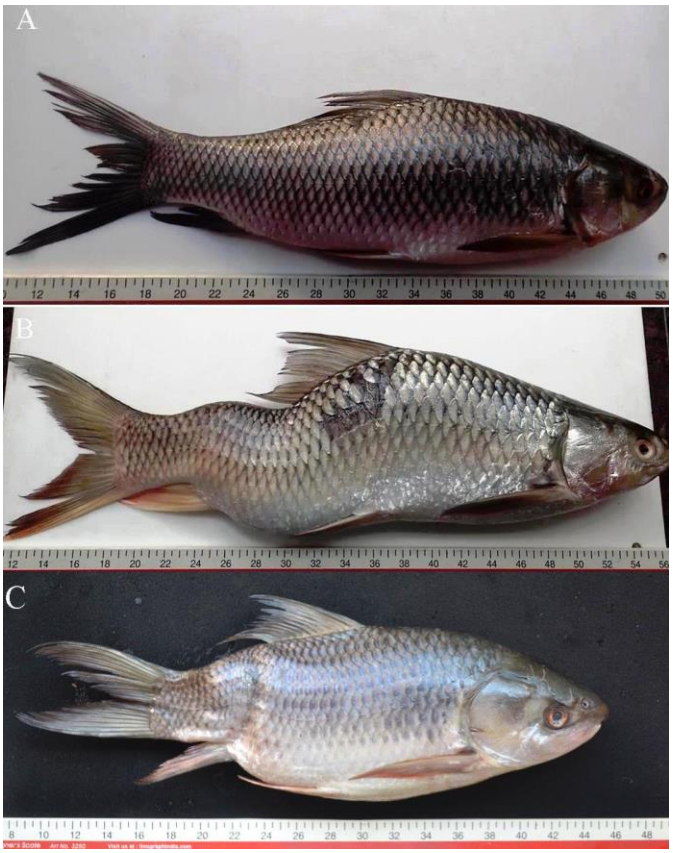

Fig 1: *Labeo rohita* (A) Normal specimen had a length of 21.3 cm and weight of 345.0 g (B - C) Deformed specimens had a average length of 20.9 ± 0.04 cm and average weight of 349.0 ± 0.5 g.

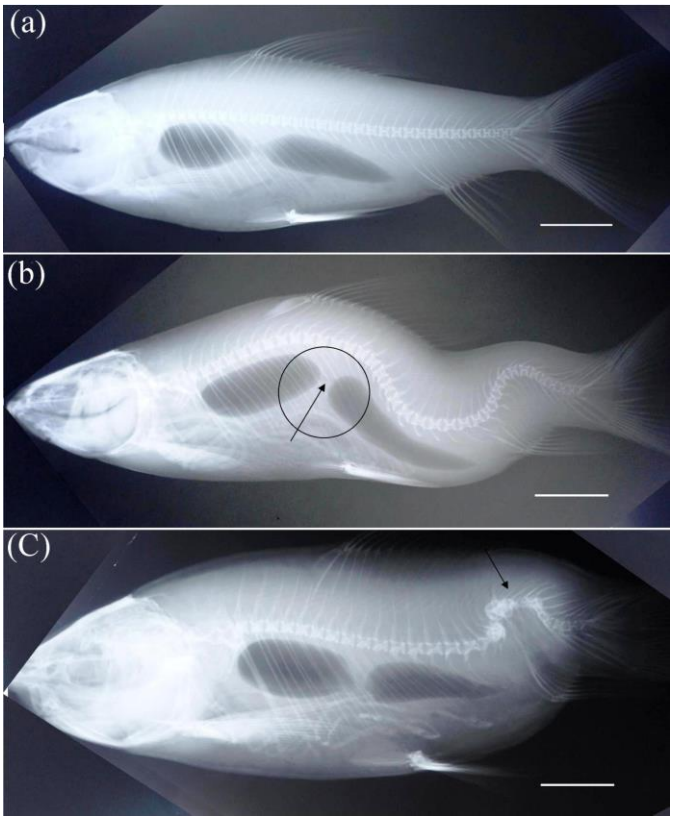

Fig 2: (A) X-radiograph of dorsal and ventral position of normal specimen (B) X-radiograph showing dorsal spinal curvature (kyphosis), ventral spinal curvature (lordosis), post dorsal spinal curvature (kyphosis) and encircle inside arrow indicates a malformation of swim bladder has the two major chambers were disconnected with absence of pneumatic ducts and (C) thin arrow showing post dorsal spinal curvature (kyphosis).

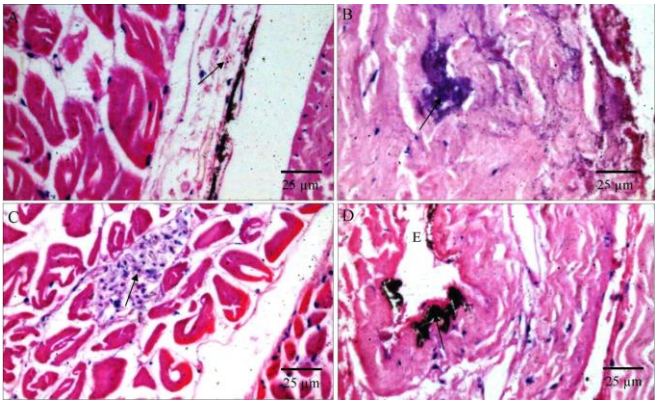

Fig 3: (A) Photomicrograph of deformed rohu muscle tissues showing (small arrow) congestion of the cutaneous blood capillaries. (B) Long arrow showing dermal edema. (C) Small arrow showing infiltrations of inflammatory cells (D) Arrow showing proliferation of melanophores and marked muscular edema **(E)** (H & E, 100x).

The newly constructed ponds required a continuous monitoring to overcome the next crop challenges. Though the study area is known for fresh water rohu cultivation, the deformities were observed for the first time by the local farmers. The noticed skeletal deformities (kyphosis and lordosis) were witnessed mostly at the dorsal, ventral and caudal region. Similar type of skeletal deformities was reported in *Atherina boyeri*, *Clarias gariepinus* and *Salmo salar* from fresh and brackish water environment (Dutta *et al*., 2013; Jawad *et al*., 2017) [1, 12].

Vitamin C deficiency, high temperature, vigorous rearing, parasite and genetic disorders are suspected as causative agents for fish skeletal deformities (Jawad *et al*., 2017) [12]. Earlier investigation stated that vitamin C deficiency might causes kyphosis, lordosis and decreased collagen content of spine in channel catfish *Ictalurus punctatus* (Lim & Lowell, 1978) [13]. Since, the current pond water and feeds were not tested at the earlier stage of rearing, the possibilities of vitamin C deficiency for the current observation cannot be ruled out. Same way the genetic disorder can be ruled out because the seedling were purchased as whole lot and no proven methodologies has been reported to test at this stage. Parasite and the disease have been ruled out from the clinical examinations. The possible reason could be either in temperature or rearing intensification.

Felizardo *et al*. (2011) [14] reported deformations in piapara, *Leporinus obtusidens* such as irregular coelomic cavity and sac like projection without exteriorization in swim bladder. However, in the present study such deformations in swim bladder were not observed instead the disconnection between two major chambers in swim bladder was observed with the absence of pneumatic ducts. Investigation carried out by Haenan *et al*. (1994) [15] suggested that contamination by worm *Auguillicola crassus* in young specimens might cause severe lesions and inflammation in swim bladder. However, in the present study showed no evidence of inflammation process but retarded development was found in swim bladder. This type of pathology was therefore ruled out as a source of swim bladder abnormality.

In the present study, the observed epidermal and muscular edema as well as inflammatory cell aggregation in rohu *L. rohita* could cause chronic irritation that could be linked to erratic movements of deformed vertebrae, manifested as lordosis or kyphosis. Previously, similar type of histological observation was reported in *Clarias gariepinus* (Eissa *et al*., 2009).

4. Conclusion

From the observation, it was clearly witnessed that the condition is not fatal and certainly affected mobility in some way. Hence, it is preliminary to conclude their impact on reproduction and other response on environmental stress for deformed fishes. To our knowledge, this present study is the first report of skeletal and swim bladder deformities in reared rohu, *L. rohita*.

5. Acknowledgements

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