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Meristic variations and skeletal deformities in natural population of mahseer fish, *Tor putitora* (Hamilton, 1822).

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Abstract

Skeletal deformities seriously relegate the biological performance of fish. The frequency of occurrence of different types of meristic variations and skeletal deformities were seen in the population of mahseer fish, *Tor putitora*, in two main reservoirs; Korang Stream, Islamabad and Dhobi Ghat Stream, Hassan Abdal. A total of ninety-five fish were caught randomly, forty-five from the unpolluted Korang Stream and fifty from the polluted Dhobi Ghat Stream by cast nets from February to July, 2009. Radiographs and dry skeletal preparation techniques were used to record meristic variations and skeletal deformities and describe various types and frequency of appearance. Deformities were studied under categories, Highest Frequency of Occurrence (HFO), Normal Frequency of Occurrence (NFO) and Lowest Frequency of Occurrence (LFO). In the deformed fishes, significant (p<0.001) anomaly was noticed in nineteenth to twenty second and forty one to forty third (p<0.05) vertebrae of the vertebral column. Deformities scoliosis (C2, 5.26%), platyspondyly (C5, 11.27%), and abnormal neural spine (TC25, 13.53) appeared to affect 70%, 60% and 50% of the deformed individuals respectively. This study concluded that skeleton deformities in *T. putitora* population in the laundry waste water of Dhobi Ghat Stream have relevance with detergent waste, as a possible causative agent.

Keywords: Meristic variations, Skeleton, Deformities, *Tor putitora*

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Introduction

Mahseer fish, *Tor putitora*, is one of the most important cyprinid fish of the South Asian subcontinent. It is also known as golden mahseer. Because of its big size and fighting properties, it is an excellent game fish (Bhatt and Pandit, 2016). Skeletal deformities seriously downgrade the biological performance of any fish (Raja *et al*., 2016). Skeletal deformities have been extensively used by fishery biologists to monitor health of fish population (Lindesjöö and Thulin, 1992) under existing water regime. Wild fish, which carry deformed morphology, face problems in competing for mates, defending their territory and low survival rate and turn out to be inferior both in intra and interspecific competition, compared to the normal conspecifics (Sato, 2006). In salmon study, a low occurrence of vertebral deformities have little effect on the growth performance (Hansen *et al*., 2010).

A wide variety of chemical, physical and biological stressors have been associated with the occurrence of skeletal deformities. These chemicals often induce neuromuscular damage that can result in skeletal deformities (Bengtsson and Larsson, 1986). Certain chemicals can also cause skeletal deformities by impairing developmental processes and bone formation. Compounds such as 2,3,7,8, tetrachlorodibenzo-p-dioxin, polyaromatic hydrocarbons, polychlorinated biphenyls, toxaphene and cadmium have been reported to cause skeletal deformities through such mechanisms (Olsson *et al*., 1999). Skeletal deformities have also been linked to water quality problems including low pH (Trojnar, 1977), low dissolved oxygen (Garside, 1959), and elevated temperature (Kwain, 1975). Other factors such as genetics (Sadler *et al*., 2001), infections (Madsen *et al*., 2001) and antibiotics (Pates *et al*., 2016) and intensive aquaculture conditions (Koumoundouros *et al*., 2001) may also induce deformities. Etiology and precise mechanism of such skeletal deformities are still unknown, which makes it difficult to achieve an effective prevention protocol in hatcheries. Skeletal deformities induced by excess VA are a popular model for studying the development of skeleton in fish larvae. Several studies suggest the importance of retinoic acid receptor (RAR) and retinoid X receptor (RXR) pathways in skeletogenesis in fish.

The majority of the studies conducted in Pakistan on mahseer fish, *T. putitora* until now are largely confined to systematics (Mirza, 2004), food and feeding (Subhan and hafeez, 1994), gonadal maturation and breeding (Subhan and Hafeez, 1998), length-weight and condition factor relationship (Zafar *et al*., 2001), distribution (Akhtar *et al*., 2016) and toxicology (Khan *et al*., 2016). Fish populations in Dhobi Ghat Stream, which receives detergent waste, have some sort of skeletal deformities compared to the

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fish population in Korang. The objective of the present study is to record meristic variations and skeletal deformities and describe their various types and frequency of appearance in crooked mahseer from polluted Dhobi Ghat Stream and unpolluted Korang Stream waters.

Materials and methods

Sampling area

Dhobi Ghat a natural stream located at 33°49.4′N, 72°41.7′E and altitude 441 m near Hassan Abdal, Punjab province of Pakistan. Korang Stream is a tributary of Soan River which is located at 33°16.4′N and 73°48.8′E and altitude 13.9 m running through mid of Islamabad city, Capital of Pakistan. The water of Dhobi Ghat natural stream is continuously polluted by the direct entry of waste water from nearby laundry units for past 15 years. This stream is the natural seasonal spawning ground of mahseer.

Collecting strategy

A total of ninety five fish were collected; fifty morphologically deformed specimens (31.90 ±2.8 cm total body length) from the fish population at Dhobi Ghat and forty five morphologically normal specimens (31.7±2.7 cm total body length) from the fish population at Korang Stream by random sampling technique.

Radiography of skeleton

The fish samples were euthanized for radiography in the lateral position on Trophy X-rays Machine 500 mAs, using Kodak green film (size 35×43 cm) at 100 mAs and 40 kV for 2 seconds. With some modification, the dry bone preparation method (Hussain, 2000) was also used to study skeleton deformities through and through.

Meristic and skeletal abnormalities

Abnormalities in meristic characters were studied by the method of Lewis *et al*. (2004). These included total number of vertebrae, trunk and caudal vertebrae and the number of deformed vertebrae. The vertebral column was distinguished as anterior trunk region and posterior caudal region. The posterior caudal vertebrae lack ribs, but possess haemal arches (Alvi, 1999). If centrum of two vertebrae were partially fused with each other, they were counted as two separate vertebra and as a single element when centrum were fused completely (Ferreri *et al*., 2000).

The alphanumeric dichotomic key of Lewis *et al.* (2004) was used for characterization of skeletal abnormalities (Table 2). The location and variation of deformities were studied by observing the structure, size and position of each vertebra in all fish samples (Madsen and Dalsgaard, 1999).

Assessment of abnormalities

The number of deformed fish were counted and classified as % deformity (Tutman *et al.*, 2000). The % frequency of abnormal individuals (FAI) and frequency of abnormalities (FA) were

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estimated as described by Lewis *et al.* (2004).

Results

During the sampling period, a total of ninety five mahseer fishes were caught, fifty from the polluted Dhobi Ghat Stream where 25% samples were observed with morphologically deformed body and forty five from unpolluted Korang Stream where none of the fish was found to be morphologically deformed.

In *T. putitora*, the vertebral column is composed of an anterior trunk region of 22 (1-22) vertebrae and a posterior caudal region of 21 (23-43) vertebrae. Fish population of Korang Stream possessed a constant total number of vertebrae in the vertebral column i.e., 43 whereas in Dhobi Ghat population, it ranged from 41-43. It was also observed that such deformity was more pronounced in the caudal region as compared to the trunk region. In the present study there were significant differences (p<0.05) in the total number of vertebrae whereas the number of caudal vertebrae showed highly significant (p<0.001) differences (Table 1).

Table 1: Meristic characters used for analysis of axial skeleton of mahseer, *Tor putitora*.

Meristic characters	Korang Stream (Normal fish, n=45)	Dhobi Ghat Stream (Deformed fish, n=50)	
	Mean (mm)	Mean (mm) ± S.E	Range
Total no. of vertebrae	43.0 ± 0.0	*42.4 ± 0.22	41.0 - 43.0
No. of vertebrae in trunk region	22.0 ± 0.0	22.0	22.0
No. of vertebrae in caudal region	21.0 ± 0.0	**20.4 ± 0.22	19.0 - 21.0
No. of affected vertebrae	-	3.8 ± 0.39	2.0 - 5.0

* Significant difference (p<0.05)
** Highly significant difference (p<0.001).

The skeletal abnormality in terms of highest frequency (>12.78%) of occurrence (HFO), was an abnormal neural spine (CT25, 13.53%) although this single abnormality affected only 50 % of deformed specimens (Table 2) while the most representative abnormalities (3.01-12.78%) were scoliosis (C2, 5.26%); vertebral ankylosis (C4, 8.27%); platyspondyly (C5, 11.27%); missed neural element (C6, 7.51%); abnormal neural canal (C11, 5.26%); and abnormal haemal spine (C26, 12.78%). The abnormalities in terms of lowest frequency (<3.01) of occurrence (LFO) was lordosis (C1, 0.75%); lordosis-scoliosis (C3, 0.75%); missed haemal element (C7, 3.01%), exposed spinal cord (C8, 2.25%); notochord distortion (C9, 1.51%); vertebral hypertrophy (C10, 1.51%); abnormal haemal canal (C12, 3.01%); fused neural elements (C13, 3.01%); fused haemal elements (C14, 1.51%); missed neural arch (T15, 0.75%); unfused neural arch (C16, 0.75%), detached neural element (C17, 0.75%); bifurcated neural spine (T18, 3.01%); supernumerary spine (T19, 0.75%); supernumerary ribs

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(T20,1.51%); missed ribs (T21, 0.75%); weberian apparatus (T22, 0.75%); overossified haemal arch (T23, 3.01%) and short haemal arch (C24, 1.51%). These abnormalities affected 10-30 % of deformed individuals (Table 2).

Table 2: Number and frequency of abnormal individuals (FAI) and frequency of abnormalities (FA).

Skeletal abnormalities[a]		Frequency (%)			
		No.[b]	FAI	No.[c]	FA
	Axial abnormalities				
C1	Lordosis	1	10	1	0.75
C2	Scoliosis	7	70	7	5.26
C3	Lordosis–scoliosis	1	10	1	0.75
	Major abnormalities				
C4	Vertebral ankylosis	7	70	1	8.27
C5	Platyspondyly	6	60	7	11.27
C6	Missed neural element	6	60	1	7.51
C7	Missed haemal element	3	30	1	3.01
C8	Exposed spinal cord	3	30	7	2.25
C9	Notochord distortion	2	20	1	1.51
C10	Vertebral hypertrophy	1	10	1	1.51
	Minor abnormalities				
C11	Abnormal neural canal	4	40	7	5.26
C12	Abnormal neural canal	4	40	4	3.01
T13	Fused neural elements	1	10	4	3.01
C14	Fused haemal elements	2	20	2	1.51
T15	Missed neural arch	2	20	1	0.75
C16	Unfused neural arches	1	10	1	0.75
C17	Detached neural element	1	10	1	0.75
T18	Bifurcated neural spine	1	10	4	3.01
T19	Supernumerary neural spine	1	10	1	0.75
T20	Supernumerary ribs	1	10	2	1.51
T21	Missed ribs	1	10	1	0.75
T22	Weberian apparatus	1	10	1	0.75
T23	Overossified haemal arch	4	40	4	3.01
C24	Short haemal arch	2	20	2	1.51
CT25	Abnormal neural spine	5	50	18	13.53
C26	Abnormal haemal spine	5	50	17	12.78
	Total anomalies events	73		88	

[a]Skeletal abnormalities location: C-Caudal and T-Trunk.
No.[b] Number of abnormal individuals.
No.[c] Number of abnormalities.

Further the observed abnormalities in *T. putitora* were categorized into three groups i.e., axial abnormalities, major abnormalities and minor abnormalities (Table II). In the first category, the axial abnormalities included scoliosis

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(C2, 5.26%) with HFO as it also affected 70 % of deformed fish, compared to lordosis (C1, 0.75%) and lordosis-scoliosis (C3, 0.75%) abnormalities that have LFO, each of these affected 10 % of deformed fish whereas only 10 % of deformed individuals did not show any sign of axial abnormalities in this category (Table 2). In the second category, the major abnormalities, HFO was observed for platyspondyly (C5, 11.27%) that affected 60 % deformed fish while LFO was observed for notochord distortion (C9, 1.51%), which affected 20 % deformed fish while vertebral hypertrophy (C10, 1.51%) affected 10 % of deformed fish (Table 2). In the third category, minor abnormalities which appeared with HFO was the abnormal neural spine (CT25, 13.53%) affecting 50 % of fish whereas unfused neural arch (C16, 0.75%); detached neural element (C17, 0.75%); supernumerary neural spine (T19, 0.75%); missed ribs (T21, 0.75%); weberian apparatus (T22, 0.75%) were the minor abnormalities which occurred with LFO and affected 10 % of deformed fish samples, except missed neural arch (T15, 0.75%) which affected 20 % of the deformed fish (Table 2).

The most serious axial abnormalities were scoliosis; lordosis and lordosis-scoliosis which appeared in the caudal region (24^{th}–31^{st}) of the vertebral column (Fig. 1). The maximum axial angle observed was 49^{o} in a sample (45 cm total body length) that has lordosis while minimum 8^{o} in the sample (31.8 cm total body length) affected with scoliosis (Fig. 1).

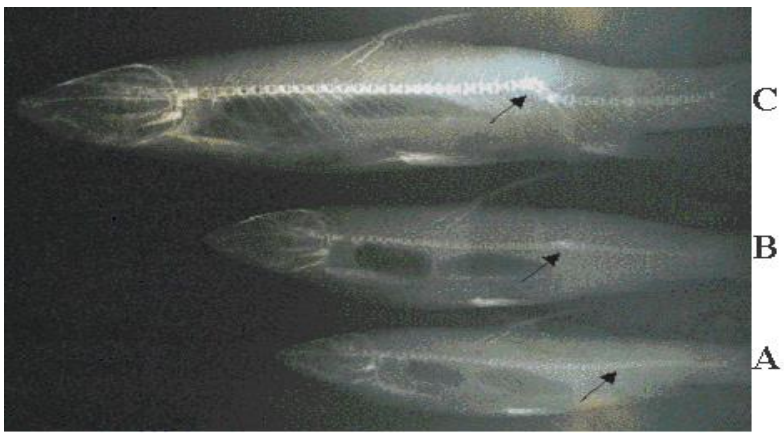


Figure 1: Radiological illustration of mahseer *Tor putitora* skeleton. (A) Normal, (B) Scoliosis, (C) Lordosis.

Discussion

In *T. putitora*, the vertebral column was composed of an anterior trunk region of 22 (1-22) vertebrae and a posterior caudal region of 21 (23-43) vertebrae. The fish population of Korang Stream possessed a constant total number of vertebrae in the vertebral column i.e., 43 whereas in Dhobi Ghat population, it ranged from 41-43. Mahseer *Naziritor*

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zhobensis is reported to have 23 vertebrae for the anterior trunk and 18 for the posterior caudal region of vertebral column (Alvi, 1999). This comparison, shows fusion of vertebrae severally causing the difference in length as compared to its normal counterparts. Such anomalies are reported due to complete vertebral ankylosis in which completely fused vertebrae are counted as one (Ferreri *et al.*, 2000). In our results the axial angle was 49^{o} relating to lordosis while the minimum angle 8^{o} based on total body length is the outcome of scoliosis (Fig. 1).

Other reports mentioned that axial angle in deformed *S. auratus* ranges from 10^{o}–120^{o} (Chatain, 1994). Vertebrae were not malformed randomly, some regions such as the caudal region were more susceptible to malformation than the trunk region. Caudal regions are comparatively more susceptible as it is involved in providing maximum propulsion against water. Caudal damage could make fish prone to be attacked by predators. No work so far is reported on vertebral abnormalities in *T. putitora*. These axial abnormalities are important as they affected both size and shape of the fish (Favaloro and Mazzola, 2000). Axial curvature ratio found in our study ranged from 1.01–1.07 which is in agreement with deformed *golden sparse* which possessed 1.01-1.03 (Gerhard *et al*., 2002).

This study concluded that *T. putitora* has skeletal deformities specifically in the caudal region. Fusion of vertebrae indicates lordosis and scoliosis which could originate due to many chemical, biological and physical factors. Further studies are required to find synergistic effects of detergent quality and concentration in relation to the development of such skeletal deformations. The present study has provided useful preliminary information on deformity in mahseer fish which would help the wild fish conservators to take due measures to save this endangered fish species.

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