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Research Article

COMPARATIVE MORPHOMETRIC ANALYSIS OF VERTEBRAE AND EXTERNAL MORPHOLOGY IN DEFORMED FISH SPECIMENS: AN OBSERVATIONAL SERIES OF CASE STUDIES

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ABSTRACT

The fish specimens sometimes are observed with abnormal morphological features, which may lead to vertebral deformities, and is the symptom of a teratogenic effect. The present study was attempted to perform comparative morphometric analysis of freshwater fish *Labeo rohita* (Hamilton-Buchanan) to identify the external morphology and vertebral structure compared to normal features. The morphometric analysis, especially external morphology and vertebral column deformities were studied on three abnormal specimens and one normal specimen of *L. rohita*. The morphological features *viz*. head length (HL), head width (HW), body length (BL), body width central position (BW), caudal fin length (CFL) and caudal fin width (CFW) were measured manually. For vertebral deformities study, radiographs were taken to know bone structure especially vertebrae and further measurements *viz*. angle (in degree), length (cms) and numbers were performed by using ImageJ tool (version, 1.49). Among 4 specimens, normal fish was found without any morphological and vertebral deformities while 3 specimens were observed abnormal morphology like tail bulging and bending, the truncated body as well as vertebral deformities especially scoliosis (bending dorsally), kyphosis (calcification) and lordosis (bending ventrally) and fused in the caudal vertebrae, which were confirmed with the 3D interactive surface plot. In conclusion, this observational study is indicated suitable information on the morphometric analysis in the *L. rohita*, but the cause of deformities is unknown. In future, it is suggested to recover these abnormalities concerning specific factors like genetic, abiotic, nutritive, etc. that can be prevented the economic loss because fish is an important diet.

Keywords: Fish specimen, *Labeo rohita*, Freshwater fish, Morphometric analysis, Vertebral deformities, Image analysis.

1. INTRODUCTION

Among several edible aquatic species, fish is a most common food for human beings in which large population of India including West Bengal depends upon fish species of freshwater and marine origin as main diet [1-2]. There are several fish species found in and around Kolkata market from freshwater, brackish water, and the marine origin and most are found in all seasons.

In many studies from national and international context, morphological anomalies were observed in fish species such as *Oreochromis* sp., *Mozambique tilapia*, *Catla catla*, *Barbus barbus*, *Barilius bendelisis*, *Cirrhinus mrigala*, *Puntius sarana*, *Tor putitora*, *Heteropneustes fossilis*, *Mystus bleekeri*, *Labeo rohita*, etc. Several studies have emphasized different types of morphological abnormalities such as fin erosion, fin deformity, lower lip protrusion, gill deformity, ocular disorders, scale deformity and disorientation, neoplasia or hyperplasia, etc. observed in the fish species due to water pollution [3-14].

To date, the abnormalities in the skeletal system are not clear and it was observed that individually or combinations of nutritional, environmental, traumatic injuries, and genetic factors [12, 14-24]. The skeletal deformities are induced during the embryonic and post-embryonic periods of life of fish [24]. As per researchers, different types of bone deformities were observed in the specific area such as skull, jaws, lordosis, scoliosis, and kyphosis in vertebrae, etc. [9, 11-14, 23, 25].

It was attempted to conduct a comparative morpho-metric analysis of freshwater fish *Labeo rohita* F. Hamilton found with abnormal morphology and vertebral deformities compared to normal features.

2. MATERIAL AND METHODS

The fish specimens, *Labeo rohita* were collected from the local fish seller of wholesale market of West Bengal. Just

died fish of 4 specimens were observed visually in which 3 specimens were found abnormal in gross morphology. In all these 4 specimens, different morphological features such as overall length, head length (HL), head width (HW), body length (BL), body width central position (BW), caudal fin length (CFL) and caudal fin width (CFW) were measured (cms) manually.

In the second part of the study, radiography was performed by using X-ray machine (ME-3010) and examined the vertebral deformities such as scoliosis (bending dorsally), kyphosis (calcification) and lordosis (bending ventrally) related to angle (in degree), length (cms) and numbers of vertebrae by using ImageJ tool (version 1J 1.49) developed by National Institute of Health, Bethesda, MD, USA [26]. Interactive three-dimensional (3D) surface plot was visualized after the selection of a vertebral column of each specimen by using the ImageJ plugin of the interactive 3D surface plot as per Barthel [27]. The luminance of each pixel of the vertebral column in each image was interpreted as the height, length, and curvature of the surface plot after selection of spectrum LUT.

3. RESULTS

The present observational study indicates the fish specimens (*L. rohita*) in which one was observed normal and three specimens were found with abnormal morphological features and vertebral deformities.

Table 1 tabulates the description of morphometric analysis of external morphology of studied 4 specimens. For normal category, specimen 1 is observed overall length of 21.2cm and body weight of 380gms and other parameters (cms) *viz.* HL (6.50), HW (4.80), BWcp (5.41), CFL (6.30) and CFW (5.40), respectively (fig. 1a). In case of abnormal categories, the specimen 2 is observed overall length of 17.0cm and body weight of 200gms and other parameters (cms) *viz.* HL (3.43), HW (2.56), BWcp (4.08), CFL (3.90) and CFW (2.42), respectively (fig. 2a) while the specimen 3 is observed overall length of 17.2cm and body weight of 210gms and other parameters *viz.* HL (2.11), HW (1.90), BWcp (3.90), CFL (3.50) and CFW (2.66), respectively (fig. 3a) and the specimen 3 is observed overall length of 17.3cm and body weight of 510gms and other parameters *viz.* HL (4.51), HW (3.90), BWcp (5.83), CFL (3.43) and CFW (2.51), respectively (fig. 4a).

Table 2 describes the morphometric analysis of the vertebral column in the X-ray images of studied 3 abnormal specimens and 1 normal specimen. In specimen 1, the normal vertebral column is containing 32nos. of vertebrae in which trunk vertebrae of 15 nos. and caudal vertebrae of 17 nos. were observed and designated as a control specimen (fig. 1b). In the case of specimen 2, deformed vertebrae (32 nos.) were observed such as scoliosis, kyphosis (calcification) and lordosis in caudal vertebrae of 13-17nos., 11-12 nos. and 6-10 nos. andit was also observed deformed upper caudal spines some degeneration (fig 2b). In case of specimen 3, deformed vertebrae (32 nos.) along with gaps formed on neural spine and caudal vertebrae of 16-32 nos. were observed "S' shaped as well as scoliosis and lordosis in caudal vertebrae of 16-25 nos. and 26-32 nos., respectively (fig. 3b). In the case of specimen 4, deformed vertebrae of 30 nos. were observed due to fusion of 31-32 nos. at the caudal region and caudal vertebrae showed one lordosis (6-12 nos.) as well as two scolioses as 1st (11-14 nos.) and 2nd (15-19 nos.) and kyphosis (24 no.), respectively (fig. 4b).

Table 1: Morphometric analysis of external morphology of fish specimens of fish specimens (*L. rohita*)

Fish specimens	Overall Length (in cm)	Body weight (in gms)	HL (in cm)	HW (in cm)	BWcp (in cm)	CFL (in cm)	CFW (in cm)
Specimen 1 (normal)	21.2	380	6.50	4.80	5.41	6.30	5.40
Specimen 2 (deformed)	17.0	200	3.43	2.56	4.08	3.90	2.42
Specimen 3 (deformed)	17.2	210	2.11	1.90	3.90	3.50	2.66
Specimen 4 (deformed)	17.3	510	4.51	3.90	5.83	3.43	2.51

Nos. = Numbers; cms = Centimeters; gms = Grams; HL = Head length; HW = Head width; BL = Body length; BWcp = body width central position; CFL = Caudal fin length; CFW = Caudal fin width

Table 2: Morphometric analysis of the vertebral column of fish specimens (*L. rohita*)

L. rohita	Total vertebrae (in nos.)	Length of VC (in cms)	Angle measurement (in degree)	
			Scoliosis	Lordosis
Specimen 1 (normal)	32	15.00	---	---
Specimen 2 (deformed)	32	10.10	180	90
Specimen 3 (deformed)	32	8.90	60	60
Specimen 4 (deformed)	30	9.00	90 and 43.15	90

Nos. = Numbers; cms = Centimeters; VC = Vertebral column

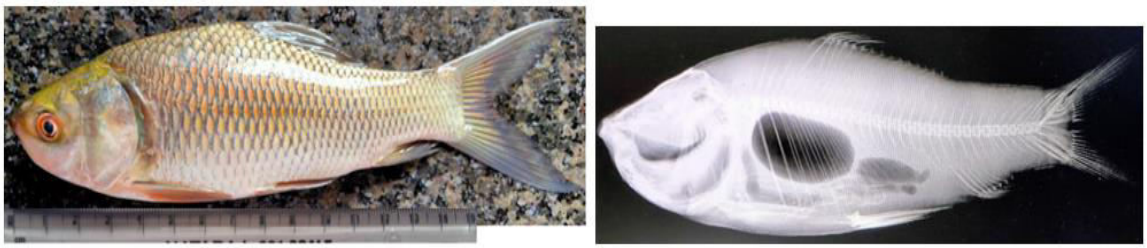

Fig 1: (a) External morphology and (b) X-ray image of *L. rohita* showing normal body featureand vertebrae

Fig 2: (a) External morphology and (b) X-ray image of *L.rohita* is showing bulging at caudal region and deformities of caudal vertebrae

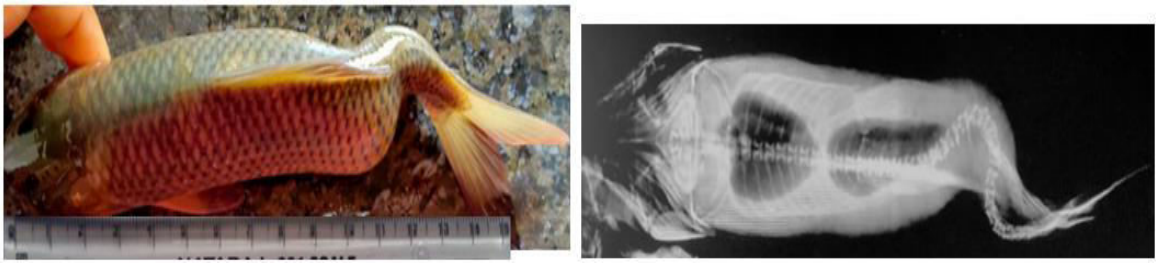

Fig 3: (a) External morphology and (b) X-ray image of *L.rohita* is showing bending at caudal region anddeformities of caudal vertebrae

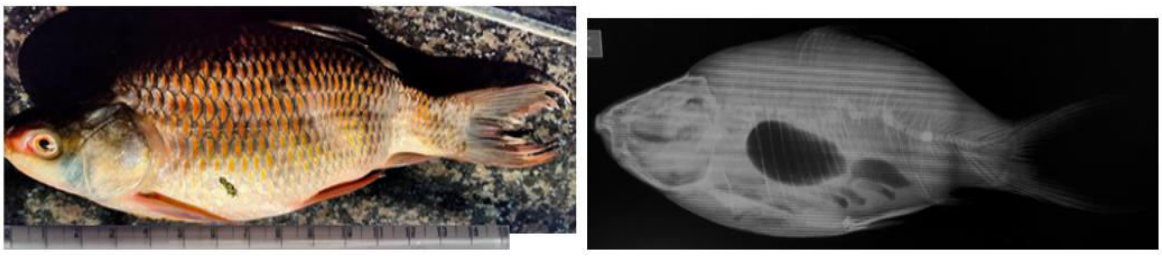

Fig 4: (a) External morphology and (b) X-ray image of *L.rohita* is showing thickening of trunk region and deformities of the trunk and caudal vertebrae

The 3D interactive surface plots are exhibited for normal vertebrae (fig. 5) and abnormal vertebrae (fig. 6). These graphs may determine the height and curvature as two independent variables (X and Z axes) and one dependent variable as length (Y-axis). Moreover, each plot estimates the surface of objects and line colours that obtained an area of the same range of values. In the present findings, the normal vertebral plot shows a smooth surface with highest values of 190-250 (fig. 5) while abnormal vertebral plots show the ranges were lesser with highest values of 160-180 (fig. 6).

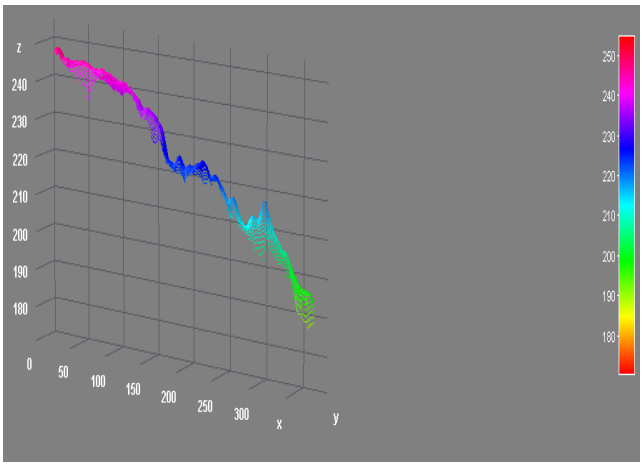

Fig 5: 3D interactive surface plot of vertebral column of normal specimen of *L. rohita*

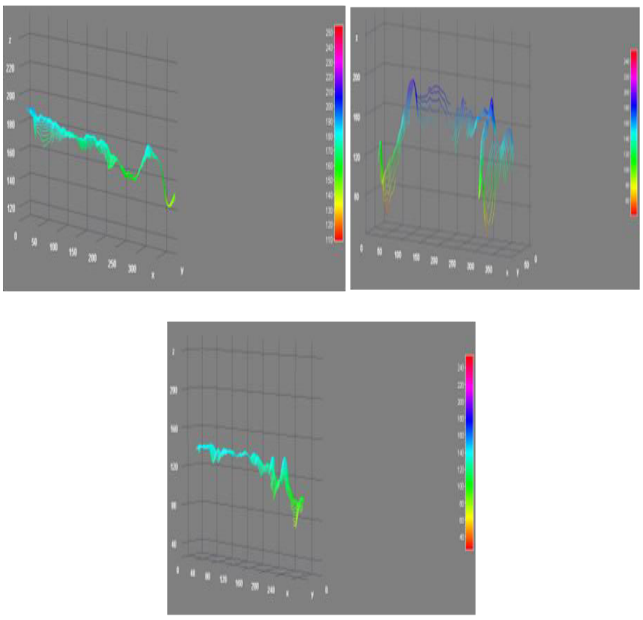

Fig 6: 3D interactive surface plots of vertebral column of abnormal specimens of *L. rohita*

4. DISCUSSION

The present observational study on morphometric analysis of external morphology and vertebral column deformities such as scoliosis, lordosis, and kyphosis in the fish specimens (*L. rohita*) found an informative teratogenic effect when compared to normal features, which may be influenced by several factors in combination or individually such as temperature variation, salinity fluctuation, radiation, heavy metals, organic compounds, pesticides, dietary vitamin deficiency, etc. The present study has some similarities related to abnormal morphology and vertebral column deformities in different fish species with earlier works in India and other parts of the globe [9, 11-14, 23, 25, 28]. Kessabi et al. [28] reported abnormalities in the spines of Mediterranean killifish, *Aphanius fasciatus* in Gulf of Gabès in Tunisia due to the cadmium pollution. Bhagat et al. [11] studied on three fish species viz. *Cirrihinus mrigala*, *Catla catla* and *Labeo rohita* collected from Ranjit Sagar reservoir built on river Ravi at Basholi (J and K) but the region of deformities is unknown as they mentioned developmental error. In another study by Alarape et al. [13] skeletal and morphological abnormalities in African catfish (*Clarias gariepinus*, Burchell 1822) have been increased from hatcheries on farms in Ibadan, Nigeria and the cause of deformities is unknown. In a recent study, it was identified that European seabass (*Dicentrarchus labrax*) had observed variation of caudal vertebrae as 24-26 nos. in all three age groups (fingerlings, juveniles and adults), as a malformation of vertebrae [29]. But in past, an interesting work carried out by Fragkoulis et al. [14] regarding recovering of lordosis after caging and maintaining the temperature and foods for the growth of juveniles (*Sparus aurata* L.). Interestingly, the measurement of height, length and curvature obtained through the 3D interactive surface plot, which cannot be possible to estimate manually all these values. In the present study, the ImageJ tool automatically evaluated the features within vertebrae in abnormal specimens in comparison with the normal specimen.

5. CONCLUSION

Through an observational study, it was recorded that abnormal morphology like tail bulging and bending, the truncated body as well as vertebral deformities especially scoliosis, kyphosis (calcification) and lordosis and fused in the caudal vertebrae of *L. rohita* may be suitable information of habitat. Still, the cause of deformities is unknown, it is suggested in future to study the recovery aspects about factors like genetic, abiotic, nutritive, etc. that can be maintained during the growth of fish in the farms to prevent the economic loss because fish is an important diet.

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Conflict of interest

Authors declare no conflict of interest.

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