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Skeletal Deformities in Invaded Population of Amazon Sailfin Catfish *Pterygoplichthys pardalis* (Castelnau, 1855) in the East Kolkata Wetland, India

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ABSTRACT

The incidence of severe vertebral deformity was reported in the specimens of the Amazon sailfin catfish *Pterygoplichthys pardalis* (Castelnau, 1855) for the first time in India. Fish samples were collected on bimonthly basis during June 2019 to December 2020, from wastewater-fed large aquaculture impoundments (locally called 'bheries') of north-western part of the East Kolkata Wetlands, India. Sampling were performed using seine nets of mesh size 15 mm–30 mm and covering more or less 5,000 sq m area each time. Deformities were found in 18 specimens of *Pterygoplichthys pardalis*. Two types of spinal deformities, scoliosis and kyphosis, were determined visually and reconfirmed by radiography using medical X-ray system. The total length and body weights of individual fish (normal and deformed) were measured and the length-weight relationships parameters were calculated and compared. A significant difference was observed between b values of deformed (b ~1.679) and normal specimens (b>2.5) of *Pterygoplichthys pardalis*. Various environmental and genetic factors could contribute to the development of this deformity in the *Pterygoplichthys pardalis*, but based on the available data, it is impossible to confidently identify the key factor(s). However, as the fishes were from a wastewater-fed fisheries system, which receive about 600 million litres of industrial and municipal discharge of the city every day, metal and other organic contaminants could be a reason of this impairment in *Pterygoplichthys pardalis*.

KEYWORDS: Vertebral deformities, alien fish, scoliosis, pollution, *Pterygoplichthys pardalis*

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Whole vertebral column deformities like scoliosis (lateral curvature), lordosis (V-shaped dorsal-ventral curvature), kyphosis (Λ-shaped dorsal-ventral curvature) and ankylosis (abnormal stiffening and immobility of joint due to fusion of bones) (Fagbuaro and Oso, 2011), have been described in many species both cultured and from wild populations (Jawad et al., 2017; Wakida-Kusunoki et al., 2017; Fjelldal et al., 2019). Lordosis, which can affect every region of the vertebral axis, is probably the most well studied and commonly occurring axis anomaly in fishes (Jawad et al., 2017). Kyphosis is generally less common than lordosis (Boglione et al., 1995) and in very severe cases only combinations of both lordosis and kyphosis occur (De Clercq et al., 2018). In addition, vertebra body deformities like compressions, fusions, vertical shift and re-modeling (cases where two or more adjacent vertebra fuse and remodel in to a "normal" enlarged vertebra) have also been reported in fish (Witten et al., 2006; Golubtsov et al., 2021). Factors such as high rearing temperatures, vitamin C and phosphorus deficiency, genetic factors, bacteria and parasites infections are among the other factors referred in the literature as possible cause of these deformities in farmed fish (Lovett et al., 2020). Deteriorated water quality (low dissolved oxygen, increase levels of unionized ammonia, hydrogen sulphide, nitrite, organic matter in water), high stocking densities and inadequate nutrition are some of the other stressors to farmed fish (Hussan et al., 2017; Suguna, 2020) that can induce deformities. In wild fish populations such abnormalities are relatively less frequent, and mostly correlated with water pollution (Jawad et al., 2017). Mostly these abnormalities are induced during the embryonic and post-embryonic periods of life (Eissa et al., 2009) and proposed to cause multi-factorial physiological imbalances in fish (Golubtsov et al., 2021). Skeletal abnormalities can either be slight or very severe, and accordingly it can affect the ability of the fishes to swim and acquire food, and/or their survival (Jawad et al., 2017).

The South American loricariid catfishes of the genus *Pterygoplichthys* (Gill, 1858) are among one of the alien fish groups most seriously threatening tropical and subtropical freshwater regions of the world (Zworykin and Budaev, 2013; Capps and Flecker, 2013; Hussan et al., 2019). Due to characteristic attractive appearance, these fishes have high popularity and introduced worldwide for the aquaria and subsequently became invasive in many parts of the world (Wu et al., 2011). These alien fishes were introduced in East Kolkata Wetlands (EKW) around 2002 or 2003 by an aquarist, who released three specimens of *Pterygoplichthys* in a sewage feeder channel and gradually became invasive in the wastewater-fed large impoundments of the wetland (Hussan et al., 2019; Hussan et al., 2020). Within the genus *Pterygoplichthys* four species viz. *Pterygoplichthys pardalis, P. disjunctivus, P. multiradiatus* and *P. anisitsi* have closely related character and are distinguished mainly based on their ventral spot patterns (Armbruster and Page, 2006). However, due to natural or artificial hybridization large number of these species with intermediate ventral spot patterns coexists in nature, which further complicated the identification of these fishes to species level (Wu et al., 2011; Nico et al., 2012; Hussan et al., 2018). It has been surmised that, two species of the genus, the Amazon sailfin catfish *Pterygoplichthys pardalis* (Castelnau, 1855) and the vermiculated sailfin catfish *Pterygoplichthys disjunctivus* (C. Weber, 1991) are present in the EKW (Hussan et al., 2018). Tonnes of these fishes were brought to the land everyday during commercial fishing in EKW and also their occurrences and catch have been reported from many other parts of the country (Hussan et al., 2019; Sinha et al., 2010). However, skeletal anomalies in *Pterygoplichthys* spp. have not yet been reported from any parts of India.

2. MATERIALS AND METHODS

East Kolkata Wetlands (EKW) located between 22°25' and 22°40' North and 88°20' and 88°35' East, is the World's largest wastewater fed aquaculture system covering ~4,000 ha, where city sewage is utilized for traditional aquaculture and agriculture (Hussan, 2016; Anonymous, 2021). An estimated 30–50% of the sewage generated by the Kolkata City is treated and reused by the fishponds of the EKW and produces over 15,000 MT fish per annum from its 264 functioning aquaculture ponds, locally called bheries (Hussan, 2016; Mandal et al., 2018). *Pterygoplichthys* spp. got entry into this system through deliberate introduction and invaded widely in EKW water bodies through the sewage feeder channels (Hussan et al., 2019; Hussan et al., 2020). All the specimens of *Pterygoplichthys* spp. were collected during scientific survey of north-western part (covering water area ~315 ha) of this wetland (Figure 1). Samples were collected in 34 fishing hauls (covering about 5,000 sq m area each time) made bimonthly using beach seines (48 m length, 5.4 m height and 15–30 mm mesh size) during June 2019–December 2020. During the course of sampling survey eighteen specimens of *Pterygoplichthys* spp. showing spinal deformities were captured.

The fishes were identified following the method provided by Armbruster and Page (2006) and Wu et al. (2011). The total length (TL) of the deformed and normal fishes was measured using digital caliper (0.10 cm accuracy) and the body weight (W) using an electronic balance (0.10 g accuracy). Fresh deformed specimens and normal specimen samples were than radio-graphed using a medical X-ray system. The lengths (L)-weight (W) relationships of the normal and deformed fish were also calculated using

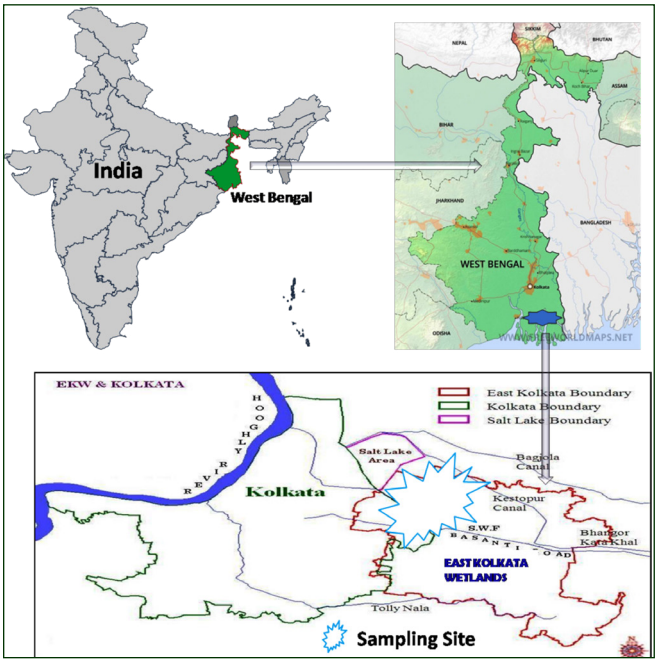

Figure 1: Collection site of *Pterygoplichthys pardalis*

the equation: log W= log *a* + *b* log L, where a and b are regression parameters (Froese, 2006). The significance of the regression between LWRs of fishes was tested by ANOVA. Differences between values of normal and deformed fish species were tested using the student's t-test.

3. RESULTS AND DISCUSSION

A total of 2432 specimens of *Pterygoplichthys* spp. were caught during the study, of which 312 specimens were identified as *Pterygoplichthys pardalis*. Spinal deformations, with humps and variable degree of curvatures in the posterior half of spinal column, were detected in eighteen specimens (5.76%) immediately after capture (Figure 2). All the eighteen deformed specimens were identified as *Pterygoplichthys pardalis*. Two types of spinal deformities, scoliosis and kyphosis, were determined. Thirteen of the specimens had severe kypho-scoliosis, while others had mainly scoliosis in the caudal region. According to Lovett et al. (2020) lordosis-kyphosis-scoliosis (LKS), the three major vertebral curvature types can develop in isolation or in various combinations within an individual. Occurrence of all three types of curvature in same individual though documented rarely; lordo-kyphosis, lordo-scoliosis and kypho-scoliosis in same individual has been well reported (Boglione et al., 1995; Jawad et al., 2017). The X–ray radiograph of highly deformed specimens showed that both the thoracic vertebrae and the whole caudal vertebrae were involved in the deflection of the shape of the specimen (Figure 3). However, it's still to be established whether these curvature types are, in fact, related, or they arises via a separate aetiology (Lovett et al., 2020). Total length of the deformed specimens was found significantly lesser compared to the normal specimens of comparable weight group, and '*b*' value (1.679) was significantly lesser than the expected (Table 1). However, in normal specimens 'b' value (2.567–2.734) were in the expected range of 2.5–3.5 (Froese, 2006), and comparable to earlier findings (Hussan et al., 2019).

Spinal anomalies in fish can be physical, genetic/heritable and/or environmentally induced (Bardon et al., 2009; Golubtsov et al., 2021). However, in natural population such abnormalities are considered linked more to pollution (Jawad et al., 2017). Organic contaminants such as organochlorines, polychlorinated biphenyls, fluorinated herbicides; metals like mercury, cadmium, lead and other pollutants can cause skeletal deformities in fish by altering bone metabolism and causing protoplasmic poisoning (Messaoudi et al., 2009). High variation in water temperature and hypoxia can also induce spinal malformations during embryonic

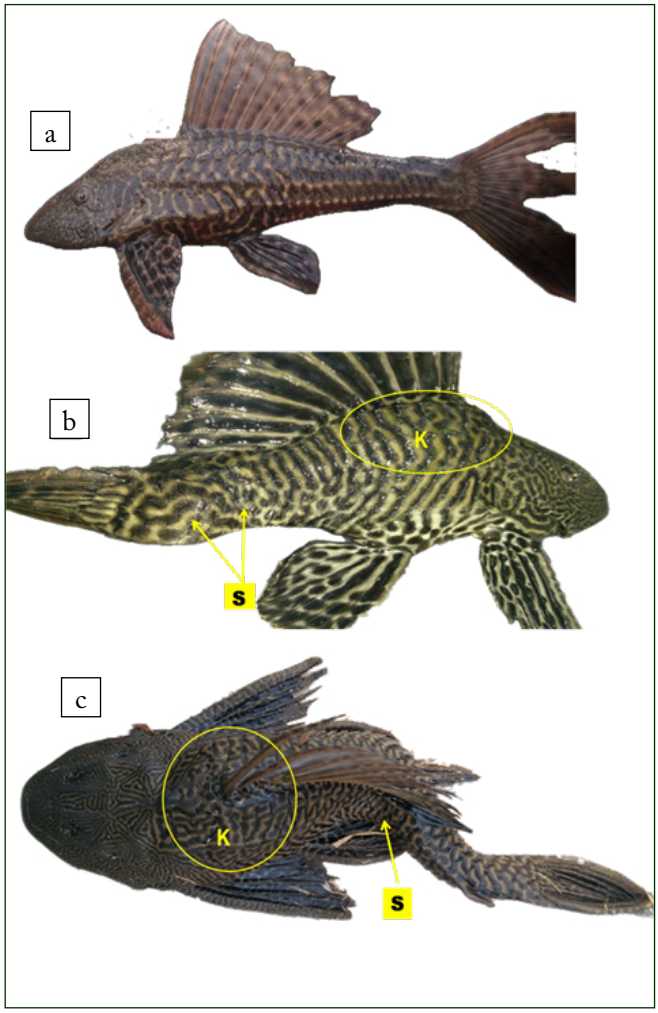

Figure 2: Dorsolateral view of normal (a) and deformed (b and c) *Pterygoplichthys pardalis* from EKW, India. K: Kyphosis, S: Scoliosis

Table 1: Descriptive statistics and length-weight relationship parameters for deformed and normal specimens of *Pterygoplichthys pardalis* collected from East Kolkata Wetlands, India

P. pardalis	n	Total length (cm)			Weight (g)			Regression parameters		95% confidence intervals		r^2
		Min	Max	Mean± SD	Min	Max	Mean±SD	a	b	a	b	
Deformed	8	23.8	29.1	26.4±2.04^{ap}	249	354	305.05± 40.29^{ap}	1.245	1.679	0.271–5.702	1.214–2.144	0.963
Normal (CW)	20	29.2	37.3	33.92±2.51^{b}	208.6	387	300.21± 62.25^{a}	0.018	2.734	0.003–0.113	2.232–3.281	0.937
Normal (CL)	20	22.1	29.2	26.12±2.21^{p}	95.8	208.6	146.16± 34.15q	0.033	2.567	0.004–0.296	1.896–3.237	0.891

Normal (CW): Normal specimens (length) of the species having comparable weight; Normal (CL): Normal specimens (weight) of the species having comparable length; n: Sample size; Min: Minimum; Max: Maximum; a: Intercept; b: slope; r^2-Coefficient of determination; Note: Superscript a, b for deformed vs Normal (CW) and superscript p, q for deformed vs Normal (CL) comparison. $p<0.05$

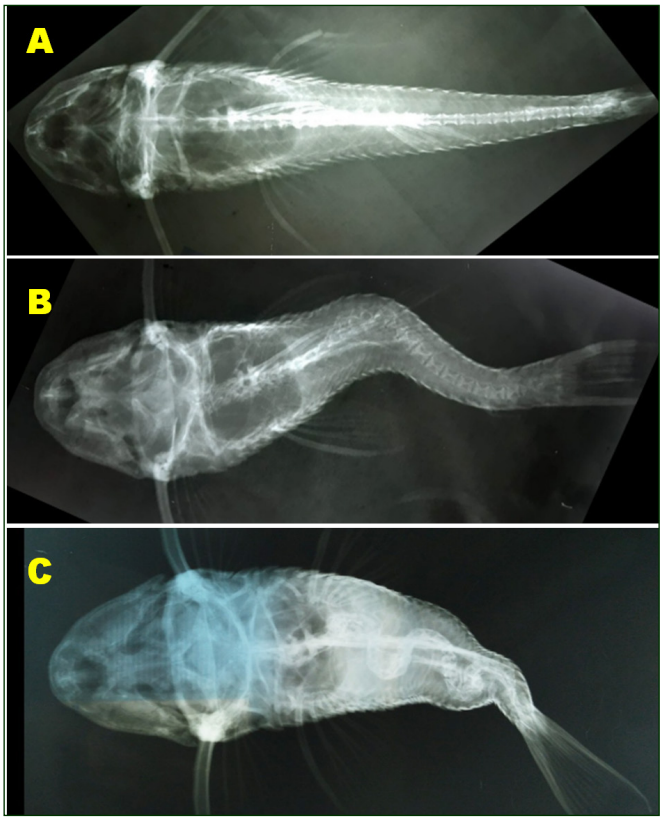

Figure 3. Dorsal view X-ray radiographs of *Pterygoplichthys pardalis* from EKW, India. A-normal specimen, B & C- sample specimen with kyphosis-scoliosis

development and the first larval stage (Castro et al., 2011). In carps and salmons, such deformities have been well reported (Grimmett et al., 2011, Fjelldal et al., 2021), however in Siluriformes such information is very limited. Only few reports are available, like, Wakida-Kusunoki et al. reported Kyphosis and scoliosis in wild population of *Pterygoplichthys pardalis* in 2014 and in hardhead catfish *Ariopsis felis* (Linnaeus, 1766) in 2017 and argued pollution as a probable reason.

Because of isolated observations, we also could not able to confirm the specific cause for the spinal deformities of *P. pardalis* noticed in the present study. However, as the specimens collected were from a wastewater-fed fisheries system, stress of metal and other organic contaminants could be suggested as the cause of this impairment. The EKW receives about 600 million litres of industrial and municipal discharge from Kolkata city every day (Hussan, 2016; Mandal et al., 2018). These wastewaters are then introduced into the fishponds in batches or continuously, through sewage feeder channel, mainly taking advantage of gravity and also used for agriculture purposes (Anonymous, 2021). However, inflowing wastewater has also given rise to the metals concentrations in the sediments of the wetlands. Khatun et al. (2016) reported metals concentrations higher than the permissible in the sediments of the wetlands. Studies also confirmed bioaccumulation of heavy metals in fish and vegetables produced in EKW (Anonymous, 2021).

4. CONCLUSION

The study described the occurrence of spinal deformities in a wild population of *P. pardalis*. Even though the exact cause of spinal deformities observed in the present study cannot be determined, skeletal deformities in wild population are frequently an indicator of exposure to different toxic substances. In this context the current information, indicate the need of re-evaluation of different pollutant levels in wastewater feed aquaculture of EKW, to avoid foreseeable public health issues.

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